File No: LTD/1769

October 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Firwood

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1769	Firmenich Limited	Firwood	Yes	≤ 1 tonne per annum	Fragrance ingredient

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 sensitisation (Category 1)	H317 – May cause an allergic skin reaction

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:

R43 May cause sensitisation by skin contact

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

Hazard classification	*Hazard statement*
Acute (Category 2)	H401 - Toxic to aquatic life
Chronic (Category 2)	H411 – Toxic to aquatic life with long lasting effects

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

When used at ≤ 1.0% in fine fragrances, ≤ 0.5% in other cosmetic products, and ≤ 1.5% in household products, the notified chemical is not considered to pose an unreasonable risk to public health.

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction

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

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemical for listing on the SUSMP.

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

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 during reformulation processes:
  - Enclosed, automated processes, where possible
  - Ventilation system, including local exhaust ventilation

- 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 :
  - Avoid contact with skin and eyes
  - Avoid inhalation

- 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 :
  - Impervious gloves, eye protection and coveralls

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

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

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

Public Health

- The following measures should be taken formulators to minimise public exposure to the notified chemical:
  - The notified chemical should only be used at ≤ 1.0% in fine fragrances, ≤ 0.5% in other cosmetic products, and ≤ 1.5% in household products.

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 importation volume exceeds one tonne per annum notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 1.0% in fine fragrances, 0.5% in other cosmetic products, and 1.5% in household products;

or

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Road
BALGOWLAH, NSW 2093

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, identities of degradation products and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: dissociation constant, particle size, flammability limits, reactivity, explosive and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
Low Volume Chemical (LVC) permit notification

NOTIFICATION IN OTHER COUNTRIES
EU (2003), Japan (2007), Korea (2006), Philippines (2005), Switzerland (2007), USA (2003)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Firwood

MOLECULAR WEIGHT
< 500 Da

ANALYTICAL DATA
Reference NMR, IR, GC, MS and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: colourless liquid.

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< - 41.3 °C	Measured
Boiling Point	289 ± 1 °C at 98 kPa	Measured
Density	926 kg/m^3 at 20 °C	Measured
Vapour Pressure	2 x 10^{-4} kPa at 25 °C	Measured
Water Solubility	5.1 × 10^{-3} g/L - 5.29 × 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ < 1 day at pH 4, 5.86 days at pH 7, and 86.2 days at pH 9 at 25 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow > 6.2	Measured
Adsorption/Desorption	log K_{OC} = 4.43	Measured
Dissociation Constant	Not determined	Contains no dissociable functional groups

Surface tension	55.3 mN/m at 20.5 °C	Measured
Flash point	96 ± 2 °C at 101.325 kPa	Measured
Auto-ignition Temperature	236 ± 5 °C	Measured
Explosive Properties	Predicted negative	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidising properties.

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 not be manufactured in Australia. The notified chemical will be imported into Australia at 100% concentration, as well as a component of compounded fragrance formulations (at concentrations ≤ 10%) and various formulated end-use cosmetic and household products (at concentrations ≤ 1.5%).

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

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

PORT OF ENTRY
Sydney, by wharf or airport.

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited.

TRANSPORTATION AND PACKAGING
The notified chemical (at ≤ 100% concentration) will be imported into Australia in lacquered drums of sizes ranging from 5 kg up to 180 kg. The end-use products (≤ 1.5% notified chemical) will be packaged in typical consumer-sized containers suitable for retail sale.

The notified chemical will be transported from the port of entry by road to the notifier's warehouse facilities for storage in its original packaging until transportation to the customer site. Alternatively, the notified chemical and products containing it will be shipped directly from the port of entry to the customer site.

USE
The notified chemical will be used as a fragrance component in a variety of cosmetic and household products. The content in the final consumer products will vary, with the following proposed usage concentrations: fine fragrances (≤ 1%), other cosmetic products (≤ 0.5%) and household products (≤ 1.5%).

OPERATION DESCRIPTION
No manufacturing, processing, reformulating or repackaging of the notified chemical will occur at the notifier's facility. The imported products containing the notified chemical will be stored at this facility until they are transported to customer facilities (in original importation packaging).

At the customer facilities, the procedures for incorporating the imported fragrance preparations (containing ≤ 100% notified chemical) into end-use products will likely vary depending on the nature of the cosmetic and household products formulated, and may involve both automated and manual transfer steps. However, in

general, it is expected that the reformulation processes will involve blending operations that will be highly automated and occur in a fully enclosed environment, followed by automated filling of the reformulated products into containers of various sizes.

Household products
Household products containing the notified chemical (≤ 1.5% concentration) may be used by consumers and professional workers. The products may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines, or open processes and manually applied by rolling, brushing, spraying and dipping, using a cloth, sponge, mop or brush and followed by wiping. In some cases the household product will be diluted with water prior to application.

Cosmetics
The finished cosmetic products containing the notified chemical at ≤ 1% concentration will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application of products could be by hand, sprayed or through the use of 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 warehouse workers	unknown	unknown
Mixer	4	2
Drum Handling	4	2
Drum Cleaning/washing	4	2
Maintenance	4	2
Quality Control worker	0.5	1
Packager	4	2
End users (professionals)	unspecified	unspecified

EXPOSURE DETAILS
Transport and storage
Transport and storage workers may come into contact with the notified chemical, at 100% concentration or as a component of the imported fragrance preparations (≤ 10% concentration) or end-use products (≤ 1.5% concentration), only in the event of accidental rupture of containers.

At the notifier facility, the primary work activity undertaken by transport and warehouse workers will include the handling, loading and off-loading of drums containing the notified chemical at ≤ 100% concentration. Exposures of these workers will be limited to situations of an accidental discharge, spill or leaking drum, requiring clean up. If such an event occurs, a worker may be exposed through dermal or ocular contact. The notifier states that such exposures will be minimised through the use of personal protective equipment (PPE) including protective clothing, chemical resistant gloves and eye protection.

Formulation of end products
During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical (at ≤ 100% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems, and through the use of PPE such as protective clothing, eye protection, impervious gloves and respiratory protection (if appropriate).

Beauty care and cleaning professionals
Exposure to the notified chemical in end-use products may occur in professions where the services provided involve the application of cosmetic products (at ≤ 1% concentration) to clients (e.g. hair dressers, workers in beauty salons) or the use of household products (at ≤ 1.5% concentration) in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. 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.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of the cosmetic and household products (≤ 1.5% concentration in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

A combined internal dose of 1.5965 mg/kg bw/day was estimated using data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used (SCCS, 2010; Cadby *et al.*, 2002; SDA, 2005; specific use details of the notified chemical are considered as exempt information). This estimation assumed a worst case scenario and is for a person who is a simultaneous user of a selection of cosmetic and household products that may contain the notified chemical.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test.	inadequate evidence of sensitisation
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Human, skin sensitisation – RIPT (20%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 216 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic

Toxicokinetics, metabolism and distribution.
Based on the water solubility (5.1 - 5.29 × 10^{-3} g/L at 20 ºC), partition coefficient (log K_{ow} > 6.2) and the low molecular weight (<500 Da) of the notified chemical, passive diffusion across the gastrointestinal (GI) tract and dermal absorption are possible. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.
The notified chemical was found to have low acute toxicity by both the oral and dermal routes in studies conducted in rats.

