LTD/1958

February 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

4-Pentene-1-one, 1-(3,3-dimethylcyclohexyl)-

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 and Energy.

This Public Report is 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/1958	International Flavours and Fragrances (Australia) Pty Ltd	4-Pentene-1-one, 1-(3,3-dimethylcyclohexyl)-	No	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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

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

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

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

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

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself. However, these should be selected on the basis of all ingredients in the formulation.

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

- A copy of the 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 of Classification and Labelling of Chemicals (GHS)*

as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent 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, ≤ 3% in fine fragrances, 0.8% in body lotion, hand and face creams and ≤ 1% in other cosmetics and 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.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
International Flavours and Fragrances (Australia) Pty Ltd (ABN: 77 004 269 658)
310 Frankston-Dandenong Road
DANDENONG VIC 3175

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, disassociation constant, and adsorption/desorption coefficient

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA (1986)
EU (1996)

2. IDENTITY OF CHEMICAL

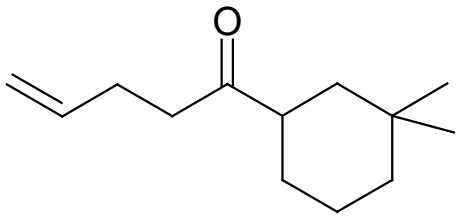
MARKETING NAME
Galbaniff

CAS NUMBER
56973-87-6

CHEMICAL NAME
4-Pentene-1-one, 1-(3,3-dimethylcyclohexyl)-

MOLECULAR FORMULA
$C_{13}H_{22}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
194.17 Da

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

3. COMPOSITION

DEGREE OF PURITY
95.91%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)

Chemical Name	2-Allyl-3,3,7-trimethyl-cycloheptanone		
CAS No.	Not assigned	*Weight %*	1.62

ADDITIVES/ADJUVANTS

Chemical Name	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-		
CAS No.	128-37-0	*Weight %*	0.1

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: clear to pale yellow liquid

Property	Value	Data Source/Justification
Freezing Point	< 25 °C	Measured
Boiling Point	243-260 °C at 101.9 kPa	Measured
Density	899.10 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.25 x 10^{-3} kPa at 25 °C	Measured
Water Solubility	0.032 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	pH=4: $t_{1/2}$ = 71 h pH=7: $t_{1/2}$ = 150 h pH=9: $t_{1/2}$ = 71 h	ECHA, 2017
Partition Coefficient (n-octanol/water)	log Pow > 3.6 at 20 °C	Measured
Surface Tension	54.3 mN/m at 19.5 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.89 and 3.11	Estimated using Pow and MCI methods (KOCWIN v2.00, US EPA 2011).
Dissociation Constant	Not determined	The notified chemical does not contain functionality that is expected to dissociate under environmental conditions.
Flash Point	111.5 °C at 101.6 kPa	Measured
Flammability in contact with water	Not flammable	Measured
Autoignition Temperature	236 °C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Estimated. The notified chemical does not contain chemical groups which confer 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 of 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 introduced into Australia as a component of finished fragrance oils at ≤ 20% concentration.

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
Melbourne

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of finished fragrance oils at ≤ 20% concentration in 205 L polypropylene-lined steel drums. The imported products containing the notified chemical will be transported to reformulation sites within Australia by road. The end-use products containing the notified chemical at ≤ 3% concentration will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient for use in cosmetic and household products. The proposed use concentration will be ≤ 3% concentration in fine fragrances, ≤ 0.8% concentration in body lotion, hand and face creams and ≤ 1% concentration in other cosmetic and household products.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. The notified chemical at ≤ 20% concentration will be introduced into Australia as a component of finished fragrance oils. These products will be sold to companies, who will reformulate the fragrance oils to make household and cosmetic consumer products containing the notified chemical at ≤ 3% concentration.

Reformulation
The procedures for incorporating the notified chemical into end-use products will likely vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. However, in general, it is expected that for the reformulation process, the notified chemical will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished cosmetic and household products. This will be followed by automated filling of the reformulated products into containers of various sizes. The blending operations are expected to be highly automated and use closed systems and/or adequate ventilation. During the reformation process, samples of the notified chemical and the finished end-use products will be taken for quality control testing.

Cosmetic products
The finished cosmetic products containing the notified chemical will be used by consumers and professionals (such as beauticians and hair dressers). Depending on the nature of the products, application could be by hand, sprayed or through the use of an applicator.

