File No: STD/1443

August 2013

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

PEG-4 Rapeseedamide

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 Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

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

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

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

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1443	Kao Australia Pty Ltd Johnson & Johnson Pte Ltd L'Oreal Australia Pty Ltd	PEG-4 Rapeseedamide	Yes	≤20 tonne/s per annum	Component of cosmetics, industrial cleaning products, lubricants and additive in photochemical production

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*
Skin Irritation (Category 2)	H315 – Causes skin irritation

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:

R38: Irritating to skin.

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

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 a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  – Skin Irritation (Category 2): H315 – Causes skin irritation

- The following should be used for products/mixtures containing the notified chemical:
  ≥ 10%: Skin Irritation (Category 2): H315 – Causes skin irritation

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced for reformulation:
  - Enclosed processes, where possible
  - Local ventilation systems, if aerosols may be generated

- 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 for reformulation:
  - Avoid contact with skin and eyes
  - Avoid breathing mists/vapours

- 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 for reformulation:
  - Goggles
  - Coveralls
  - Impervious gloves
  - Respiratory protection if aerosols are expected to be generated

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

- Formulators should take the following measures to minimise occupational exposure to the notified chemical:
  - Take account of the irritation potential of the notified chemical when formulating industrial rinse-off cleaning products.

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

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

Public Health

- The following measures should be taken by formulators to minimise public exposure to the notified chemical:
  - Take account of the irritation potential of the notified chemical when formulating cosmetic products.

- The following measures should be taken by suppliers and formulators to minimise public exposure to hazardous impurities in the notified chemical:
  - Take account of the level of 1,4-dioxane in the notified chemical and in products available to the public, noting that products containing >100 mg/kg or >100 mg/L of 1,4-dioxane are listed in Schedule 6 of the Poisons Schedule (SUSMP).

Disposal

- The notified chemical should be disposed of 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 notified chemical is to be used in leave-on cosmetic products;
- the notified chemical is to be used in spray cosmetic products;
- the concentration of the notified chemical exceeds or is intended to exceed:
  - 8% in rinse-off cosmetic products, except hair dyes;
  - 15% in hair dye products;
- information on the repeated dose toxicity potential of the notified chemical via the inhalation route becomes available;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from component of cosmetics, industrial cleaning products, lubricants and additive in photochemical production 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.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Johnson & Johnson Pte Ltd (ABN: 24 922 851 374)
45 Jones Street
ULTIMO NSW 2007

Kao Australia Pty Ltd (ABN: 59 054 708 299)
1A The Crescent
KINGSGROVE NSW 2208

L'Oreal Australia Pty Ltd (ABN: 40 004 191 673)
564 St Kilda Road
MELBOURNE VIC 8008

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: other names, molecular and structural formulae, molecular weight, analytical data, degree of purity, polymer constituents, residual monomers, impurities, additives/adjuvants, use details, import volume, identity of testing facilities, and identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: adsorption/desorption, and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Amidet N (product containing the notified chemical)
Amidet TEC N (product containing the notified chemical)

CAS NUMBER
85536-23-8

CHEMICAL NAME
Amides, rape oil, *N*-(hydroxyethyl), ethoxylated

OTHER NAME(S)
PEG-4 Rapeseedamide (INCI name)

MOLECULAR WEIGHT
<600 Da

ANALYTICAL DATA
Reference NMR, IR and LCMS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY 60–80%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 ºC AND 101.3 kPa:

Property	Value	Data Source/Justification
Melting Point/Freezing Point	5–10 °C	Measured
Boiling Point	>262 °C at 102.3 kPa	Measured. Product decomposed before boiling.
Density	997 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.5 × 10^{-4} kPa at 25 °C	Measured
Surface Tension	50.9 mN/m at 22 °C	Measured
Pour Point	7 °C	Measured
Water Solubility	9.0 × 10^{-4} g/L at 23 °C	Reported as the critical micelle concentration
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year, pH 4 $t_{1/2}$ = 77.6 days, pH 7 $t_{1/2}$ > 1 year, pH 9	Measured
Partition Coefficient (n-octanol/water)	log Pow > 2.57	Measured. The notified chemical is surface active and is expected to partition to phase boundaries
Adsorption/Desorption	log Koc = 3.32–4.11	Calculated. The notified chemical is surface active and thus the estimation result may not entirely characterise adsorption/desorption behaviour of the notified chemical. The notified chemical is expected to partition to phase boundaries due to its surface activity.
Dissociation Constant	Not determined	Not expected to be ionised under environmental conditions given it contains no readily dissociable functional groups.
Flash Point	>200 °C at 101 kPa	Measured
Flammability	Not determined	Not expected to be flammable in air based on high flash point and low vapour pressure
Autoignition Temperature	378 °C	Measured
Explosive Properties	Predicted Negative	Does not contain explosophores
Oxidising Properties	Predicted Negative	Does not contain oxidising groups

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 as a component of formulated products (≤15% concentration). The notified chemical may also be imported as the raw material form at ≤60–80% purity, for reformulation into products at ≤15% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	10-20	10-20	10-20	10-20	10-20

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The notified chemical and products containing the notified chemical will be imported to Australia by ship. Subsequent transport within Australia will be by road. The notified chemical when imported as a component of a formulated product will be packaged in bottles or tubes, usually HDPE, up to 500 mL volume. Industrial end use containers will be packaged in containers up to a volume of 20 L.

USE
The notified chemical will be used in rinse-off cosmetics, hair dyes and industrial cleaning rinse-off products as a mild foam booster, solubiliser, liquid emulsifier, thickener and surfactant. The notified chemical will be formulated into products (≤15% concentration). Uses of the notified chemical will include cosmetic rinse-off products (≤8% concentration) such as shampoos, conditioners, hand soaps, shaving products and shower gels; rinse-off industrial creams and lotions (≤15% concentration); and hair dyes (≤15% concentration). Other industrial uses (≤15% concentration) will include as an additive in metal working fluids, as an emulsifier and metal corrosion protector, and in photochemicals as an emulsifier.

OPERATION DESCRIPTION
Reformulation
At the reformulation site, compounders will weigh the notified chemical into a separate container and then add directly to a flame-proof mixing tank. The tanks are closed systems and the attached pump equipment is designed to not create aerosols or dusts and is earthed for static discharge. After formulation, the products will be dispensed into end-use containers via pumping equipment. Exhaust ventilation is fitted when appropriate. Small spills of the notified chemical are absorbed with a suitable material, while larger spills will involve bunding.

QA chemists will scoop samples of the notified chemical for further analysis at multiple stages of production including the raw material as imported, bulk formulation and end use products. Samples will be kept for reference in appropriate storage.

Industrial end-uses
As an additive in metal working fluids or lubricants at industrial sites
The ready-to-use formulated mixtures are delivered to a larger number of final end users. The concentrated pre-mixture may contain the notified chemical (≤15% concentration) and is diluted before use by the end users. The concentration of the notified chemical in the final working fluid is typically ≤1.8%. During the use of metal working fluids emissions into the environment can occur. The metal working fluid flows on metallic surfaces to facilitate processes like drilling, cutting, forming and milling.

According to the BREF document for lubricants (OECD, 2004), the primary function of a lubricant is the reduction of friction. Lubricants can perform a number of additional functions, depending on the process and working environment, for example the removal of heat and wear particles from the load carrying zone. A lubricant may act as a sealant to prevent the ingress of foreign materials into the lubrication clearance zone or as protection agent against corrosion. After use the greatest fraction of metal working fluids and lubricants is collected, while a smaller fraction of <10% may remain on the metal surfaces. At the end of the life-time of the metal working fluids the emulsions are collected and acidified to obtain an oil-layer and a water layer. The oil-layer is disposed of as dangerous waste and incinerated. The water-layer may be treated further, for example by applying an oil interceptor, and then discharged into the sewerage system. The thin layer of metal working fluid remaining on the surfaces can be removed by dipping the respective metal parts into cleaning baths or treating surfaces with high-pressure cleaners.

Photochemical additives
The notified chemical may be used as an additive in photochemicals production. Industrial workers may use such photochemicals by blending, mixing, spraying, brushing, rolling, pouring or dipping articles into it.

Industrial cleaning and washing products.
Cleaning and washing agents containing the notified chemical (≤15% concentration) will be used by industrial and professional workers. They will be supplied as liquid products that may need to be diluted in water before use. Transfer processes of the notified chemical by workers at non-dedicated or dedicated facilities will vary. Industrial and professional cleaners may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines, or open processes and manually by rolling, brushing, spraying and dipping. The cleaning and washing liquids are completely discharged into industrial sewerage systems after use.