No acute inhalation toxicity data were provided for the notified chemical.

Irritation
In a rabbit eye irritation study, very slight conjunctival redness and chemosis were observed in all three animals on day 1 and persisted in one animal on day 2 (24 hour observation). A very slight redness of the conjunctiva remained evident in one animal up to an including the 72 hour observation.

In an acute dermal irritation study using three male New Zealand white rabbits, a single 4-hour, semi-occluded application of the notified chemical resulted in very slight to well-defined erythema in all three animals from day 1, lasting up to day 7 (resolving for two animals) and day 8. Dryness of the skin was observed in all animals. The irritation scores did not warrant classification of the chemical as a skin irritant according to the *GHS*, as adopted for industrial chemicals in Australia, but did warrant classification of the chemical according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Sensitisation.

The potential for skin sensitisation to be induced by the notified chemical was assessed in a Maximisation Test in guinea pigs. After both the first (at 100% concentration) and second (at 30% concentration) challenge applications, discrete to moderate erythema was noted in both the control and test group animals. As the reactions observed in the treated group animals were of a similar incidence and severity to the reactions in the control animals, the study authors attributed the observations to the irritant properties of the test substance, not to induced delayed contact hypersensitivity.

The notified chemical was subsequently tested in an LLNA study in mice (tested at 1, 5, 10, 20 and 40% concentration, with 40% producing a stimulation index of 7.9) and found to be a skin sensitiser. The EC_3 value was calculated to be 23.5%.

In a human repeat insult patch test (HRIPT) completed on 101 subjects, the notified chemical (at 20% concentration) did not induce skin sensitisation.

Repeated dose toxicity.
In a 28 day repeat dose dietary study, rats were administered the notified chemical at 0, 24, 216 and 1,486 mg/kg bw/day. Effects attributed to the notified chemical seen in male and/or female animals of the highest dose group included an increase in clotting (pro-thrombin) time, reduction in plasma bilirubin, increase in plasma cholesterol and protein, increases in relative adrenal, kidney and liver weights and reductions in relative ovary and spleen weights.

Some effects seen in a few select mid dose group animals were considered not of toxicological significance. This included globular accumulations of eosinophilic material that were observed in the tubular epithelium of male rats in the highest dose group and the mid group. The study authors found this observation consistent with the appearance of hydrocarbon nephropathy, resulting from excessive accumulation of α2-microglobulin in renal proximal tubular epithelial cells. As this is exclusively found in adult male rats, this finding was considered not indicative of a hazard to human health.

Treatment related hyperplasia of the transitional epithelium was observed in animals of both sexes in the highest dose group, with some animals showing associated sub-epithelial inflammatory cell infiltrates. Epithelial hyperplasia was also observed in the urinary bladder of one control group female, one low dose group male and one mid dose group female as spontaneous findings. The study authors presumed this change was associated with excretion of an irritant test material metabolite in the urine.

Overall, based on the observation of adverse effects at the highest dose tested, a No Observed Adverse Effect Level (NOAEL) of 216 mg/kg bw/day was established for the notified chemical in this study.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in two separate bacterial reverse mutation studies and was not clastogenic in an in vitro mammalian chromosome aberration test.

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 sensitisation (Category 1)	H317 – May cause an allergic skin reaction

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):
R43 May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation
Exposure of workers to the notified chemical (at ≤ 100% concentration) may occur during blending operations. The notified chemical has the potential to cause skin irritation and slight eye irritation effects and is considered

to be a skin sensitiser. In addition, harmful effects following inhalation exposure to the notified chemical cannot be ruled out. Therefore, caution should be exercised when handling the notified chemical during reformulation processes.

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

End-use
Cleaners and beauty care professionals will handle the notified chemical at ≤ 1.5% concentration, similar to public use. Therefore, the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser 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

Repeated-dose toxicity
Members of the public may experience repeated exposure to the notified chemical (at ≤ 1.5% concentration) through the use of the cosmetic and household products.

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products of 1.5965 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 216 mg/kg bw/day, which was established in a 28-day repeated dose toxicity study on the notified chemical. A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences. Using the abovementioned NOAEL, a MoE of 135 was estimated, which is considered to be acceptable.

Irritation
The notified chemical has the potential to cause skin irritation. However, skin irritation effects are not expected from use of the notified chemical at the proposed concentrations in cosmetic and household products.

Sensitisation
The notified chemical is considered to have the potential to cause skin sensitisation. Methods for the quantitative risk assessment of dermal sensitisation have been proposed and been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). Using a fine fragrance (containing 1.0 % notified chemical) as an example product that may contain the notified chemical, as a worst case scenario, the Consumer Exposure Level (CEL) is estimated to be 37.5 µg/cm^2 (Cadby *et al.*, 2002).

When tested in an LLNA study, the notified chemical was a skin sensitiser with an EC_3 value of 23.5%. Consideration of the study details (and consideration of the available HRIPT study) and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 54.40 µg/cm^2. In this instance, the factors employed included an interspecies factor (1), intraspecies factor (10), a matrix factor (3.16) and a use and time factor (3.16), giving an overall safety factor of ~100.

As the AEL>CEL, the risk to the public of the induction of sensitisation that is associated with the use of fine fragrances (a worst case example of a leave-on cosmetic product) at ≤ 1.0% concentration is not considered to be unreasonable. Based on the significantly lower expected exposure level from other cosmetic products (containing ≤ 0.5% notified chemical), and household products (≤ 1.5% notified chemical), by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on the aggregate exposure has not been conducted.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 1.0% in fine fragrances, ≤ 0.5% in other cosmetic products and ≤ 1.5% in household products, is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia at 100% concentration or as a fragrance component of compounded formulations and various formulated end-use cosmetic and household products. Environmental release of the notified chemical during transportation and storage is expected to be minimal and will be limited to accidental spills or leaks of drums.

It is expected that the reformulation processes will involve blending operations that will be highly automated. It is expected to occur in a fully enclosed environment, followed by automated filling of the reformulated products into containers of various sizes. A total of 0.2% of waste is expected to be generated each from blending or formulation activities as a result of spills and residues.

All the above releases are expected to be collected for disposal, which is most likely to landfill.

RELEASE OF CHEMICAL FROM USE

The notified chemical is expected to enter the aquatic compartment during use of the various end-use products into which it will be incorporated. Cosmetic products will be washed off the hair and skin and will be released to sewers. Cleaning products will also be diluted in water and will be released to sewers. It is estimated that a maximum of 3% of the consumer products will remain in the consumer containers once the consumer product is used up. These containers are expected to be sent to landfill or to be recycled.

RELEASE OF CHEMICAL FROM DISPOSAL

Containers containing residual notified chemical are expected to be released to landfill or to be recycled.

7.1.2. Environmental Fate

The notified chemical is not expected to be readily biodegradable, but it may have potential for biodegradation under certain conditions. It can hydrolyse in the aquatic environment with a half-life from < 1 day to 86 days, depending on the pH conditions. For the details of the environmental fate studies please refer to Appendices A and C. The notified chemical has a high log K_{OW} (> 6.2) and a low molecular weight of < 500 Da. Hence, it has potential to bioaccumulate in aquatic organisms.