Household products
Household products containing the notified chemical may be used by consumers and professional workers (i.e., cleaners). The products may be used in either closed systems with episodes of controlled exposures, for example automatic washing machines, or open processes and manually by rolling, brushing, spraying and dipping.

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
Plant operators-mixing/compounding	4	250
Plant operators-drum handling	1	250
Plant operators-drum cleaning/washing	2	200
Plant operators-equipment cleaning/washing	2	250
Plant operators-quality control	1	250

EXPOSURE DETAILS

Transport and storage

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

Reformulation

Reformulation workers may be exposed to the notified chemical at ≤ 20% concentration during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The primary routes of exposure to the notified chemical (at ≤ 20% concentration) are dermal and ocular. Exposure to the notified chemical is expected to be minimised through the use of automated processes and PPE such as coveralls, goggles and impervious gloves. Exposure through inhalation is also possible, but not expected given the low vapour pressure of the notified chemical and the use of adequate local ventilation.

End-use

Exposure to the notified chemical in end-use products at ≤ 3% concentration may occur in professions where the services provided involve the application of cosmetic products to clients (i.e., hair and beauty salons) or the use of household products 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 at ≤ 3% concentration through the use of a wide range of cosmetic and household products. The principal route of exposure will be dermal, while ocular and inhalation exposure (e.g. through the use of spray products) are also possible.

Data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used are shown in the following tables (SCCS, 2012; Cadby *et al.,* 2002; ACI, 2010; Loretz *et al.,* 2006). For the purposes of the exposure assessment *via* the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed for the notified chemical (ECHA, 2014). For the inhalation exposure assessment, a 2-zone approach was used (Steiling *et al.,* 2014; Rothe *et al.,* 2011; Earnest, Jr., 2009). An adult inhalation rate of 20 m^3/day (enHealth, 2012) was used and it was conservatively assumed that the fraction of the notified chemical inhaled is 50%, which accounts for a number of other exposure considerations (e.g., the amount ending up on the hair, as intended). A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Cosmetic Products (direct dermal exposure)

Product type	Amount (mg/day)	C (%)	RF (unitless)	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	0.8	1	0.97750
Face cream	1540	0.8	1	0.19250
Hand cream	2160	0.8	1	0.27000
Fine fragrance	750	3	1	0.35156
Deodorant	1500	1	1	0.23438
Shampoo	10460	1	0.01	0.01634
Conditioner	3920	1	0.01	0.00613
Shower gel	18670	1	0.01	0.02917
Facial cleanser	800	1	0.01	0.00125
Hand wash	20000	1	0.01	0.03125
Hair styling	4000	1	0.1	0.06250
Total				**2.1726**

C = concentration (%); RF = retention factor.
Daily Systemic Exposure = (Amount × C × RF × Dermal Absorption)/Body Weight

Household Products (indirect dermal exposure from clothes)

Product type	Amount (g/use)	C (%)	Product retained (%)	Product transferred (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	1	0.95	10	0.0341
Fabric softener	90	1	0.95	10	0.0134
Total					**0.0475**

Daily Systemic Exposure = (Amount × C × PR × PT × Dermal Absorption)/Body Weight

Household Products (direct dermal exposure)

Product type	Frequency (use/day)	C (%)	Contact area (cm^2)	Product usage (g/cm^3)	Film thickness (cm)	Time scale factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	1	1980	0.01	0.01	0.007	0.0003
Fabric softener	3	1	1980	0.009	0.01	0.03	0.0025
All-purpose cleaner	1	1	1980	1	0.01	0.007	0.0217
Total							**0.0245**

Daily systemic exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale Factor x dermal absorption)/Body Weight

Aerosol Products (inhalation exposure)

Product type	Amount (g/use)	C (%)	Inhalation rate (m^3/day)	Exposure duration zone 1 (min)	Exposure duration zone 2 (min)	Fraction inhaled (%)	Volume zone 1 (m^3)	Volume zone 2 (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	10	1	20	1	20	50	1	10	**0.0322**

Daily Systemic Exposure = [(Amount × C × Inhalation rate × Fraction inhaled × 0.1) / Body Weight × 1440)] × (Exposure Duration Zone 1 / Volume Zone 1 + Exposure Duration Zone 2 / Volume Zone 2)

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table that contain the notified chemical. This would result in a combined internal dose of 2.2768 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products (in addition to hair spray) may occur. However, it is considered that the combination of the conservative (screening level) hair spray inhalation exposure assessment parameters, and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% absorption rate, is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with lower exposure factors.