Cosmetics
The finished rinse-off cosmetic products containing the notified chemical (≤8% concentration) and hair dyes (≤15% concentration) may be used by consumers and professionals such as hairdressers or beauty salon workers. Depending on the nature of the product, these could be applied in a number of ways, such as by hand or by using an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and Storage	4	12
Chemist	3	12
Compounder	8	12
Packers	8	12
End users	8	365
Metal working users	8	220
Metal working (blending)	8	100
Photochemical workers	8	30
Beauty care professionals	6	220

EXPOSURE DETAILS
Note: The stated concentrations of the notified chemical are as a per cent of the raw material form of the notified chemical.

Transportation and Packaging
Worker exposure to the notified chemical during the importation, transport and storage of the notified chemical and products containing the notified chemical is not expected, except in the event of an accident where packaging may be breached.

Reformulation
Workers may be exposed to the notified chemical during collection of samples, formulation of products and packaging products into end-use containers. Dermal and ocular exposure to the notified chemical is expected. Such exposure will be minimised by the use of safe work practices and wearing personal protective equipment (PPE) including impervious gloves, coveralls and goggles. Inhalation exposure to the notified chemical may occur due to formulation occurring at elevated temperatures. However, this is expected to be minimised by the employment of closed production processes, local exhaust ventilation and respirator equipment where ventilation is inadequate.

As an additive in metal working fluids or lubricants at industrial sites
Workers may be exposed to the notified chemical (≤15% concentration) during blending and mixing processes. Dermal and ocular exposure to the notified chemical at lower concentrations may occur during application of the formulated products to substrates with roller, brush or dipping. Inhalation exposure may occur with industrial spraying of the blended formulations or through formation of mists during processing. Dermal and ocular exposure to workers would be mitigated through the use of personal protective equipment (PPE) including protective coveralls, impervious gloves and goggles. Inhalation exposure will be minimised by the use of local exhaust ventilation in areas around the machinery where appropriate; generation of aerosols

containing the notified chemical may vary depending on the processes carried out at individual workplaces.

Photochemical additives
Dermal, ocular and perhaps inhalation exposure to the notified chemical (≤15% concentration) may occur during the photochemical mixing process, the transfer of photochemicals to equipment, maintenance and service tasks.

Dermal and ocular exposure to workers would be mitigated through the use of personal protective equipment (PPE) including protective coveralls, impervious gloves and goggles. Inhalation exposure will be minimised by the use of local exhaust ventilation in areas around the machinery; generation of aerosols containing the notified chemical may vary depending on the processes carried out at individual workplaces.

Industrial cleaning and washing products
There will be widespread and repeated exposure of worker to the notified chemical (≤15% concentration) through the use of rinse-off industrial creams and lotions and washing products. The principal routes of exposure will be dermal and inhalation, while ocular exposure is also possible.

Beauty care professionals
Exposure to the notified polymer in end-use products (≤15% concentration) may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hair dressers, beauty salon workers). Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical (≤15% concentration).

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical (≤8% concentration in rinse off cosmetics, ≤15% concentration in hair dyes). The principal routes of exposure will be dermal and inhalation; ocular exposure is also possible, as is the potential for oral exposure, during facial use.

Data on typical use patterns of cosmetic product categories in which the notified chemical may be used are shown in the following table (SCCS, 2010; Cadby *et al.*, 2002). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. A dermal absorption of 100% was assumed for the notified chemical. An adult bodyweight of 60 kg was used for calculation purposes.

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Makeup remover	5000	8	0.1	0.67
Shower gel	18670	8	0.01	0.25
Hand wash soap	20000	8	0.01	0.27
Shampoo	10460	8	0.01	0.14
Hair conditioner	3920	8	0.01	0.05
Facial cleanser	800	8	0.01	0.01
Total				**1.38**

C - concentration; RF - retention factor.
Daily systemic exposure = Amount × C × RF × dermal absorption/body weight

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all rinse-off cosmetic products listed in the above table that contain the notified chemical. This would result in a combined internal dose of 1.38 mg/kg bw/day.

The notified chemical will also be used in hair dyes. Typical exposure to hair dyes includes application of 20 mg/cm^2 product to a scalp surface area of 580 cm^2 (SCCS, 2012). Given the proposed concentration of up to 15% in hair dye products, an amount of 1740 mg notified chemical is estimated to be used per application. Using a retention factor of 0.1, body weight of 60 kg, dermal absorption of 100%, and frequency of use of one application per 28 days, the equivalent daily systemic exposure is 0.10 mg/kg bw/day.

The potential systemic exposure to members of the public from the combined use of rinse-off cosmetics and hair dyes is 1.48 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. All testing was performed with the raw material where the notified chemical is of 60–80% purity. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity (Test 1)	LC50 1–5 mg/L/4 hour; harmful
Rat, acute inhalation toxicity (Test 2)	LC50 > 6 mg/L/4 hour; low toxicity
Rabbit, skin irritation	severely irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – non-adjuvant test	no evidence of sensitisation
Human, skin sensitisation – RIPT (0.5%)	no evidence of sensitisation
Rat, repeat dose (oral gavage) toxicity – 28 days	NOAEL 150 mg/kg bw/day (female) NOAEL 15 mg/kg bw/day (male)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro (micronucleus)	non genotoxic
Genotoxicity – in vivo (chromosomal aberration)	non clastogenic
Rat, reproductive and developmental toxicity	NOEL 500 mg/kg bw/day (parental and progeny toxicity)

Toxicokinetics, metabolism and distribution.
The commercial form of the notified chemical contains significant proportions of impurities (purity is 60–80%). The notified chemical is expected to have potential for dermal absorption, based on its low molecular weight and log Kow of 2.6. Furthermore, it is a surfactant, which may enhance the dermal absorption of other compounds. The repeated dose study indicates that the notified chemical is distributed systemically.

Acute toxicity.
The notified chemical was of low acute toxicity via the oral and dermal routes. In two acute inhalation studies carried out to OECD Test Guidelines, the notified chemical was harmful in one study and of low toxicity in a later study, based on the differences in mortality in the two studies. Adverse clinical signs were observed in both studies, and macroscopic effects in organs were seen in the first study only. The first study was conducted to OECD TG403 ($C \times t$ protocol) using 2 male rats per exposure time but did not have a GLP certificate. The second study was conducted to OECD TG 436 (acute toxic class method) and had a GLP certificate. Therefore, on a weight of evidence basis, as OECD TG 436 requires a larger test cohort, it is likely that the notified chemical does not meet the criteria for classification for acute toxicity via inhalation. However, adverse effects as a result of acute inhalation exposure cannot be ruled out.

Irritation and sensitisation.
The notified chemical is a severe skin irritant to rabbits at the tested concentration (~70%, raw material) and is a slight eye irritant to rabbits. Under the GHS, the notified chemical is classified as a skin irritant at concentrations of 10% and above. Data on respiratory tract irritation was not available. There was no evidence of sensitisation in a guinea pig study, or in a repeat insult patch test in humans at 0.5%.

Repeated Dose Toxicity.
In a 28-day oral gavage repeated dose toxicity study in rats, the NOAEL was determined to be 150 mg/kg bw/day in females and 15 mg/kg bw/day in males. A NOAEL of 150 mg/kg bw/day was chosen for the public health risk assessment, as the effects at 15 mg/kg bw/day were minimal. The primary adverse effect of the study was irritation of the forestomach leading to acanthosis and hyperkeratosis of the forestomach, occasionally with associated subepithelial inflammatory cell infiltrates. Cortical and thyroid hypertrophy were observed in some animals at the high dose level. Data on repeated dose toxicity via the dermal and inhalation routes was not available.

Mutagenicity/Genotoxicity.
The notified chemical was non mutagenic in a bacterial reverse mutation test. There was no evidence of clastogenicity in an *in vitro* mammalian chromosome aberration test in cultured human lymphocytes or in an *in vivo* mouse micronucleus test in bone marrow cells of mice. Based on these studies, the notified chemical is not expected to be genotoxic.

Toxicity for reproduction.
In a 55-day oral gavage reproduction development toxicity study in rats the NOEL was determined to be 500 mg/kg bw/day. No significant macroscopic findings on necropsy or adverse clinical observations were recorded during the study for test animals or rat litters. The significance of changes in the surface righting reflex in the filial generation is not clear. In this study only reproductive tissues were analysed at necropsy.