The vapour pressure of the notified chemical of 0.2 Pa at 25°C provided by the notifier indicates a moderate volatility. Based on the calculated Henry's Law Constant of 1.0 Pa m^3/mole, it is moderately volatile also from water or moist soil surfaces. Based on a calculated (AOPWIN v 1.92; US EPA, 2011) half-life of 2.7 hours through atmosphere oxidation, it is not considered to be persistent in the air.

Most of the notified chemical is expected to be released into sewer systems after use of the associated products. A small amount of the notified chemical may be released to landfill as container residues or spills or thermally decomposed during containers' recycling, forming water and oxides of carbon. In landfill, the notified chemical is not expected to leach given the high adsorption/desorption constant. In sewage treatment plants (STPs), the majority of the notified chemical is expected to be removed by adsorption to sludge sediment and be disposed of to landfill or fields, with a small proportion being released into public waters. In water or soil/landfill, the notified chemical is expected to undergo biotic or abiotic degradation processes, forming water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated assuming 100% release of the notified chemical into sewer systems nationwide. Based on the SimpleTreat model (EC, 2003), 86% of the notified chemical is expected to be removed from water surface by evaporation (1%) and sludge adsorption (85%). The removal of notified chemical from STPs via hydrolysis is not considered in this calculation.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year

Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	86%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.08	µg/L
PEC - Ocean:	0.01	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 5.15 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.034 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.17 mg/kg and 0.34 mg/kg, respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.085 µg/L may potentially result in a soil concentration of approximately 0.57 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 2.8 µg/kg and 5.7 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EC50 = 1.3 mg/L	Toxic to fish (acute)
Daphnia Toxicity	48 h EC50 = 1.1mg/L	Toxic to *Daphnia* (acute)
	21 day NOEC = 0.029 mg/L	Very toxic to *Daphnia* (chronic)
Algal Toxicity	72 h EC50 > 1.9 mg/L	Not harmful to algae (acute)
Inhibition of Bacterial Respiration	EC50 > 1000 mg/L	Not inhibitory to sludge bacteria (acute)

The notified chemical is considered to be toxic to aquatic organisms based on the acute endpoints for fish and daphnids. It is also considered to be very toxic based on the chronic endpoint for daphnids. Based on the acute toxicity, the notified chemical is formally classified as "Acute Category 2; toxic to aquatic life" under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009). The notified chemical is not readily biodegradable. However, it can hydrolyse readily at acidic to neutral pH conditions, which may cover the common pH range of the sewage in the treatment plants. Based on the chronic endpoint for daphnids and the likely readily degradability, the notified chemical is classified as "Chronic Category 2; Toxic to aquatic life with long lasting effects".

PBT consideration
The notified chemical is not readily biodegradable, has potential to bioaccumulate based on the log P_{OW} of > 6.2, and is very toxic chronically to daphnids (21 day NOEC = 0.029 mg/L). However, this is not considered to be a PBT concern based on the following considerations:

The hydrolysis study indicates that it has a half-life of < 1 at pH 4, 5.86 days at pH 7, and 86 days at pH 9. It is noted that the half-life of 86 is longer than two months, indicating persistence of the notified chemical at this pH

for PBT consideration. However, pH of 9 is considered an extreme condition; the aquatic environment is mostly expected to have a pH of around 7. Therefore, the notified chemical is expected to be hydrolysed in STPs. According to the notifier, the notified chemical may degrade into water soluble or readily biodegradable chemicals that are not considered to be PBT chemicals.

In addition, the readily biodegradability studies indicated that the notified chemical has potential to biodegrade under certain conditions (Appendix C).

Based on the above, the notified chemical is not considered to have a PBT concern.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) was calculated using the chronic daphnid endpoint 21 d NOEC = 0.029 mg/L. A safety factor of 100 was used considering acute endpoints for three trophic levels and a chronic endpoint for one species is available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
Daphnia (21 d NOEC)	0.029 mg/L
Assessment Factor	100
PNEC:	0.29 µg/L

7.3. Environmental Risk Assessment

Risk Quotient Table (PEC/PNEC)

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.08	0.29	0.28
Q - Ocean	0.01	0.29	0.03

The risk quotient (Q = PEC/PNEC) was calculated to be < 1 for discharge of effluent containing the notified chemical to the aquatic environment, indicating that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual use quantity.

The notified chemical is not considered to have PBT potential given the potential to hydrolyse in the environment.

Based on the calculated risk quotient and the assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point - 41.3 ± 0.2 °C

Method OECD TG 102 Melting Point/Melting Range.
EEC Commission Directive 92/69 A.1 Melting/Freezing Temperature.
Remarks The study authors noted that the test item was hard to homogenise during the test, and that only 1 assay from 4 was validated.
Test Facility SEPC (2000)

Boiling Point 289 ± 1 °C at 98 kPa

Method OECD TG 103 Boiling Point.
EEC Directive 92/69 A.2 Boiling Temperature.
Remarks Determined according to the Siwoloboff method.
Test Facility RCC (2001)

Density 926 kg/m^3 at 20 ± 0.5 °C

Method OECD TG 109 Density of Liquids and Solids.
Commission Directive 92/69/EEC A.3 Relative Density.
Remarks Determined using the pycnometer method.
Test Facility Safepharm (2002a)

Vapour Pressure 2.0 x 10^{-4} kPa at 25 °C

Method Commission Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Determined using a vapour pressure balance.
Test Facility Safepharm (2001a)

Water Solubility 5.1 × 10^{-3} g/L at 20 °C

Method OECD TG 105 Water Solubility
Remarks Column Elution Method. Two experiments were conducted with a total elution time of 122 hours at 20 °C . Samples were collected at 1 hour intervals. The mean water solubility of the two experiments was determined to be 5.1 × 10^{-3} g/L.
Test Facility RCC (2000)

Water Solubility 5.29 × 10^{-3} g/L at 20 °C

Method OECD TG 105 Water Solubility
Remarks Flask Method. Mixtures of notified chemical and distilled water were added to four separate flasks. They were shaken at approximately 30 °C and, allowed to stand at 20 °C for not less than 24 hours. The contents of the flasks were centrifuged and the concentration of the notified chemical was determined using gas chromatography (GC). Decline in concentration over shaking time was observed. This may be from hydrolysis of the notified chemical in water. The water solubility was determined to be 5.29 × 10^{-3} g/L. This was based on the samples that were shaken for the shortest time.
Test Facility Safepharm (2001b)

Hydrolysis as a Function of pH $t_{½}$ < 1 day at pH 4, 5.86 days at pH 7, and 86.2 days at pH 9 at 25 °C

Method Method C7 specified in Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548IEEC)

pH	*T (°C)*	*$t_{½}$ (day)*
4	25	< 1
7	25	5.86
9	25	86.2

Remarks	The test was conducted at 50 °C at pH 4, 7, and 9, and 40 °C at pH 7 and 9. Based on the test results, the half-life at 25 °C were estimated as shown in the table above. The notified chemical is not considered to be hydrolytically stable.
Test Facility	Safepharm (2002a)