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 = 5055 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	non-irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Human, skin sensitisation – RIPT (1% v/v)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL 250 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non clastogenic
Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test	non genotoxic

Toxicokinetics

Dermal absorption is expected to be limited given the low water solubility (32.3 x 10^{-3} g/L) and high lipophilicity (log Pow > 3.6) of the notified chemical, limiting penetration of the hydrophilic epidermis. Given the low molecular weight (194.17 Da) of the notified chemical, absorption across the gastrointestinal and respiratory tract may occur.

Acute toxicity

The notified chemical was found to be of low acute oral and dermal toxicity in studies conducted in rats.

Irritation and sensitisation

Based on studies conducted in rabbits, the notified chemical is slightly irritating to skin and non-irritating to eyes.

In the skin irritation study, very slight to well defined erythema and very slight oedema was observed. Erythema was resolved in all animals at 6 day observation period. Very slight oedema was resolved by the 48 hour reading. Slight skin exfoliation was present in 2/6 tested animals from day 5 to the end of the study period (i.e. day 7).

In the guinea pig maximisation test, the notified chemical did not show evidence of skin sensitisation when tested up to 100% concentration. During the human repeated insult patch test, slight erythema was observed in 1/49 subjects 24 h after challenge with the notified chemical. This effect was resolved by the 48 h reading. No other adverse responses were noted at challenge.

Repeated dose toxicity

In a 28-day repeated dose oral toxicity study in rats the No Observed Adverse Effect Level (NOAEL) for the notified chemical was established as 250 mg/kg bw/day based on no test substance related adverse effects at any of the doses tested.

Mutagenicity/Genotoxicity

The notified chemical tested negative in a bacterial reverse mutation assay, an *in vitro* chromosomal aberration assay using human lymphocytes and an *in vivo* mouse micronucleus test. The notified chemical is not expected to be genotoxic.

Health hazard classification

Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical is expected to be of low hazard presenting as a slight skin irritant.

Reformulation

During reformulation, workers may be exposed to the notified chemical at ≤ 20% concentration. At this proposed use concentration significant skin irritation effects are not expected. Furthermore, it is anticipated by the notifier that engineering controls such as enclosed and automated processes and local ventilation will be implemented where possible, and appropriate PPE (coveralls, imperious gloves, eye protection and respiratory protection) will be used to limit worker exposure.

Therefore, under the occupational settings described, 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 up to 3% 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 experience 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

Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic and household products (containing the notified chemical at ≤ 3% in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if the products are applied by spray.

Irritation

The notified chemical is slightly irritating to the skin. Given the low proposed use concentration (≤ 3%) irritation effects are not expected.

Repeat dose toxicity

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 2.2768 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 250 mg/kg bw/day derived from a 28 day repeated dose oral toxicity study, the margin of exposure was estimated to be 110. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 3% in fine fragrances, ≤ 0.8% in body lotion, hand and face creams and ≤ 1% in other cosmetics and 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 as a component of fragrance formulations at ≤ 20% concentration for reformulation into finished cosmetic and household products. Environmental release during importation, transport and distribution may occur as a result of accidental spills. In the event of a spill, the notified chemical is expected to be contained and collected with an inert absorbent material and disposed of in accordance with local regulations.

The fragrance formulations containing the notified chemical will be blended with other ingredients in the manufacture of cosmetic and household products within a fully enclosed environment. The process is expected to be followed by automated filling of the formulated products into containers of various sizes suitable for retail and use. Wastes containing the notified chemical generated during reformulation include equipment wash water, empty import containers and spilt materials. These are expected to be collected and recycled during subsequent

blending processes. Empty import containers and wash waters are expected to be recycled or disposed of through licensed waste management services. In the event of a spill, the notified chemical is expected to be contained and collected with an inert absorbent material and disposed of in accordance with local regulations.

RELEASE OF CHEMICAL FROM USE
The notified chemical is expected to be released to the aquatic compartment through sewers during its use in various cosmetic formulations and household products across Australia.

RELEASE OF CHEMICAL FROM DISPOSAL
It is expected that some of the product containing the notified chemical will remain in end-use containers. Wastes and residue of the notified chemical in empty containers are likely to either share the fate of the container and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system before potential release to surface waters on a nationwide basis. The submitted biodegradation study indicates that the notified chemical is not expected to be rapidly degraded in sewage treatment plants (STPs). For the details of the environmental fate studies, please refer to Appendix C. The notified chemical is hydrolysable under the environmental conditions (pH = 4-9, $t_{1/2}$ ~3-6 days) (ECHA 2017), however, the full study report was not provided.