Impurities
The notified chemical contains low levels of a hazardous impurity, 1,4-dioxane, which is included in Schedule 6 of the Poisons Standard (SUSMP) at levels down to 100 mg/kg or 100 mg/L. The level of 1,4-dioxane is higher in the marketed product Amidet TEC N than in Amidet N.

Based on the structure of the notified chemical, it is not expected to contain hazardous nitrosamine impurities.

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***
Skin Irritation (Category 2)	H315 – Causes skin irritation

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(s):
R38: Irritating to skin

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is expected to have irritation potential via all routes of exposure and is classified as a skin irritant. Based on acute inhalation studies, adverse systemic effects may occur after inhalation exposure. Adverse systemic effects were seen after repeated dose exposure.

Reformulation
Workers may experience dermal and accidental ocular and perhaps inhalation exposure to the notified chemical (≤80% purity) during formulation processes. At such concentrations, skin and eye irritation effects may occur. The use of enclosed, automated processes and PPE (impervious gloves, goggles, coveralls, and respiratory protection if significant inhalation exposure is expected) should minimise the potential for exposure.

Therefore, provided that adequate control measures are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use
Workers with highest potential for exposure to products containing the notified chemical (<15% concentration) include metal workers and photochemical workers, when conducting manual processes (e.g. machining, mixing and servicing). Exposure is most likely to occur via the dermal route, although ocular and inhalation exposure to the notified chemical may also occur if mists or aerosols are generated.

Use of engineering controls to minimise manual handling, safe work practices to avoid contact with the notified chemical, and use of PPE would minimise occupational exposure to the notified chemical. Suitable PPE would include impervious gloves, goggles and protective coveralls. If mists or aerosols are expected to be generated, local exhaust ventilation in the areas surrounding the machinery and enclosed/automated processes (where possible) would minimise inhalation exposure of workers to the notified chemical. Respiratory

protection should be worn by workers if local exhaust ventilation cannot be employed and/or the general ventilation is inadequate. The risk to workers is not considered to be unreasonable if such controls are in place.

Cleaning workers and beauty care professionals will handle the notified chemical at ≤15% concentration, similar to public use. The risk to cleaning workers who regularly use products containing the notified chemical is expected to be of a similar extent than that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment, see Section 6.3.2.

6.3.2. Public Health

The general public will be repeatedly exposed to the notified chemical during the use of rinse-off cosmetic products containing the notified chemical (proposed to be used at ≤8% concentration in rinse off products and ≤15% in hair dyes).

Local effects

Based on the information available, the notified chemical is considered to be a severe skin and slight eye irritant and is likely to be irritating to the respiratory system. As the notified chemical will be present in cosmetic and hair dye products at concentrations up to 15%, irritation effects may occur. However these effects are expected to be lessened as the products will be rinsed off the skin.

Systemic effects

The potential systemic exposure to the public from the use of the notified chemical in cosmetic products was estimated to be 1.48 mg/kg bw/day (see Section 6.1.2.). Using a NOAEL of 150 mg/kg bw/day, which was derived from a repeated dose toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 101. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences, therefore, the MOE is considered to be acceptable.

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 into Australia for use as a component in rinse-off cleaning products, cosmetics, metal working fluids, photochemicals and other industrial products. Reformulation of the notified chemical into the finished products is likely to take place locally. During any formulation and mixing, release of the notified chemical to the environment is expected to be negligible as these processes occur in closed systems in industrial settings. Some residual notified chemical (1% of the total import volume) may remain in empty import containers. Empty containers are expected to be washed with water and be disposed of to landfill with rinsate being disposed of to sewers.

RELEASE OF CHEMICAL FROM USE

The products containing the notified chemical, such as detergent, shampoos and hand soaps, are either washed off the hair and skin of consumers, or disposed of following cleaning activities. Therefore, when it is used as a component in rinse-off cleaning products and cosmetics, the entire amount of notified chemical is expected to be released to the sewer in Australia.

When it is used as an additive in metal working fluids, the metal working fluids containing the notified chemical will be circulated through contained systems until they are spent. Spent metal working fluids are expected to be collected for disposal by an approved waste management company.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in end-use containers for rinse-off cleaning products and cosmetics (3%) are expected to share the fate of the container and be disposed of to landfill, or to be washed to sewer when containers are rinsed before recycling.

Residues in empty containers for metal working fluids, spills and spent metal working fluids are expected to be collected for disposal by an approved waste management company. The waste metal working fluids are likely to be finally disposed of to sewer via wastewater treatment at approved waste management company sites.

7.1.2. Environmental Fate

The notified chemical is expected to be readily biodegradable based on the biodegradation studies conducted on the notified chemical or the products containing the notified chemical. The majority of the notified chemical is expected to be released to the sewerage system. In waste water treatment processes in sewage treatment plants, a high proportion of the notified chemical is expected to be removed from influent due to its expected sorption to sludge and sediment, and rapid biodegradation. The notified chemical that partitions to sludge will be removed for disposal to landfill or used on land for soil remediation. In sludge, soil and water, the notified chemical is expected to be degraded by abiotic and biotic processes to form water and oxides of carbon and nitrogen.

Due to its dispersibility, a small proportion of the notified chemical may be discharged in treated effluent to receiving waters where the chemical is expected to partition to suspended solids and organic matter and is anticipated to disperse and rapidly biodegrade. Based on its surface activity, the notified chemical is not expected to bioaccumulate. For the details of the environmental fate studies please refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical is expected to be released to sewers following its use as cosmetic products or rinse-off cleaning products. The wastewater streams receiving the spent notified chemical may also be directed to sewers after onsite treatment. Therefore, under a worst case scenario, it is assumed that 100% of the total import volume of the notified chemical will be used as additive in metal working fluids and the entire volume will be discharged into sewers over 220 days per year corresponding to release only on working days. Assuming no removal of the notified chemical in the sewage treatment processes, 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	20,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	20,000	kg/year
Days per year where release occurs	220	days/year
Daily chemical release:	90.91	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:	20.1	µg/L
PEC - Ocean:	2.01	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 20.1 µg/L may potentially result in a soil concentration of approximately 0.134 mg/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 0.67 mg/kg and 1.34 mg/kg, respectively. These are estimated maximum values assuming no removal of the notified chemical from STPs. However, the actual level of the notified chemical from STP effluent is expected to be much lower due to sorption to sludge and removal by biodegradation.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical or the products containing 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		
Zebra fish	96 h LC50 = 4.5 mg/L	Toxic to fish
Rainbow trout	96 h LC50 = 2.9 mg/L	Toxic to fish
Daphnia Toxicity	48h EC50 = 3.8 mg/L	Toxic aquatic invertebrates
Algal Toxicity		
Scenedesmus subspicatus	72 h E_rC50 = 410 mg/L	Not harmful to algae
	NOE_rC = 4.9 mg/L	No long lasting effects to aquatic life
		Not harmful to algae
Skeletonema costatum	72 h E_rC50 > 100 mg/L	No long lasting effects to aquatic life
	NOE_rC > 10 mg/L	
Inhibition of Bacterial Respiration	EC50 > 1000 mg/L	Not inhibitory to microbial respiration

Classification should be based only on toxic responses observed in the soluble range. The dispersibility limit, the limit at which phase separation takes place, may be an appropriate parameter in lieu of solubility for toxicity classification for surface active substances. For surface active substances toxic effects should be compared to the dispersibility limit or critical micelle concentration (CMC) rather than its water solubility limit. Toxic responses were observed at concentrations higher than the reported CMC. However, the dispersibility limit was not observed in the fish and daphnia studies. Moreover, the microscopic inspection of the dead fish and immobilised daphnia in the fish and daphnia studies indicated that physical toxicity was not a factor at the pivotal test concentrations. Therefore, the fish and daphnia toxicity values reported here are considered acceptable for the purpose of the classification.

Based on acute toxicity to fish and daphnia, the notified chemical is formally classified as 'Acute Category 2; toxic to aquatic life' under the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS). The notified chemical is not formally classified for long term hazard under the GHS based on its low chronic toxicity to algae and limited potential for bioaccumulation.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the acute fish toxicity (96 h LC50 = 2.9 mg/L) of the notified chemical. An assessment factor of 100 was used as acute 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 (Fish).	2.9	mg/L
Assessment Factor	100	
PNEC:	29	µg□L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC, the following Risk Quotient has been calculated.