Partition Coefficient (n-octanol/water) log Pow > 6.2

Method	OECD TG 117 Partition Coefficient (n-octanol/water)
Remarks	HPLC Method. The log P_{OW} for the notified chemical was determined to be > 6.2, as the notified chemical eluted after the last standard (DDT, log P_{OW} = 6.2).
Test Facility	Safepharm (2001b)

Adsorption/Desorption – screening test log K_{oc} = 4.43

Method	OECD TG 121 Adsorption - Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC).
Remarks	HPLC method. The test was carried out at pH 6.2, the pH at which the notified chemical was estimated to be unionised. The adsorption coefficient (K_{OC}) of the notified chemical was determined to be 2.68×10^4, or a log K_{OC} of 4.43.
Test Facility	Safepharm (2001a)

Surface Tension 55.3 mN/m at 20.5 °C

Method	ISO 304, Method A5 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC)
Remarks	A ring method was used. The surface tension was determined at a concentration of 13.3 mg/L to be 55.3 mN/m at 20.5 ± 0.5 °C. The notified chemical is considered to be surface-active.
Test Facility	Safepharm (2002a)

Flash Point 96 ± 2 °C at 101.325 kPa

Method	Commission Directive 92/69/EEC A.9 Flash Point.
Remarks	Determined using a closed cup equilibrium method.
Test Facility	Safepharm (2000)

Autoignition Temperature 236 ± 5°C

Method	Commission Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Determined by heating aliquots of the test material in a flask and observing any ignition.
Test Facility	Safepharm (2002b)

Explosive Properties Predicted negative

Method	Commission Directive 92/69/EEC A.14 Explosive Properties.
Remarks	Observation of functional groups that would imply explosive properties.
Test Facility	Safepharm (2002b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Sprague-Dawley ICO: OFA-SD (IOPS Caw)
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 M	200	0/3
2	3 per sex	2,000	1/6

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Signs of toxicity observed during the observation period included hypoactivity, piloerection, stiff gait and dyspnea. These effects were noted in all 3 male animals dosed at 2,000 mg/kg bw/day on days 1 and/or 2. No clinical signs were seen in the other test animals.
Effects in Organs	No macroscopic findings were observed at necropsy in any of the test animals.
Remarks - Results	No deaths occurred for test animals dosed at 200 mg/kg bw/day. One female animal dosed at 2,000 mg/kg bw/day died on day 1 within 30 minutes post administration. All surviving animals gained weight over the course of the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	CIT (2000a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. Commission Directive 92/69/EEC Method B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Sprague-Dawley CD (Crl: CD (SD) IGS BR)
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	Crust formation was noted on day 4 and 5 at the treatment site of one female. No other signs of dermal irritation were seen during the study period.
Signs of Toxicity - Systemic	No systemic clinical signs were observed during the observation period.

Effects in Organs	None - no abnormalities detected at necropsy.
Remarks - Results	No deaths occurred during the study. It is noted that while all animals gained weight during week 1 of the observation period (23-94 g gain), smaller gains (9-43 g gain; 6/10 animals) or weight losses (7-48 g loss, 4/10 animals) were noted in week 2 of the study. The study authors considered that there were no toxicologically significant effects on bodyweight.

CONCLUSION The notified chemical is of low toxicity via the dermal route.

TEST FACILITY Safepharm (2001c)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Commission Directive 93/21/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations. GLP Compliance.

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	1.3	2	2	< 8days	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	No clinical signs of systemic toxicity were observed in the animals during the study. Very slight to well-defined erythema was noted in all three animals from day 1, lasting up to day 7 (resolving for two animals) and day 8. Dryness of the skin was observed in all animals from day 5 (seen in one animal) or 6 up to day 9, 10 or the end of the observation period. No other cutaneous reactions were observed during the study.

CONCLUSION The notified chemical is irritating to the skin. The study authors found the results were sufficient to classify the chemical as an Irritant with the risk phrase R38, however the notified chemical is below classification thresholds under the GHS.

TEST FACILITY CIT (2000b)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M

Observation Period	5 days
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	1	1	< 4 days	0
Conjunctiva: chemosis	0	0	0.33	1	< 48 hours	0
Conjunctiva: discharge	0	0	0	0	0	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	Very slight conjunctival redness and chemosis were observed in all three animals on day 1 and persisted in one animal on day 2 (24 hour observation). A very slight redness of the conjunctiva remained evident in one animal up to and including the 72 hour observation.. No corneal opacity or iridial inflammation was observed at any of the measuring intervals.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	CIT (2000c)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – Maximisation Method of Magnusson and Kligman. EC Directive 96/54/EC B.6 Skin Sensitisation - Maximisation Method of Magnusson and Kligman.
Species/Strain	Guinea pig/Hartley Crl: (HA) BR
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 10 per sex Control Group: 5 per sex
INDUCTION PHASE	Induction Concentration: intradermal: 25% (w/w) in corn oil topical: 100%
Signs of Irritation	The study authors note that signs of irritation were observed at the treatment site in animals of the control and treated groups, following removal of the dressings.
CHALLENGE PHASE	
1st challenge	topical: 100%
2nd challenge	topical: 30% (w/w) in corn oil
Remarks - Method	No significant protocol deviations. GLP Compliance. Prior to treatment with the test substance (topical induction), the animals were treated with sodium lauryl sulphate (0.5%; 10% in vaseline). A concurrent positive control study was not run, but had been conducted previously in the laboratory using mercaptobenzothiazole.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	100%	10/19	10/19		
	30%			13/19	2/19
Control Group	100%	8/10	7/10		
	30%			10/10	1/10

Remarks - Results

One male animal of the treated group was found dead on day 12. No clinical signs were observed prior to death. The study authors suggest that spontaneous mortality can be seen in the test species and hence did not attribute this death to the treatment.

After the first challenge application, a discrete to moderate erythema was noted in eight (8/10) animals of the control group and thirteen (13/19; noting reactions in 3/19 animals after 48 hours that were not present after 24 hours) animals of the treated group. Dryness of the skin was observed in one animal of each group.

After the second challenge application, a discrete to moderate erythema was noted in all ten animals of the control group and in thirteen (13/19) animals of the treated group. The study report also indicates that erythema was evident in 4/10 animals in the control group and 3/19 animals of the treated group prior to the second challenge application (substance application site for first challenge; vehicle site for second challenge).

As the cutaneous reactions observed in the treated group animals were of similar incidence and severity compared to that of the control group, the study authors attributed the observations to the irritant properties of the notified chemical and not to delayed contact hypersensitivity.

All surviving animals gained weight over the course of the study.

CONCLUSION

The notified chemical may have skin sensitising ability, but the test conditions are inadequate. Therefore, on the basis of inadequate evidence, no conclusion is made.

TEST FACILITY

CIT (2000d)

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

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain: Mouse/ CBA/J
Vehicle: Acetone/olive oil (AOO; 4:1)
Positive Control: Isoeugenol (in AOO)
Remarks - Method: No significant protocol deviations.
GLP Compliance.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	27.6	-

1%	27.9	1.0
5%	29.9	1.1
10%	36.8	1.3
20%	60.4	2.2
40%	217.5	7.9
Positive Control		
0.5%	44.8	1.6
1%	61.0	2.2
5%	208.2	7.5

Remarks - Results

No signs of systemic toxicity were noted in the test or control animals.