Volatilization of the notified chemical is not rapid but may be significant based on Henry's Law constant (7.42 × 10^{-5} atm-m^3/mole) (US EPA 2011). The half-life of the notified chemical in air is calculated to be 2.84 h based on reactions with hydroxyl radicals (AOPWIN v1.92, US EPA 2011). Therefore, in the event of release to atmosphere, the notified chemical is not expected to persist in the atmospheric compartment.

In STPs the notified chemical is expected to be efficiently removed (based on its adsorption and partition coefficients) from effluent by adsorption to sludge and only a small portion may be released to surface waters. A proportion of notified chemical may be applied to land when effluent is used for irrigation, or disposed of to landfill as waste. The notified chemical residues in landfill and soils are expected to have low mobility based on its calculated soil adsorption coefficient (log Koc = 2.89 - 3.11). The notified chemical has low potential to bioaccumulate as it is expected to be readily hydrolysable. In the aquatic and soil compartments, the notified chemical is expected to slowly degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. Based on the reported use in cosmetics and household cleaning products, it is assumed that 100% of the total import volume of the notified chemical is released to the sewer. The release is assumed to be nationwide over 365 days per year. It is conservatively assumed that there is no removal of the notified chemical during sewage treatment processes.

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

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.61 µg/L may potentially result in a soil concentration of approximately 4.04 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 20.2 µg/kg and 40.4 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 LC50 = 5.7 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 2.7 mg/L	Toxic to daphnia
Algal Toxicity	72 h EC50 > 9 mg/L[¥§]	Not harmful to algae up to solubility limit in test medium
Inhibition of Bacterial Respiration	3 h EC50 > 10 mg/L[¥]	Not inhibitory to microbial respiration up to solubility limit in test medium

[¥] Solubility of the notified chemical in the test medium (~12 mg/L) was lower than true water solubility (32.2 mg/L).
[§] The results of algal toxicity test should be treated with caution due to substantial losses of the test compound in both inoculated and non-inoculated test vessels possibly due to volatilisation.

Based on the above ecotoxicological endpoints, the notified chemical is toxic to fish and aquatic invertebrates. Therefore, the notified chemical is classified as 'Acute Category 2: Toxic to aquatic life' according to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). On the basis of acute toxicity data and low biodegradation rate, the notified chemical is formally classified as 'Chronic Category 2: Toxic to aquatic life with long lasting effects'.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentrations (PNEC) for the notified chemical has been calculated from the acute Daphnia toxicity and an assessment factor of 100 is applied as three measured toxic endpoints are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	2.70	mg/L
Assessment Factor	100.00	
Mitigation Factor	1.00	
PNEC:	27.00	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.61	27	0.022
Q - Ocean	0.06	27	0.002

The Risk Quotients (Q = PEC/PNEC) for discharge of treated effluents containing the notified chemical have been calculated to be < 1 for both river and ocean compartments indicating that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual importation quantity. On the basis of the PEC/PNEC ratio and assessed use pattern in cosmetic formulations and household products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -25°C

Method EC Council Regulation No 440/2008 A.1 Freezing Temperature.
Remarks The notified chemical appeared as a pourable liquid at -25 °C both times the test was performed.
Test Facility Huntingdon (1996a)

Boiling Point 243-260 °C at 101.9 kPa

Method EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Test Facility Huntingdon (1996a)

Density 899.10 kg/m^3 at 20°C

Method EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Pycnometer Method was used.
Test Facility Huntingdon (1996a)

Vapour Pressure 1.25 x 10^{-3} kPa at 25 °C

Method EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Vapour Pressure Isoteniscope Method was used.
Test Facility Huntingdon (1996a)

Water Solubility 32.3 mg/L at 20 °C

Method EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method
Test Facility Huntingdon (1996a)

Partition Coefficient (n-octanol/water) log Pow > 3.6 at 20 °C

Method EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Shake Flask Method. Concentrations of the notified chemical were below detection limits in the aqueous phase test solutions.
Test Facility Huntingdon (1996a)

Surface Tension 54.3 mN/m at 19.5 °C

Method EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks Concentration: 90% saturated aqueous solution
Surface tension was determined with a surface tension/torsion balance using the OECD harmonised ring method.
Test Facility Huntingdon (1996a)

Flash Point 111.5 °C at 101.6 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks Flash point determined using the Pensky-Martens closed cup method.
Test Facility Huntingdon (1996a)

Flammability Not flammable

Method EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water).
Remarks Flammability was determined by observing the reaction between the notified chemical and water for evolution of flammable gases. The notified chemical did not evolve gas during the study.