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	20.1	29	0.693
Q - Ocean:	2.01	29	0.069

The risk quotient for discharge of effluents containing the notified chemical to the aquatic environment, assuming a worst case with no removal during sewage treatment plant (STP) processes, indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual use quantity. Based on the calculated risk quotient, the safety margin is relatively narrow in this case. However, the worst case scenario for aquatic exposure assumes no removal of the notified chemical at STPs although efficient removal of the notified chemical is expected due to the sorption to sludge and removal by biodegradation. The notified chemical has a low potential for bioaccumulation and is not expected be persistent in the environment, due to its biodegradability. On the basis of the PEC/PNEC ratio, maximum annual use volume and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point 5–10 °C

Method ASTM 57C
Remarks
Test Facility Test Facility D (2012)

Boiling Point (Test 1) >262 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
Remarks The product decomposed before boiling.
Test Facility Test Facility D (2012)

Boiling Point (Test 2) >262 °C at 102.3–102.8 kPa

Method OECD TG 103 Boiling Point.
Remarks The product decomposed before boiling. DSC measurement.
Test Facility Test Facility J (2003a)

Density (Test 1) 997 kg/m^3 at 20 °C

Method ISO 758.
Remarks Pycnometer method conforming to ISO 3507.
Test Facility Test Facility D (2012)

Density (Test 2) 997 kg/m^3 at 20 °C

Method OECD TG 109 Density.
Remarks Pycnometer method.
Test Facility Test Facility J (2003a)

Vapour Pressure 2.5×10^{-4} kPa at 25 °C

Method EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Vapour pressure balance method. Average of 10 runs.
Test Facility Test Facility C (2011a)

Pour Point 7 °C

Method OECD TG 102 Pour Point.
Remarks Glass jacket water bath method.
Test Facility Test Facility J (2003a)

Water Solubility CMC = 9×10^{-4} g/L

Method OECD TG 105 Water Solubility.
Remarks Flask Method. The test material formed an emulsified solution upon shaking with the distilled water. Therefore, the flask method may not be applicable to the test substance.
Visual estimation was also carried out after the test solutions were ultrasonicated for 15 minutes. At the test concentration of 4.0×10^{-3} g/L, the solution was clear colourless and no excess test material. At the test concentration of 9.4×10^{-3} g/L, the solution was slightly hazy with excess test material (Safepharm, 2003a)

The notified chemical is surface-active and has a complex solubility behaviour. The critical micelle concentration (CMC) is therefore an appropriate parameter for the solubility value. The critical micelle concentration (CMC) of the notified chemical was reported to be 0.9 mg/L (Krüss, 2009).
Test Facility Test Facility J (2003a) and Test Facility E (2009)

n-Octanol Solubility 652–702 g/L at 20 °C

Method Based on OECD TG 105.

Remarks Flask Method. The test substance is highly soluble in n-octanol and it is impossible to prepare sample at five times the estimated saturation concentration as per protocol requirments. The n-octanol solubility was determined by visual assessment.

The mixtures of test substance and n-octanol were shaken for 24 hours at 30 °C. Visual estimation was carried out after equilibrating the solution for 24 hours at 20 °C. At the test concentration of 652 g/L, the solution was clear and no undissolved particles observed. At the test concentration of 702 g/L, the solution was translucent and undissolved particles were observed.

Test Facility Test Facility C (2011b)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$*
4	25	> 1 year
7	25	77.6 days
9	25	> 1 year

Remarks Test samples were prepared at nominal concentration of 2.0×10^{-3} g/L in the three buffer solutions with 1% of co-solvent of methanol or acetonitrile to increase solubility.
Less than 10% hydrolysis was observed at pH 9.0 after 5 days at 50 °C in the preliminary test, equivalent to a half-life of greater than 1 year at 25 °C. No further test at pH 9 was conducted as per protocol requirements. Results from the preliminary test indicated that further tests need to be conducted at pH 4 and 7 to estimate the half-lifes at 25 °C. Tier 2 tests were conducted and the half-lifes were calculated to be 433 days and 77.6 days at 25 °C for pH 4 and 7, respectively.

At pH 1.2 and 37 °C the test substance did not show pseudo first order kinetics, therefore the half life was not calculated.

Test Facility Test Facility J (2008a)

Partition Coefficient (n-octanol/water) log Pow = 5.57–5.73 at 30 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).

Remarks HPLC Method. In the preliminary test, the log Pow of the test substance was estimated to be > 4.14 based on the individual solubilities in water and n-octanol. Therefore, the HPLC method was selected and carried out without pH adjustment to the mobile phase as the test substance is not expected to dissociate at the experimental pH of 8.1. The determined log Pow was reported to be 5.57-5.73. However, as the test substance shows surface activity, the HPLC method used here may not be applicable. Moreover, multiple peaks were observed in the HPLC chromatography with only partial interpretation in the report. Therefore, the results reported here should be treated with caution.

Test Facility Test Facility J (2003a)

Partition Coefficient (n-octanol/water) log Pow > 2.57 at 25 °C

Method OECD TG 123 Partition Coefficient (n-octanol/water).

Remarks In the preliminary test, log Pow of the test substance was estimated to be > 5.63 based on the approximate solubilities in water and n-octanol, respectively.

The slow stirring method was used to reduce contamination if the water phase with micro-droplets of the organic phase. On sampling the aqueous phase, the samples were

observed to be contaminated with the organic phase, however, it was reported that the organic phase contamination could be removed from the aqueous phase by centrifugation. Therefore, it was considered that the formation of micro-droplets would not have significant effects on the study. However, the test substance has surfactant activity and its critical micelle concentration (CMC) was reported to be 0.9 mg/L. As the concentration of the test substance in the organic phase was required to be 50% of the CMC in the partition coefficient test, it was not possible to achieve the sensitivity of analysis required for determination of the expected levels of test substance in the aqueous phase. Based on the limit of quantification of the analytical methodology, the log Pow was reported to be > 2.57.

At a nominal concentration of 1.05 µg/L, the recovery of the analysis sample for the aqueous phase was between 63.3–186%. Due to the presence of interfering species in the recovery and aqueous matrix blanks, the recovery data was considered to be acceptable.

This method applies to pure substances that do not display significant interfacial activity, and as the test substance has surface active properties, these results should be treated in caution.

Test Facility Test Facility C (2011b)

Surface Tension 50.9 mN/m at 22 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks Concentration: 1×10^{-2} g/L
The material was considered a surface active agent.
Test Facility Test Facility J (2008a)

Adsorption/Desorption log K_{oc} = 3.32-4.11

Method Calculated using Quantitative Structure Activity Relationship (QSAR).
Remarks The organic carbon-water partition coefficients were calculated to be 3.32–4.11 for the major components of the product containing the notified chemical using estimation software, KOCWIN v 2.0, EPI suite 4.0 (US EPA, 2009).

QSAR calculation may not entirely characterise adsorption/desorption coefficient for surface active agents. However, it is expected that the notified chemical will partition to phase boundaries.

Test Facility Test Facility C (2010a)

Dissociation Constant Not expected to ionise at environmental pH (4-9)

Method Calculated using estimation software Advanced Chemistry Development (ACD) I-Lab Web Service (ACD/pKa 8.03)
Remarks The nitrogen groups of the notified chemical were estimated to dissociate at pH of greater than 15 and the hydroxyl groups were estimated to be in it ionised form at pH of great than 14. The notified chemical is not expected to be ionised under environmental condition given it contains no readily dissociable functional groups.
Test Facility Test Facility C (2010b)

Flash Point (Test 1) >200 °C at 101 kPa

Method ASTM D6450-99
Remarks Test substance did not flash
Test Facility Test Facility D (2012)

Flash Point (Test 2) None below its boiling temperature

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Closed cup method.
Test Facility	Test Facility J (2008b)

Autoignition Temperature 378 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases). EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks	
Test Facility	Test Facility J (2008b)

Explosive Properties Predicted Negative

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	
Test Facility	Test Facility J (2008b)

Oxidising Properties Predicted Negative

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	
Test Facility	Test Facility J (2008b)

Reactivity Stable

Method	In-house method
Remarks	Stable in the organic solvents acetone, n-octanol and methanol at 10 g/L for at least 28 days at 25 °C
Test Facility	Test Facility C (2010c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 401 Acute Oral Toxicity – Fixed Dose Method. EEC Directive 84/449/EEC Part B.1 Acute Toxicity (Oral Gavage).
Species/Strain	Rat/Wistar
Vehicle	None
Remarks – Method	No deviations from the protocol was recorded