The positive control elicited an EC-3 of 1.55%. The notified chemical produced an EC-3 of 23.5%.

Mean ear thickness measurements did not increase by ≥ 10% (days 1-3) at any tested concentration, either for the test substance or isoeugenol.

CONCLUSION

There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY

BRT (2003)

B.7. Skin sensitisation – human volunteers

TEST SUBSTANCE

Notified chemical (20% concentration in DEP)

METHOD

Repeated insult patch test with challenge

Study Design

Induction Procedure: patches containing 0.2 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 hours and graded after an additional 24 hours (or 48 hours for patches applied on Friday).
Rest Period: 10-15 days
Challenge Procedure: a patch was applied to a naïve site. Patches were removed by the applicants after 24 hours. Sites were graded 24 and 48 hours post-patch removal.

Study Group

89 F, 24 M; age range 18 - 71 years

Vehicle

DEP

Remarks - Method

A pilot study (17 subjects), using 20% notified chemical, was conducted prior to the main study.

The test substance was spread on a 2 cm × 2 cm occluded patch.

A panel of 113 healthy human subjects (devoid of any physical or dermatological conditions) was amassed. Of these, 101 (81 female and 21 male) test subjects completed the study (11 subjects were lost to follow up, 1 subject voluntarily withdrew; 0-8 induction observations recorded).

RESULTS

Remarks - Results

One test subject was observed with definite erythema at induction readings 4 and 5. The same subjects and six other test subjects were also noted to have minimal or doubtful responses (presented with slightly different surrounding skin) at 1-3 induction observations. No reactions were evident in any test subject during the challenge phase.

CONCLUSION

The notified chemical was non-sensitising under the conditions of the test.

TEST FACILITY

TKL (2004)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral (Dietary) Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/CD (Crl:CD SD IGS BR)
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Mortality*
		Nominal (ppm)	*Actual (mg/kg bw/day)*	
control	5 per sex	0	0	0/10
low dose	5 per sex	225	24	0/10
mid dose	5 per sex	2,250	216	0/10
high dose	5 per sex	15,000	1,486	0/10

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

No clinically observable signs of toxicity were detected in test or control animals throughout the study. A missing tail tip was observed in one female of the low dose group, but the study authors considered this a physical injury unrelated to treatment. No treatment-related effects on test animal behaviour, functional performance and sensory reactivity were detected during the study.

A slight reduction in mean weekly food consumption was noted throughout the study period in females of the high dose group. A slight reduction in food efficiency and a statistically significant reduction in bodyweight gain were noted in male and female animals of the high dose group compared to control findings, but these findings were confined to week 1 of observations only. No adverse effects were detected in the mid and low dose groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

A statistically significant increase in clotting (pro-thrombin) time was detected in male animals in the highest dose group with all individual values reportedly outside the normally expected range for rats of the age and strain used. There were no statistically significant increases in this parameter detected in the other dose groups. No other haematological parameters rendered intergroup differences of note.

A statistically significant reduction in plasma bilirubin and increase in plasma cholesterol were detected for males in the highest dose group, together with an increase in plasma total protein for females treated at the highest dose. While statistically significant, these were considered by the study authors to be of no toxicological importance.

Effects in Organs

Both male and female test animals in the highest dose group showed an increased relative liver weight (statistically significant in males only). Relative kidney weight was elevated (statistically significant) in the highest dose group male animals only. Females treated at the highest dose showed a statistically significant increase in relative adrenal weight and statistically significant reductions in relative ovary and spleen weights. However, in the absence of any histopathological correlation between weight change and degenerative change, these intergroup differences were considered by the study authors not to be of toxicological significance. No treatment related effects were seen in the mid and low dosed groups.

No treatment related macroscopic abnormalities were detected. A grossly enlarged thymus was seen in one control group female animal which was attributed to a thymic tumour, unrelated to treatment.

On microscopic examination of the test animals, tissue sections revealed treatment-related liver, kidney and urinary bladder changes. Centrilobular hepatocyte enlargement was observed in animals of both sexes in the highest dose group, but not at lower dose levels. As hepatocyte enlargement is often seen in the rodent liver following treatment with xenobiotics, in the absence of any associated degenerative or inflammatory changes, the study authors considered this to be an adaptive change.

Globular accumulations of eosinophilic material were observed in the tubular epithelium of three male rats in the highest dose group and for two male rats in the mid dose group. The study authors found this observation consistent with the appearance of hydrocarbon nephropathy, resulting from excessive accumulation of α_2-microglobulin in renal proximal tubular epithelial cells. As this is exclusively found in adult male rats, this finding was considered not indicative of a hazard to human health.

Treatment related hyperplasia of the transitional epithelium was observed in animals of both sexes in the highest dose group, with some animals showing associated sub-epithelial inflammatory cell infiltrates. Epithelial hyperplasia was also observed in the urinary bladder of one control group female, one low dose group male and one mid dose group female as spontaneous findings. The study authors presumed this change was associated with excretion of an irritant test material metabolite in the urine.

All other morphological changes observed were discounted by the study authors as common to the strain and age of the rats and/or as having no differences in incidence or severity of effects between the control and treatment groups and were considered to be of no toxicological significance.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 216 mg/kg bw/day for the notified chemical in this study, based on adverse effects at the highest dose tested.

TEST FACILITY	Safepharm (2002c)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Commission Directive No. 92/69/EEC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure/Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 312.5 – 5,000 µg/plate b) Without metabolic activation: 312.5 – 5,000 µg/plate
Vehicle for test item	Ethanol (at 100 mg/mL)
Vehicle for Positive controls	Dimethyl sulfoxide
Remarks - Method	No significant protocol deviations. GLP Compliance. A preliminary toxicity test (0-5000 µg/plate) was performed to determine the toxicity of the test material (TA98, TA100 and WP2uvrA strains). Test 1 and Test 2 (without metabolic activation) were conducted as plate incorporation assays. Test 2 (with metabolic activation) was undertaken as a pre-incubation assay. Positive control tests were conducted in parallel to the main test using sodium azide, 9-aminoacridine, 2-nitrofluorene, 4-nitroquinoline 1-oxide and 2-anthramine.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1,000	≥ 625	≥ 5,000	negative
Test 2		≥ 1,250	≥ 5,000	negative
Present				
Test 1	> 5,000	≥ 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 5,000	negative

Remarks - Results

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose material, either with or without metabolic activation.

The positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

CONCLUSION

The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY

CIT (2000e)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 471 Bacterial Reverse Mutation Test.
Commission Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure/Pre incubation procedure

Species/Strain

S. typhimurium: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System

S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver

Concentration Range in Main Test

a) With metabolic activation: 50 – 5,000 µg/plate
b) Without metabolic activation: 50 – 5,000 µg/plate

Vehicle for test item

Acetone

Remarks - Method

Ames Test - No significant protocol deviations.
GLP Compliance.

A preliminary toxicity test (0-5,000 µg/plate) was performed to determine the toxicity of the test material (TA100 and WP2uvrA strains).

Positive control tests were conducted in parallel to the main test using N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), 9-Aminoacridine (9AA) and 4-Nitroquinoline 1-oxide (4NQO) in the absence of S9-mix, and 2-Aminoacridine (2AA) and Benzo(a)pyrene (BP) with S9-mix.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 5,000	negative
Present				

Test 1	> 5,000	> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 5,000	negative

Remarks - Results

The notified chemical caused no visible reduction in the growth of the bacterial background lawn at any dose level, with and without metabolic activation. An oily precipitate was observed in the 5,000 µg plate.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose material, either with or without metabolic activation.

The positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

CONCLUSION

The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY

Safepharm (2006)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Commission Directive 92/69/EEC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain: Human
Cell Type/Cell Line: Lymphocytes
Metabolic Activation System: S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Vehicle: Acetone
Remarks - Method: No significant protocol deviations.
GLP Compliance.
A preliminary toxicity study was performed (4 hr exposure, with and without activation and 24 hr exposure without activation) at concentrations 10.02 – 2,564 µg/mL.
Vehicle and positive controls (Mitomycin C without metabolic activation and cyclophosphamide with metabolic activation) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 5, 10*, 20*, 40*, 60, 80	4 h	24 h
Test 2	0*, 5.01, 10.02, 20.04*, 40.07*, 60.10*, 80.13	24 h	24h
Present			
Test 1	0*, 10*, 20*, 40*, 60, 80, 120	4 h	24 h
Test 2	0*, 10.02, 20.04*, 40.07*, 80.13*, 120.19, 160.25	4 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 80.13	≥ 60	≥ 80	negative
Test 2		≥ 60.1	≥ 60.1	negative
Present				
Test 1	≥ 80.13	≥ 60	≥ 120	negative
Test 2		≥ 80.13	≥ 160.25	negative

Remarks - Results	No toxicologically significant increases in the number of cells with aberrations were noted, with or without metabolic activation. The test material did not induce a statistically significant increase in the number of polyploidy cells at any dose in either of the exposure groups. The cultures from the 120.19 µg/ml dose level (Test 2, with metabolic activation) were lost due to technical error, however, the study authors considered it likely that this dose level would not have provided metaphase cells for analysis. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Safepharm (2001d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: A method very similar to the ISO test No 14593, Water quality/evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium/method by analysis of inorganic carbon in sealed vessels (CO_2, headspace test).

Inoculum	Domestic sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	The degradation was assessed by the measurement of the carbon dioxide produced on days 5, 8, 13, 16, 20, 23 and 28
Remarks - Method	According the test report, the notified chemical failed all good laboratory practice (GLP) biodegradation tests. The notified chemical is recognised to be hydrolytically unstable depending on the pH and has a low aqueous solubility. It is also not suitable for biodegradability test with continuous aeration due to the moderately volatile property. Its high log P_{OW} value can lead to a high non-specific adsorption. In addition, the biodegradability for the parent chemical of the notified chemical (through abiotic degradation) has been estimated. A method very similar to the ISO test No 14593 has been carried out mainly because this procedure fits quite well the physico-chemical properties of the notified chemical (solubility, vapour pressure, oxygen demand, adsorption, etc.). The only modification was to replace the air in the headspace with pure oxygen to ensure no oxygen transfer limitation. In addition, in the ISO test, only the carbon dioxide in the headspace is normally measured after acidification at pH 3. At this pH, the equilibrium constant for the distribution of carbon dioxide between the liquid phase and the headspace is approximately equal to one. In this case, tests were normally carried out in small size vessels which were for each analysis. A reference compound, methyl dihydrojasmonate, different than the usual reference compounds, has been used. According to the report, this is a readily biodegradable compound widely used in the perfumery industry. This molecule has a closer elementary composition to the test substances and has no endogenous nitrogen. The test materials at a concentration of 25 mg/L (18.75 mg carbon/L) for the notified chemical and 25.25 mg/L (19.73 mg carbon/L) for the parent chemical were exposed to an activated sewage sludge with culture medium at 24 ± 2°C.

RESULTS

Notified chemical		*Parent chemical*		*Methyl dihydrojasmonate*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
13	3.1	5	41.3	5	65.2
20	42.5	8	64.3	8	73.9
28	82.2	28	86.9	28	79.9

Remarks - Results: No toxicity control test was arranged, which is acceptable given the high degree of degradation detected by day 28.
The notified chemical showed a lower biodegradability than the parent chemical. The study author explained this may be due to the higher absorption of the notified chemical to test vessel wall (log P = 4.58 for the

parent chemical), which delays the biodegradation. It is unclear if the 10 day window criterion was met. The notified chemical may be readily biodegradable based on the reported test results. Since the test was conducted at pH 3, the notified chemical may hydrolysis readily based on the results of the hydrolysis study above. The test result needs to treated with caution.

CONCLUSION The notified chemical is not stable under the test conditions.

TEST FACILITY Firmenich (2003)

C.1.2. Ready biodegradability

TEST SUBSTANCE Notified chemical

METHOD ISO test No 14593, Water quality/evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium/method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test).

Inoculum	Domestic sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	The degradation was assessed by the measurement of the carbon dioxide produced and dissolved organic carbon (DOC, for reference control test)
Remarks - Method	No significant protocol deviations. GLP Compliance. The test material, at a concentration of 20 mg C/L, was exposed to activated sewage sludge micro-organisms with culture medium in sealed culture vessels in the dark at 20 ± 1°C and pH 7.4 for 28 days. The test material was a poorly water soluble and non-viscous liquid. Following the recommendations of the International Standards Organisation (ISO 1996) the notified chemical was added directly to the test vessels using a high precision volumetric syringe. Control solutions with inoculums and the standard material, sodium benzoate, together with a toxicity control were used for validation purposes.

RESULTS

Notified chemical		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	3	2	62
16	-2	10	79
28	1	28	94

Remarks - Results All the test validity criteria were met. The toxicity control test attained 40% degradation after 28 days. Therefore, the notified chemical was not considered to be toxic to sewage micro-organisms.
The notified chemical attained 1% degradation after 28 days, therefore, it is not considered to be readily biodegradable.
The test outcome is different from the test summarised above. Since the test was conducted at pH 7.4, the test outcome is considered reliable for judgment of biodegradability.

CONCLUSION The notified chemical is not readily biodegradable.

TEST FACILITY Safepharm (2003a)

C.1.3. Ready biodegradability

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	The CO_2 generated was measured for calculation of the degree of biodegradation
Remarks - Method	No significant protocol deviations. GLP Compliance. The test solutions were prepared at a concentration of 10 mg/L total organic carbon (TOC) at 20°C to 23°C.