RESULTS

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

LD50	2000 mg/kg bw
Signs of Toxicity	No mortality or clinical signs of ill health or behavioural changes were observed. Body weight gain was considered to be similar to that of untreated animals.
Effects in Organs	No abnormalities identified at necropsy.
Remarks - Results	

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Test Facility I (1991a)

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 CD
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No deviations from the protocol were recorded

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Dermal irritation was not observed.
Signs of Toxicity - Systemic	No signs of systemic toxicity were observed. All animals showed the expected increases in bodyweight.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	

CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Test Facility J (2007a)

B.3. Acute toxicity – inhalation (Test 1)

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity ($C \times t$ protocol). EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation).
Species/Strain	Rat/Wistar (Crl:(WI) BR strain)
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	½, 1, 2 and 4 hours
Physical Form	liqud aerosol
Particle Size	2.14 μm (GSD 2.31)
Remarks - Method	The second group was started before the end of the first group because of the deficit of the test item, due to change in legislation. The study did not have a GLP certificate.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Exposure time (hours)*	*Mortality*
		Nominal	*Actual*		
1	2 M	24.34	4.92	½	0
2	2 M	24.34	4.92	1	0
3	2 M	24.34	4.92	2	1
4	2 M	24.34	4.92	4	1

LC50	1–5 mg/L/4 hours
Signs of Toxicity	Clinical signs on the day of exposure comprised laboured and noisy respiration and sneezing. All animals from Group 1 and 2 recovered from Day 1 such that no significant clinical signs were noted. Two animals died of Group 3 and 4 at 1-day post exposure. The 2 surviving animals from Group 3 and 4 showed laboured and noisy respiration and sneezing on Day 1 post exposure.
Effects in Organs	Amongst animals that died during the study the following abnormalities were observed during necropsy. Lungs showed dark discoloration (red, diffuse, all lobes); non-collapsed (mottled, red-black all lobes). The liver was mottled (pale, all lobes). The right kidneys showed dilatation. The small and large intestines shows dilatation and a gelatinous yellow material.
Remarks - Results	

CONCLUSION	The notified chemical is harmful via inhalation.
TEST FACILITY	Test Facility F (2009)

B.4. Acute toxicity – inhalation (Test 2)

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar (Albino RccHan)
Vehicle	None
Method of Exposure	Oro-nasal exposure.
Exposure Period	4 hours
Physical Form	liquid aerosol
Particle Size	2.05–2.14 μm (GSD 2.33–2.48)
Remarks - Method	No deviations to the protocol were recorded.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
1	3 M, 3 F	119	6	0

LC50	6 mg/L/4 hours
Signs of Toxicity	Salivation was noted in all animals during exposure. After exposure decreased activity, ruffled fur, laboured breathing and breathing noises were noted in all males and persisted in all males until Day 2. Breathing noises persisted in 2 males up to Day 7. Breathing noises were recorded on Day 2 only in one female.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	Test Facility C (2012)

B.5. Skin sensitisation – Human volunteers

TEST SUBSTANCE	Notified chemical (applied at 0.5% dilution)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: 9 induction applications made on Monday, Wednesday, Friday of three consecutive weeks. Skin was assessed 24 hours after patch removal (or 48 hours for patches applied on Friday). Rest Period: Approximately 2 weeks. Challenge Procedure: 1 challenge application to a naïve site, followed by skin assessment 24, 48 and 72 hours after application.
Study Group	56 (45 F, 11 M) ranging in age from 18–71 years.
Vehicle	Water
Remarks - Method	Occlusive 2×2 cm patches containing 0.2 mL of diluted test material were held in place for 24 hours before removal by the applicants. Six female subjects withdrew for non-treatment related reasons.
RESULTS	
Remarks - Results	Scores of zero were noted at almost all induction and challenge observations. Three subjects had barely perceptible or spotty erythema on one occasion each during the induction phase. As the effects were slight and were not repeated, they were not considered significant..
CONCLUSION	The test substance was non-irritating and non-sensitising under the conditions of the test.
TEST FACILITY	Test Facility H (1998)

B.6. Irritation – skin

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EEC Directive 84/449/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Female
Vehicle	None
Observation Period	22 days
Type of Dressing	Semi-occlusive.
Remarks - Method	The skin area of two animals was re-shaved on multiple occasions to facilitate observations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2	4	3	4	>21 days	1
Oedema	2.7	2	2.3	3	>21 days	2

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

Remarks - Results: The skin irritation consisted of both erythema and oedema. There was no corrosive effect on the skin, and no signs of systemic toxicity. The skin irritation had not resolved within the study period in all animals. New skin formation, oedema and scaliness was seen in 2 animals at the last observation, on day 22.

CONCLUSION: The notified chemical is irritating to the skin.

TEST FACILITY: Test Facility I (1991b)

B.7. Irritation – eye

TEST SUBSTANCE: Notified Chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EEC Directive 84/449/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 Female
Observation Period: 7 days
Remarks - Method:

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	1.3	1.7	1.3	2	<7 days	0
Conjunctiva: chemosis	0	0.7	0.3	1	48 h	0
Conjunctiva: discharge	0.3	0.3	0.3	1	48 h	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results: Irritation of the conjunctivae had resolved in all three animals within 7 days.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: Test Facility I (1991c)

B.8. Skin sensitisation

TEST SUBSTANCE: Notified Chemical

METHOD: OECD TG 406 Skin Sensitisation – Maximisation Test.
EC Directive 96/54/EC B.6 Skin Sensitisation – Maximisation Test.

Species/Strain: Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY: Maximum Non-irritating Concentration:
intradermal: 0.01%
topical: 0.01%

MAIN STUDY

Number of Animals	Test Group: 10 Male Control Group: 5 Male
INDUCTION PHASE	Induction Concentration: intradermal: 0.2% in sesame oil topical: 10% in sesame oil
Signs of Irritation	Slight irritation observed in all animals
CHALLENGE PHASE	
1st challenge	topical: 0.01% in sesame oil
Remarks - Method	There were no significant deviations to protocols. One animal died prematurely in the test group during the main study.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*		
		24 h	*48 h*	*72 h*
Test Group	0.01%	0/10	0/10	0/9
Control Group	0%	0/5	0/5	0/5

Remarks - Results

CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Test Facility G (1999)

B.9. Repeat dose toxicity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley (Crl:CD (SD) IGS BR strain)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 1 h and 5 h (weekdays) after dosing
Vehicle	Arachis oil (BP)
Remarks - Method	Functional observations were not performed during week 3 due to technical error.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 M, 5 F	-	0
low dose	5 M, 5 F	15	0
mid dose	5 M, 5 F	150	0
high dose	5 M, 5 F	1000	0

Mortality and Time to Death

No treatment related deaths occurred during the study.

Clinical Observations

Increased salivation was detected up to 10 minutes after dosing, for animals of either sex treated with the high dose from day 4 onward. No treatment related behavioural, functional performance or sensory reactivity changes were detected during the study. Males in the high dose group showed a non-statistically significant reduction in bodyweight gain and food consumption during the study when compared with controls. A statistically significant ($p < 0.05$) reduction in body weight was detected in females in the mid dose group during week 2. No adverse bodyweight development was observed for the other groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Males in the high dose groups showed a statistically significant increase in albumin and reduction in plasma phosphorus. All individual values were within the normal range and in the absence of any consistent change in plasma calcium or renal function, the change was considered not toxicologically significant. Males in the high dose and low dose groups showed a non-dose dependent statistically significant reduction in absolute thymus weight.

Effects in Organs

No macroscopic abnormalities were detected in animals at necropsy. Cortical hypertrophy of the adrenal glands was observed in 3 females in the high dose group but not at any other dose level. Treatment related follicular cell hypertrophy of the thyroid gland was observed in male rats in the high dose group. Acanthosis and hyperkeratosis of the forestomach, occasionally with associated subepithelial inflammatory cell infiltrates, were observed as effect of treatment for rats of either sex in the high dose group. Subepithelial inflammatory cell infiltrates were observed in the forestomach of 2 males in the mid dose group.

Remarks – Results

The study authors noted that increased salivation is often reported following oral administration of unpalatable or slightly irritant test material formulation. They considered that the microscopic forestomach changes and reduced bodyweight were due to slight irritancy of the test material causing abdominal discomfort, and that the adrenal changes may reflect a non-specific stress response to the irritancy of the test material.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 15 mg/kg bw/day for males and 150 mg/kg bw/day for females in this study.