RESULTS

Notified chemical		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
8	3.0	4	16.3
14	14.1	14	70.7
28	55.9	28	91.7

Remarks - Results	All the test validity criteria were met. Biodegradation of the notified chemical totalled 42% at the end of the 10-day window and 55.9 % at the end of the test. The notified chemical is biodegradable but is not considered to be readily biodegradable based on the reported test results.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	CIT (2000f)

C.1.4. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	The CO_2 generated was measured for calculation of the degree of biodegradation
Remarks - Method	No significant protocol deviations. GLP Compliance. The test was conducted at a concentration of 5 or 10 mg C/L with activated sewage sludge micro-organisms with culture medium in sealed culture vessels in the dark at 21°C. A total of four separate experiments were performed in order to determine the biodegradability of the test material. Different methods of preparation were used to determine the effect of different inoculums sources on the biodegradability of the test material. Experiment 1 utilised a test concentration of 10 mg C/L. The test material was adsorbed onto granular silica gel prior to dispersion in the test medium in order to aid dispersion of the test material in the test medium and to increase the surface area of the test material exposed to the test organisms. The inoculum used in Experiment 1 was obtained from Loughborough Sewage Treatment Works, Leicestershire, UK. Experiment 2 utilised a test concentration of 5 mg C/L. All other conditions were the same as experiment 1. Experiment 3 utilised a test concentration of 10 mg C/L. The method of preparation of the test material vessels followed that employed in a

previous biodegradation study conducted on the test material (CIT, 2000) whereby the notified chemical was adsorbed onto glass fibre filter papers prior to addition to the test vessels. The inoculum used in experiment 3 was the same as in experiments 1 and 2.
Experiment 4 was conducted using the same test concentration and method of preparation as experiment 3, however the inoculum was obtained from Belper Sewage Treatment Works, Derbyshire, UK.

RESULTS

Notified chemical		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
8	3.0	4	16.3
14	14.1	14	70.7
28	55.9	28	91.7

Remarks - Results

All the test validity criteria were met.

Experiment 1 was terminated after 14 days as the results obtained indicated that under the definition given in the OECD Guidelines, the test material was exhibiting an inhibitory effect on the test organisms.

In experiment 2 the test material was also shown to exhibit an inhibitory effect on the test organisms at the reduced concentration of 5 mg C/L, and no significant degradation of the test material was observed after 28 days.

The test material therefore cannot be considered to be readily biodegradable under the strict terms and conditions of OECD Guideline No 301B.

These experiments were conducted as screening tests using a reduced number of control, standard and test material vessels and hence care should be taken in the interpretation of the results obtained.

In experiment 3 the test material attained 36% degradation after 28 days. The toxicity control vessel attained 0% degradation after 14 days and 22% degradation after 28 days. Greater than 25% degradation in the toxicity control vessel was not obtained after 14 days. Therefore, under the strict terms and conditions of the OECD test guideline the test material would be classed as having caused an inhibitory effect. However, given that significant degradation of the test material occurred in the test material vessel, the results obtained for the toxicity control should be treated with caution.

Experiment 4 was conducted using a different source of inoculum, and the test material attained 11 % degradation after 28 days. The toxicity control attained 36% degradation after 14 days and 45% degradation after 28 days thereby confirming that the test material was not toxic to the sewage treatment micro-organisms used in this experiment.

The above experiments indicate that the use of granular silica gel was not suitable for this test material as it resulted in an increase in the inhibitory effect that the test material exerted on sewage treatment micro-organisms and as such no significant biodegradation of the test material occurred. The use of glass fibre filter papers was shown to lower the inhibitory effect of the test material. This may have been due to there being a lower bioavailable test material concentration when using this method of preparation. The use of inocula from different sources may affect the biodegradation rate of the test material.

According to the study author, data supplied by the sponsor showed that the test material degrades to volatile chemicals (exempt information). The volatile degradates would be removed from the test system in a standard CO_2 evolution test. It is therefore considered that a standard CO_2 evolution

test may not be the most applicable method of determining the ready biodegradability of the test material.

When using test methods based on the OECD Guideline No 301B the test material was shown not to be readily biodegradable. This conclusion complies the conclusion from the studies summarised above.

CONCLUSION The notified chemical is not readily biodegradable.

TEST FACILITY Safepharm (2003b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE

METHOD OECD TG 203 Fish, Acute Toxicity Test - Semi-static.
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - Semi-static.

Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Test concentrations were analysed using gas chromatography (GC) at i0 hour and after 24 hours of the test
Remarks – Method	No significant protocol deviations. GLP Compliance. Following a preliminary range-finding test, fish were exposed to an aqueous solution of the notified chemical at nominal concentrations of 0.28, 0.50, 0.90, 1.6 and 2.8 mg/L. The test material solutions were prepared by stirring an excess (100 mg/L) of test material in dechlorinated tap water and then removing any undissolved test material by filtration (0.2 µm). This "saturated" solution was found to have a concentration of 2.8 mg/L, and was further diluted to provide target test concentrations. The mortality data were analysed by the trimmed Spearman-Karber method using ToxCalc to give LC50 values.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	10	0	0	0	0	0
0.28	0.21	10	0	0	0	0	0
0.50	0.35	10	0	0	0	0	0
0.90	0.69	10	0	0	0	0	0
1.6	1.2	10	0	1	1	1	4
2.8	2.0	10	6	10	10	10	10

LC50	1.3 mg/L at 96 hours.
NOEC	0.35 mg/L at 96 hours.
Remarks – Results	All the test validity criteria were met. Chemical analysis indicated decline of the test concentrations over time. A stability study included in the report indicated the notified chemical is stable over a period of 24 hours (pH condition not provided). However, the notified chemical showed hydrolysis in water at pH 4-9 (Appendix A for test results). The concentration decline may be relevant to hydrolysis, evaporation, adsorption to test glassware, and accumulation in test organisms of the notified chemical. Time-weighted mean measured values were used as the actual concentrations. The 96 hour LC50 based on the time-weighted mean measured test

concentrations was 1.3 mg/L with 95% confidence limits of 1.1 - 1.5 mg/L. Sublethal effects, such as swimming at surface or bottom, increased pigmentation and loss of equilibrium, etc. were observed at the level including and above 0.69 mg/L. Therefore, the no observed effect concentration was determined to be 0.35 mg/L.

CONCLUSION The notified chemical is toxic to fish.

TEST FACILITY Safepharm (2002d)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia sp*. Acute Immobilisation Test - Static test.
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - Static test.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Test concentrations were analysed using gas chromatography (GC) at 0 hour and after 48 hours of the test
Remarks - Method	Following a preliminary range-finding test, daphnids were exposed to an aqueous solution of the notified chemical at nominal concentrations of 0.025, 0.045, 0.080, 0.14, 0.25, 0.45, 0.80, 1.4 and 2.5 mg/L. The test material solutions were prepared by stirring an excess (100 mg/L) of test material in dechlorinated tap water and then removing any undissolved test material by filtration (0.2 µm). This "saturated" solution was found to have a concentration of 2.5 mg/L, and was further diluted to provide target test concentrations. The data were analysed by the trimmed Spearman-Karber method using ToxCalc to give EC50 values.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	20	0	0
0.025	0.024	20	0	0
0.045	0.037	20	0	0
0.08	0.064	20	0	0
0.14	0.13	20	0	0
0.25	0.19	20	0	0
0.45	0.37	20	0	0
0.80	0.67	20	0	0
1.4	1.19	20	4	13
2.5	2.0	20	17	20

EC50	1.1 mg/L at 48 hours
NOEC	0.67 mg/L at 48 hours
Remarks - Results	All the test validity criteria were met. Chemical analysis indicated decline of the test concentrations over time. This may be relevant to hydrolysis, evaporation, adsorption to test glassware, and accumulation in test organisms of the notified chemical. Time-weighted mean measured values were used as the actual concentrations. The 48 hour EC50 based on the time-weighted mean measured test concentrations was 1.1 mg/L with 95% confidence limits of 0.97 mg/L to 1.2 mg/L. The no observed effect concentration was determined to be 0.67 mg/L.