TEST FACILITY Test Facility J (2003b)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE Notified Chemical

METHOD

	OECD TG 471 Bacterial Reverse Mutation Test. EEC Directive 92/69/EEC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
Metabolic Activation System	S9-mix from Aroclor induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 312.5–5,000 µg/plate b) Without metabolic activation: 312.5–5,000 µg/plate
Vehicle	DMSO
Remarks - Method	The bacterial strains used did not include one of the groups recommended in the OECD TG (E. coli WP2 uvrA, or E. coli WP2 uvrA [pKM101], or S. typhimurium TA102) One strain only (TA100 without metabolic activation) was used for the preliminary toxicity test.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	>5000	None	Negative
Test 2		>5000	None	Negative
Present				
Test 1		>5000	None	Negative
Test 2		>5000	None	Negative

Remarks - Results	No toxicity or significant increases in the numbers of revertant colonies of bacteria were observed (all strains). The positive controls gave the expected increase in revertants, confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Test Facility J (1993)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EEC Directive 92/69/EEC B.10 Mutagenicity – In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human
Cell Type/Cell Line	Cultured peripheral lymphocytes
Metabolic Activation System	Microsomal enzymes (S9-mix) from Aroclor-1254 induced rat liver
Vehicle	DMSO
Remarks - Method	There were no deviations from the protocol.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 39*, 78.1*, 156.25*, 312.5, 625, 1250, 5000	4	20
Test 2	0, 39, 78.1*, 156.25*, 234.34*, 625, 1250, 5000	4	20
Test 2	0, 39, 78.1*, 156.25*, 234.34, 625, 1250, 5000	4	44
Present			
Test 1	0, 39*, 78.1*, 156.25*, 312.5, 625, 1250, 5000	4	20
Test 2	0, 39, 78.1, 156.25*, 234.34, 625, 1250, 5000	4	20
Test 2	0, 39, 78.1, 156.25, 234.34, 625*, 1250, 5000	4	44

*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		>156	>156	Negative
Test 2 (20h)		>234	>234	Negative
Test 2 (44h)		>156	>156	Negative
Present				
Test 1		>156	>156	Negative
Test 2		>156	>156	Negative
Test 2 (44h)		>625	>625	Negative

Remarks - Results	Both in the presence and absence of S9-mix, the notified chemical did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations. A slight increase in aberrations was seen in Tests 1 and 2 (20h) at the highest dose in the presence of metabolic activation, however the increase was small in comparison with the positive controls, and was not statistically significant. No increase in the number of polyploid cells was seen. Positive controls performed as expected, verifying 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 Test Facility J (1994)

B.12. Genotoxicity – in vivo

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EEC Directive 2000/32/EC B.12 Mutagenicity – Mammalian Erythrocyte Micronucleus Test.
Species/Strain Mouse/albino Crl:CD-1 (ICR) BR strain
Route of Administration Oral – gavage
Vehicle Arachis oil (BP)
Remarks - Method There were no deviations from the protocol. Doses were chosen on the basis of a preliminary toxicity test.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	14	-	24
I (vehicle control)	14	-	48
II (low dose)	7	100	24
III (mid dose)	7	200	24
IV (high dose)	7	400	24
IV (high dose)	7	400	48
V (positive control, CP)	5	50	24

CP=cyclophosphamide.

RESULTS
Doses Producing Toxicity Clinical signs were observed with the test material at and above 200 m/kg in both the 24 and 48-hour groups including hunched posture, ptosis and pallor of the extremities.
Genotoxic Effects None observed
Remarks - Results The notified chemical was found not to produce a significant increase in the frequency of micronuclei in polychromatic erythrocytes. No statistically significant decreases in the PCE/NCE ratio were observed in the 24 or 48 hour test dose groups when compared to controls, however marked decrease in the PCE/NCE ratio was observed in Group III and in Group IV at the48 h sacrifice time. These effects along with the clinical signs after dosing suggest the occurrence of systemic absorption.

CONCLUSION The notified chemical was not clastogenic to mice under the conditions of this in vivo mouse micronucleus test.

TEST FACILITY Test Facility J (2003c)

B.13. Reproduction/Developmental toxicity

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 421 Reproduction/Developmental Toxicity Screening Test
Species/Strain Rat
Route of Administration Oral – gavage
Exposure Information Exposure days: 55 days
Post-exposure observation period: 1 h and 5 h (weekday), 1 h (weekend)
Vehicle Arachis oil
Remarks - Method Dose levels were chosen on the basis of a previous toxicological study. There were no deviations from the protocol. On day 15 all animals were paired (1M, 1F). Following evidence of mating the males were returned to original cages and were killed on day 43. Females were allowed to give birth and maintain offspring until day 5 *post partum*, at which all females

and surviving offspring were killed and examined.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	10 M, 10 F	-	0
Low dose	10 M, 10 F	15	0
Mid dose	10 M, 10 F	150	0
High dose	10 M, 10 F	500	0

Mortality and Clinical Observations

There were no unscheduled deaths. Episodes of increased salivation were evident in animals of either sex in the high dose group. Isolated episodes of noisy respiration were detected in the males of the high dose group. Isolated episodes of increased salivation and noisy respiration were evident in some males of the mid dose group. No adverse effect on bodyweight development was detected for treated animals when compared to controls. No adverse effect on mating performance, fertility or gestation length was detected when compared to controls.

Effects on Dams

No female rats were found to be non-pregnant. Glandular hyperplasia was observed in the mammary tissues of all female animals examined consistent with pregnancy and lactation. No histopathological changes were observed in the ovaries or vagina. Observed changes in peripheral uterine tissues are a commonly encountered consequence of pregnancy and parturition in the female rat. One mid dose group female had a mass on the abdomen whilst one low dose group female had a mass on the left horn of the uterus.

Effects on Filial animals

No obvious effect on litter size, sex ratio and offspring viability was detected from treatment animals, in comparison to controls. No adverse effect on total litter weights or offspring bodyweight development was detected in treatment animals, in comparison to controls. The interim death offspring from the mid dose group revealed no milk in the stomach. There were no other macroscopic abnormalities or unscheduled deaths in the other groups. Lower results for the surface righting reflex were seen in the mid and high dose group. As the variability of results within these groups also increased, the significance of this change is not clear.

Other histopathology

No other significant histopathology was detected in the pituitary or seminal vesicles/coagulating glands. Changes observed in the testes and prostate of 3 animals are commonly seen among laboratory animals maintained to this age and were not considered treatment related.

Remarks - Results

Increased salivation often recorded following oral administration of an unpleasant tasting test material formulation and may not be indicative of systemic toxicity.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 500 mg/kg bw/day in this study.

TEST FACILITY Test Facility C (2009)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Product containing notified chemical (50–70%)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	TOC analyser for the measurement of dissolved organic carbon (DOC) TOC analyser for CO_2 analysis
Remarks - Method	Test was conducted according to the test guideline above without significant deviation from the protocol.

RESULTS

Test substance (CO_2 evolution)		*Sodium benzoate (CO_2 evolution)*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
6	60	6	70
10	90	10	77
20	88	20	92
28	96	28	100

Remarks - Results	The test substance attained 90% and 96% degradation after 10 and 28 days, respectively, indicating that the test substance satisfied the 10-day window. The test substance can be considered to be readily biodegradable. The degradation of toxicity control is 79% over 28 days, implying that the test substance was not toxic to the sewage treatment microorganisms used in the study. All validity criteria for the test were satisfied.
CONCLUSION	The test substance is readily biodegradable
TEST FACILITY	Test Facility J (2003d)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Product containing notified chemical (50–70%)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Chemical titration for CO_2 measurement
Remarks - Method	Test was conducted according to the test guideline above without significant deviation or the protocol.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation**
2	6	2	33
7	40	7	68
17	62	17	86
28	70	28	90

* Raw data interpreted from the degradation curve.

Remarks - Results	The test substance attained 71% degradation after 28 days and the 10-day window was met. The test substance is considered to be readily biodegradable. All validity criteria for the test were satisfied.

CONCLUSION The test substance is readily biodegradable

TEST FACILITY Test Facility B (1996)

C.1.3. Ready biodegradability

TEST SUBSTANCE Product containing the notified chemical (50–70%)

METHOD OECD TG 301 F Ready Biodegradability: Manometric Respirometry test.

Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD system OxiDirect
Remarks - Method	Test was conducted according to the test guideline above without significant deviation or the protocol.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
1	15.1	1	-2.8
7	49.8	7	64.1
10	62.6	10	72.4
14	62.6	14	76.0
21	77.6	21	76.8
28	77.6	28	81.2

Remarks - Results	The 10-day window began from day 1 and the degradation reached 62.6% after 10 days during the test. Thus the test substance met the 10-day window and is considered to be readily biodegradable. The toxicity control attained 53.7% degradability after 5 days, indicating that test substance is not inhibitory to microorganisms. All validity criteria for the test were satisfied.

CONCLUSION The test substance is readily biodegradable

TEST FACILITY Test Facility K (2009a)

C.1.4. Anaerobic biodegradability

TEST SUBSTANCE Notified chemical

METHOD EPA TG CG-2050 Anaerobic Biodegradation.
OECD TG 311 Anaerobic Biodegradability by Measurement of Gas Production

Inoculum	Activated sludge
Exposure Period	56 days
Auxiliary Solvent	None
Analytical Monitoring	Gas-tight syringe for the measurement of biogas production
Remarks - Method	Test was conducted according to the test guideline above without significant deviation or the protocol.

RESULTS

Test substance (biogas)		*Ethanol (biogas)*	
Day	*% Degradation*	*Day*	*% Degradation*
1	1.9	1	8.6
7	34.3	7	69.5
14	52	14	82
21	61	21	83
39	75	39	86
50	80	50	92
56	81	56	95

Remarks - Results: The test substance was transformed 81% into biogas. Inhibition of > 20% was observed in the toxicity study at the beginning of the test. However, the overall mean inhibition was 7.6% during the test, indicating that the test substance is not considered to inhibit microorganism respiration. All validity criteria for the test were satisfied.

CONCLUSION: The notified chemical has potential for anaerobic biodegradation

TEST FACILITY: Test Facility B (1999)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Product containing notified chemical (50–70%)

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

Species: Zebra fish (*Brachydanio rerio*)

Exposure Period: 96 hours

Auxiliary Solvent: None

Water Hardness: 108 mg $CaCO_3$/L

Analytical Monitoring: None

Remarks – Method: The test substance was mixed with water and stirred for 1 hour at 23 °C. No granules were observed in the test solutions at the nominal concentrations. The actual concentration of the test substance was not determined during the test. The test media were renewed every 24 hours. The test was conducted according to the test guideline above without significant deviations from the protocol.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
control	-	10	0	0	0	0	0
1.0	N/A	10	0	0	0	0	0
1.8	N/A	10	0	0	0	10	10
3.2	N/A	10	0	0	0	20	20
5.6	N/A	10	0	0	40	50	60
10	N/A	10	0	10	20	80	100

LC50: 4.5 mg/L at 96 hours (95% confidence limit: 3.5–5.7 mg/L).

NOEC: 1.0 mg/L

Remarks – Results: The results were based on nominal concentration as the actual concentration for the test substance was not measured.

No abnormal behaviours were observed for the fish at the test concentration of 1.0 mg/L during the 96-hours exposure period. At 1.8 mg/L and the higher concentrations, death and toxic signs were observed, e.g. slowly swimming, loss of equilibrium and pigment.

CONCLUSION The test substance is toxic to fish

TEST FACILITY Test Facility K (2009b)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE Product containing notified chemical (50–70%)

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

Species	Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	133 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks – Method	The required amount of test substance was added directly to each test vessel and dispersed in water. At the nominal concentration of 1.0, 1.8 and 3.2 mg/L, the test solutions were clear and colourless. At the nominal test concentration of 5.6 and 10 mg/L, the test solutions were homogenous dispersions and cloudy. The test media were renewed every 24 hours. The number of mortalities and any sub-lethal effects of fish in each test and control vessel were determined 3 and 6 hours after the exposure and then daily throughout the test. The test was conducted according to the protocol and without significant deviation from the protocol.

RESULTS

*Concentration mg/L		Number of Fish	Mortality (%)				
Nominal	*Time-weighted mean measured concentration*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
control	-	20	0	0	0	0	0
1.0	0.466	20	0	0	0	0	0
1.8	0.766	20	0	0	0	0	0
3.2	1.64	20	0	0	0	0	0
5.6	5.2	20	0	0	100	100	100
10	8.68	20	0	10	100	100	100

* Based on test concentration for the active ingredient in the test substance.

LC50	2.9 mg/L at 96 hours (95% confidence limit: 1.6–5.2 mg/L); based on the time-weighted mean measured test concentration.
NOEC	0.77 mg/L
Remarks – Results	At the nominal concentration of 3.2 mg/L, 5 fish were observed to swim at the surface after exposure for 24 hours. At the nominal concentration of 5.6 mg/L, all fish were observed to swim at bottom of the test vessel or at the surface after exposure for 24 hours and 11 fish were observed to be moribund after exposure for 46 hours. At the nominal concentration of 5.6 and 10 mg/L the test substance was observed to form a homogenous dispersion in the test media. As a precautionary measurement, microscopic inspection of the dead fish was performed and the results indicated that physical toxicity was not a factor at these two concentration levels. The concentration of the test substance in freshly prepared solution was determined to be 80–118% of the nominal concentration apart from the

lowest prepared concentration of 1.0 mg/L, for which the test concentration is 79% of the nominal concentration. This low value was considered to be due to sampling and/or analytical variation given that all other freshly prepared test media shown measured test concentration in excess of 80% of the nominal concentration.

Significant declines in the measured test concentrations for the old or expired test media were observed for most of the test concentrations. This decline was considered to be due to the unstable nature of the test substance under the test conditions. Therefore, the fish ecotoxicity endpoints were reported based on the time-weighted mean measured test concentration of the test media.

Based on the time-weighted mean measured test concentration, the highest test concentration resulting in 0% morality was 1.6 mg/L and the lowest test concentration resulting in 100% mortality was 5.2 mg/L.

CONCLUSION The test substance is toxic to fish

TEST FACILITY Test Facility J (2003e)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Product containing notified chemical (50–70%)

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Semi-static

Species *Daphnia magna*

Exposure Period 48 hours

Auxiliary Solvent None

Water Hardness 146 mg $CaCO_3$/L

Analytical Monitoring Gas chromatography

Remarks - Method The required amount of test substance was dispersed into dechlorinated water to prepare the stock solution at nominal concentration of 100 mg/L. The test substance was observed to form a homogenous dispersion in the test media at the nominal concentration of 100 mg/L. The reminder of the test solutions was prepared by serial dilution of the stock solution. The test media were renewed daily. Any immobilisation or adverse reactions were recorded at the exposure time period of 24 and 48 hours.

The test was conducted according to the protocol and without significant deviation from the protocol.

RESULTS

*Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Time-weighted mean measured concentration*		*24 h*	*48 h*
Control	-	20	0	0
0.10	0.0317	20	0	0
0.18	0.0535	20	0	0
0.32	0.0802	20	0	0
0.56	0.152	20	0	0
1.0	0.193	20	0	0
1.8	0.312	20	0	0
3.2	1.13	20	0	0
5.6	2.20	20	0	0
10	4.06	20	11	11

*Based on test concentration for the active ingredient in the test substance.

EC50	3.8 mg/L at 48 hours (95% confidence limit: 3.0–4.8 mg/L); based on the time-weighted mean measured test concentration.
NOEC	2.2 mg/L
Remarks - Results	At the nominal concentration of 100 mg/L the test substance was observed to form a homogenous dispersion in the test media. A precautionary measure microscopic inspection of immobilised daphnid was performed and the results indicated that physical toxicity was not a factor at this concentration level. The concentration of the test substance in freshly prepared solution was determined to be 87–114% of the nominal concentration. However, significant declines in the measured test concentration for the old or expired test media were observed at 24 and 48 hours. This decline was considered to be due to the unstable nature of the test substance under the test conditions. Therefore, the daphnia ecotoxicity endpoints were reported based on the time-weighted mean measured test concentration of the test media.
CONCLUSION	The test substance is toxic to aquatic invertebrates.
TEST FACILITY	Test Facility J (2003f)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Scenedesmus subspicatus* CHODAT
Exposure Period	72 hours
Concentration Range	Nominal: 0, 2.5, 5, 10, 20, 40 and 80 mg/L Actual: 0, 2.9, 6.5, 9.7, 31.3, 54.4 and 80.9 mg/L (determined at exposure time of 0 hour).
Auxiliary Solvent	None
Water Hardness	122 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometer
Remarks - Method	The test was conducted according to the protocol and without significant deviation from the protocol.