CONCLUSION The notified chemical is toxic to *Daphnia.*

TEST FACILITY Safepharm (2002e)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna,* Reproduction Test (1998)
Species *Daphnia magna*
Exposure Period 21 d
Auxiliary Solvent None
Water Hardness 250 mg/L
Analytical Monitoring Test concentrations were analysed using gas chromatography (GC) throughout the test
Remarks - Method No significant protocol deviations.
GLP Compliance.

Daphnids were exposed to an aqueous solution of the notified chemical at nominal concentrations of 0.008, 0.025, 0.080, 0.25 and 0.80 mg/L. The test material solutions were prepared by stirring an excess (100 mg/l) of test material in dechlorinated tap water and then removing any undissolved test material by filtration (0.2 µm). This "saturated" solution was found to have a concentration of 2.2 mg/L, and was further diluted to provide target test concentrations.

The EC50 for immobilisation was calculated by the trimmed Spearman-Karber method using ToxCalc. The EC50 for reproduction was estimated by inspection of the data due to the unsuitable nature of the data for statistical analysis.

For the estimation of the no observed effect concentration (NOEC) the numbers of live young produced per adult over the duration of the test for the control, 0.0080, 0.025 and 0.080 mg/L test groups were compared using one way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control.

Nominal loading tested, cumulative total number of offspring released, mean length and standard deviations and survival of parental daphnids.

	Nominal loading Rate (mg/L)					
Test Day 21	*Control*	*0.008*	*0.025*	*0.08*	*0.25*	*0.80*
Total no. of offspring released by survived *Daphnia*	898	940	919	903	207	0
Mean length (standard deviation) of survival parent daphnids (mm)	4.1 (0.2)	4.1 (0.1)	4.0 (0.3)	4.0 (0.0)	-	-
No. of adult *Daphnids* Immobilised	0	0	1	1	0	0
% Survival	100	100	90	90	0	0

21 day EC50 (Immobilisation) 0.044 mg/L
21 day EC50 (Reproduction) 0.029 mg/L to 0.11 mg/L
21 day NOEC 0.029 mg/L

Remarks - Results The test validity criteria wet met.
Marked decline (up to 93% of the nominal for the most decline by day 5) in measured test concentrations were observed. The endpoints were calculated on the basis of time-weighted measured concentrations (according to the study report).

The 0.25 and 0.80 mg/L test groups data were not included in the statistical analysis as the effects of exposure eliminated all the daphnids prior to termination of the test.

The 21 day EC50 for immobilisation for the parental *Daphnia* generation (P_1) was calculated to be 0.044 mg/L (nominal concentration 0.11mg/L) with 95% confidence limits of 0.033 mg/L to 0.06 mg/L (nominal concentrations of 0.083 – 0.15 mg/L).

The 21 day EC50 for reproduction was estimated to be 0.029 to 0.11 mg/L (nominal concentrations 0.08 – 0.25 mg/L) on the basis that at 0.029 mg/L there was no significant difference ($P \geq 0.05$) in terms of the number of young produced per adult when compared to the control and that the adult daphnids at 0.11 mg/L were eliminated from the test by day 19 due to a prolonged toxic effect of the test material.

The NOEC was considered to be 0.029 mg/L (nominal 0.08 mg/L) on the basis that at this test concentration there were no significant mortalities (immobilisation) observed in the parental generation (P_1) and that there were no significant differences ($P \geq 0.05$) between the control and the 0.029 mg/L test group in terms of numbers of live young produced per adult by day 21.

CONCLUSION The notified chemical is very toxic to daphnids on a chronic basis.

TEST FACILITY Safepharm (2003c)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
EC Council Regulation No 440/2008 C.3 Algal Inhibition Test.

Species	Green alga (*Scenedesmus subspicatus*)
Exposure Period	72 hours
Concentration Range	Nominal: 2.3 mg/L (0 hour) Actual: 1.9 mg/L (mean of 0 hour and 72 hour)
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	Test concentrations were analysed using gas chromatography (GC) at initial and after 72 hours of the test. Cell densities were determined using Coulter® Multisizer II Particle Counter.
Remarks - Method	No significant protocol deviations. GLP Compliance. Following preliminary range-finding tests, algae were exposed to an aqueous solution of the notified chemical at a time mean measured concentration of 1.9 mg/L. The test material solutions were prepared by stirring an excess (100 mg/L) of test material in dechlorinated tap water and then removing any undissolved test material by filtration (0.2 µm). Statistical analysis of the area under the growth curve data was carried out using a Student t-test.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72h	*NOEC mg/L*	*E_rC50 mg/L at 72h*	*NOEC mg/L*
> 1.9	1.9	> 1.9	1.9

Remarks - Results All the test validity criteria were met. Decline in test concentration (from

	2.3 mg/L at 0 hour to 1.55 mg/L at 72 hour) over time was observed. The study author commented that this may be from the instability of the notified chemical in the test medium and the adsorption of the notified chemical to algae. Considering the test was conducted in saturated solutions and no effects were observed, the notified chemical is considered not to be harmful up to the limit of solubility.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	Safepharm (2002f)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1, 3.16, 10, 31.6, and 100 mg/L
Remarks – Method	No significant protocol deviations. GLP Compliance. The IC50 of the notified chemical and the reference substance was determined considering the oxygen consumption of the controls to be 100%.
RESULTS	
IC50	> 100 mg/L
NOEC	100 mg/L
Remarks – Results	All the test validity criteria were met. The respiration rate of the test solution of highest concentration (100 mg/L) was equivalent to the respiration rate of the first control (i.e. these rates were within 15% of each other). Therefore, the oxygen consumption rate of the rest test solutions (1 to 31.6 mg/L) was not determined. The 3 hour IC50 of the notified chemical is > 100 mg/L for activated sludge. The notified chemical is considered as not inhibitory to the sludge micro-organisms.
CONCLUSION	The notified chemical is not inhibitory to the sludge micro-organisms.
TEST FACILITY	CIT (2000g)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1, 3.16, 10, 31.6, and 100 mg/L
Remarks – Method	No significant protocol deviations. GLP Compliance. Following a preliminary range-finding test, the definitive test was conducted using two methods of dispersing the test material in the test system. One used a single test concentration of 5.29 mg/L (the limit of the water solubility), prepared using a preliminary solution in an auxiliary solvent, the other used a test concentration of 1000 mg/L prepared by direct

	dispersion on the notified chemical in water with the aid of ultrasonication.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	All the test validity criteria were met. No solvent control was conducted, which is considered acceptable since no effects were observed in the test. No significant inhibition of the respiration rate of activated sewage sludge was observed in each of the two tests. These results show that the notified chemical does not have an effect on the respiration rate of activated sewage sludge up to the limit of the water solubility. The notified chemical is considered as not inhibitory to the sludge micro-organisms.
CONCLUSION	The notified chemical is not inhibitory to the sludge micro-organisms.
TEST FACILITY	Safepharm (2002g)

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