RESULTS

Biomass		*Growth*	
E_bC50	*NOE_bC*	*E_rC50*	*NOE_rC*
mg/L at 72h	*mg/L*	*mg/L at 72h*	*mg/L*
22	3.2	410	4.9

Remarks - Results	The actual concentration of the test substance was determined according to the method described in DIN 38412, part 23 for quantification of the non-ionic surfactant. The recovery of the test substance at 72 hours was determined to be 61–85%, close to the required stability level of 80%. Therefore, the results based on the nominal concentration. All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not harmful to algae
TEST FACILITY	Test Facility B (2000)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE	Product containing the notified chemical (50–70%)
METHOD	N/A
Species	*Skeletonema costatum*
Exposure Period	72 hours
Concentration Range	Nominal: 0, 1, 10, 100 and 1000 mg/L Actual: Not reported
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Not reported
Remarks - Method	The test was conducted on a marine species with 4 control and 2 test substance replicates using 3,5-dichcloro phenol as a reference. The tests were carried out in natural sea water at 20 °C with light intensity of 25–30 $\mu E.m^{-2}.s^{-1}$. Only a study summary was provided.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72h	*NOE_bC mg/L*	*E_rC50 mg/L at 72h*	*NOE_rC mg/L*
> 100	> 10	> 100	> 10

Remarks - Results	The results were based on nominal concentrations. The validity for the test is unable to be confirmed since the full study report and method was not provided.
CONCLUSION	The test substance is not harmful to algae
TEST FACILITY	Test Facility A (2008)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Product containing notified chemical (50–70%)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 0, 1000 mg/L Actual: Not reported
Remarks – Method	A limit test was conducted according to the test guideline without significant deviation from the protocol.
RESULTS	
EC50	> 1000 mg/L
NOEC	> 100 mg/L
Remarks – Results	All validity criteria for the test were satisfied. In the range-finding test, no effects were observed at the test concentration of 1.0, 10 and 100 mg/L while 4% inhibition was observed at the highest concentration of 1000 mg/L for the test substance. Therefore, definitive test was conducted only at the concentration of 1000 mg/L and the inhibition was determined to be 3, 10 and 13% for three replicates, respectively.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration
TEST FACILITY	Test Facility J (2007b)

BIBLIOGRAPHY

European Commission (EC, 2003). Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market – Part II. Institute for Health and Consumer protection, European Chemicals Bureau, European Communities.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia.

OECD (2004) Series on Emission Scenario Documents No. 10 – Emission Scenario Document on Lubricants and Lubricant Additives. Environment Directorate, Organisation for Economic Co-operation and Development, December 2004. Accessed at:http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2004)21&doclanguage=en

SCCS (2010) The SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 7th Revision. Adopted by the SCCS during the 9th plenary meeting of 14 December 2010 (SCCS/1416/11).

Test Facility A (2008) *Notified chemical*: Alga Growth Inhibition Test with Sceletonema costatum 72h (Study No SSC12416N, July, 2008) (unpublished report submitted by the notifier).

Test Facility B (1996) *Notified chemical*: Test for Ready Biodegradability of Products (Study No. 96TE075975, August, 1996) (unpublished report submitted by the notifier).

Test Facility B (1999) Study on the Anaerobic Biodegradability of Product A (Sample LD 512) (Study No. IF-99/05581-00, August, 1999) (unpublished report submitted by the notifier).

Test Facility B (2000) Study on the Toxicity toward Algae of Products of *notified chemical* according to OECD 2011 (Study No. IF-100/01979-00, April, 2000) (unpublished report submitted by the notifier).

Test Facility C (2009) *Notified chemical*: Oral (Gavage) Reproduction/Developmental Toxicity Screening Test in the Rat (Project Number 1539/0018, October, 2009) (unpublished report submitted by the notifier).

Test Facility C (2010a) *Notified chemical*: Calculation of the Organic Carbon-Water Partitioning Coefficient Koc using KOCWIM (v2.0 and the Bioconcentration Factor using BCFBAS (v3.0) (November, 2010) (unpublished report submitted by the notifier).

Test Facility C (2010b) Determination of Dissociation Constant (Study No. 41003070, October, 2010) (unpublished report submitted by the notifier).

Test Facility C (2010c) *Notified chemical*: Determination of Stability in Organic Solvents (Project Number 41003069, October, 2010) (unpublished report submitted by the notifier).

Test Facility C (2011a) *Notified chemical*: Determination of Vapour Pressure (Project Number 41004898, March, 2011) (unpublished report submitted by the notifier).

Test Facility C (2011b) *Notified chemical*: Determination of Partition Coefficient and n-Octanol Solubility (Study No. 1539/0019, March, 2011) (unpublished report submitted by the notifier).

Test Facility D (2012) AMIDET TEC N: Analysis Report (Batch 320062 LF322A, September, 2012) (unpublished report submitted by the notifier).

Test Facility E (2009) Use of Precessor Tensiometer K100 in Combination with the Double Dosing Unit for Characterisation of Critical Micelle Concentration (March, 2009) (unpublished report submitted by the notifier).

Test Facility F (2009) *Notified chemical*: Acute Inhalation Toxicity Study (Nose-only) in the Rat (Study 08/764-004PE, January, 2009). (unpublished report submitted by the notifier).

Test Facility G (1999) Examination of *notified chemical* in the Skin Sensitisation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) (Report 11767/98, January, 1999) (unpublished report submitted by the notifier).

Test Facility H (1998) *Notified chemical*: Human Repeat Insult Patch Test (HRIPT) (Order No 200295/F, July, 1998) (unpublished report submitted by the notifier).

Test Facility I (1991a) Assessment of Acute Oral Toxicity with *Notified chemical* in the Rat (Project 060953, November, 1991) (unpublished report submitted by the notifier).

Test Facility I (1991b) Primary Skin Irritation/Corrosion Study with *Notified chemical* in the Rabbit (4 Hour Semi Occlusive Application) (Project 060931, November, 1991) (unpublished report submitted by the notifier).

Test Facility I (1991c) Acute Eye Irritation/Corrosion Study with *Notified chemical* in the Rabbit (Project 060942, December, 1991) (unpublished report submitted by the notifier).

Test Facility J (1993) *Notified chemical*: Reverse Mutation Assay "Ames Test" Using Salmonella Typhimurium (Project Number 590/15, December, 1993) (unpublished report submitted by the notifier).

Test Facility J (1994) *Notified chemical*: Chromosome Aberration Test in Human Lymphocytes in Vitro (Project Number 590/18, June, 1994) (unpublished report submitted by the notifier).

Test Facility J (2003a) *Notified chemical*: Determination of General Physio-Chemical Properties (Study No. 1539/002, April, 2003) (unpublished report submitted by the notifier).

Test Facility J (2003b) *Notified chemical*: Twenty Eight Day Repeated Dose Oral (Gavage) Toxicity Study in the Rat (Project Number 1539/003, October, 2003) (unpublished report submitted by the notifier).

Test Facility J (2003c) *Notified chemical*: Micronucleus Test in the Mouse (Project Number 1539/004, June, 2003) (unpublished report submitted by the notifier).

Test Facility J (2003d) *Notified chemical*: Assessment of Ready Biodegradability CO_2 Evolution Test (Study No. 1539/007, May, 2003) (unpublished report submitted by the notifier).

Test Facility J (2003e) *Notified chemical*: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) (Study No.1539/005, October, 2003) (unpublished report submitted by the notifier).

Test Facility J (2003f) *Notified chemical*: Acute Toxicity to *Daphnia Magna* (Study No. 1539/006, October, 2003) Derbyshire, UK, SafePharm Laboratories Limited (unpublished report submitted by the notifier).

Test Facility J (2007a) *Notified chemical*: Acute Dermal Toxicity (Limit Test) in the Rat (Project Number 1539/015, December, 2007) (unpublished report submitted by the notifier).

Test Facility J (2007b) *Notified chemical*: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge (Study No 1539/016, November, 2007) (unpublished report submitted by the notifier).

Test Facility J (2008a) *Notified chemical*: Determination of General Physio-Chemical Properties (Study No. 1539/0013, February, 2008) (unpublished report submitted by the notifier).

Test Facility J (2008b) *Notified chemical*: Determination of Hazardous Physio-Chemical Properties (Project Number 1539/014, January, 2008) (unpublished report submitted by the notifier).

Test Facility K (2009a) Biodegradation Test of *Notified chemical* (301F) (Study No. R0840A0199, January, 2009) (unpublished report submitted by the notifier).

Test Facility K (2009b) Acute toxicity study of *Notified chemical* to Zebra Fish (*Brachydanio Rerio*) (Study No. G0823J0179, February, 2009) (unpublished report submitted by the notifier).

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.