Test Facility Huntingdon (1996a)

Autoignition Temperature 236 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids).
Test Facility Huntingdon (1996a)

Explosive Properties Not explosive.

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The Koenen test apparatus was used to determine the thermal sensitivity (effect of a flame) of the notified chemical. A fall hammer was used to determine the mechanical sensitivity (effect of shock) of the notified chemical. The notified chemical was found not to have the explosive properties of mechanical and thermal sensitivity.
Test Facility Huntingdon (1996a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 401 Acute Oral Toxicity – Limit Test.
Species/Strain	Rat/Sprague Dawley
Vehicle	None
Remarks - Method	Rats dosed at 5000 mg/kg bw were used for the limit test. The remaining rats were used for LD50 determination test.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M/5F	3500	0/10
2	5M/5F	4000	1/10
3	5M/5F	4500	1/10
4	5M/5F	5000	6/10
5	5M/5F	5500	7/10

LD50	5055 mg/kg bw
Signs of Toxicity	3500 mg/kg bw: salivation, abnormal gait, ataxia, decreased activity and muscle tone, ptosis, poor grooming, abnormal stance, piloerection and chomodacryorrhea. 4000 mg/kg bw: signs of toxicity at 3500mg/kg bw plus diarrhoea, lacrymation, loss of righting reflex and semi-prostration. 4500 mg/kg bw: signs of toxicity at 3500mg/kg bw plus diarrhoea. 5000 mg/kg bw: signs of toxicity at 4000mg/kg bw plus semi-prostration, hypersensitivity and cyanosis. 5500 mg/kg bw: signs of toxicity at 3500mg/kg bw plus diarrhoea, loss of righting reflex, semi and complete prostration.
Effects in Organs	Necropsy of animals dosed with 4000, 5000 and 5500 mg/kg bw that died during the study revealed stomach haemorrhages, discoloured and fluid-filled intestines and bladder and discoloured liver and kidneys.
Remarks - Results	Most animals gained weight during the study. The majority of animals which lost weight had died before study completion.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Pharmakon (1983a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	
METHOD	OECD TG 402 Acute Dermal Toxicity (February 24, 1987). EEC B.3 directive 92/69/EEC (December 29, 1992).
Species/Strain	Rat/Hsd/Ola:Sprague-Dawley (CD)
Vehicle	None
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Slight erythema with or without slight oedema was observed at the treatment sites of all or a number or rats 3-6 days after treatment. These dermal effects were reversible and no longer evident 7 days after treatment.
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks - Results	No clinical signs or mortality was observed during the study period. All female rats and 2/5 male rats showed slightly low body weight gains at Day 8; one male also showed a slightly low gain on Day 15. This was not considered to be treatment related. No macroscopic abnormalities were recorded during post mortem examination.

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

TEST FACILITY Huntingdon (1996b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 404 Acute Dermal Irritation/Corrosion

Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Vehicle	None
Observation Period	7 days
Type of Dressing	Occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Erythema/Eschar	1.00	0.00	0.33	1.67	0.33	0.67	2.00	< 6 days	0.00**
Oedema	0.00	0.00	0.00	0.00	0.00	0.33	1.00	< 48 hours	0.00

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
** Slight exfoliation of skin at application site.

Remarks - Results	At 30 - 60 minutes after treatment with the test substance, a very slight erythema was noted in all animals and persisted in 5/6 animals until the 24-hour reading. This slight erythema developed into a well-defined erythema by the 48-hour reading in one animal and persisted till the Day 4 reading. Erythema was reversible and not evident in all animals 6 days after treatment. Very slight oedema was observed in 5/6 animals at the 30 - 60 minute reading which fully resolved in all animals by the 48 hour reading. Slight skin exfoliation was observed in 2 animals from Day 5 until Day 7.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Pharmakon (1983b)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 405 Acute Eye Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White

Number of Animals	6
Observation Period	7 days
Remarks - Method	No significant protocol deviations.

RESULTS

Remarks - Results	Slight to moderate conjunctival irritation was observed in all animals at the 1 hour reading. At the 24 hour reading all signs of irritation were resolved except for slight conjunctival irritation in one animal. No signs of irritation were noted from the 48 hour reading in all animals.
CONCLUSION	The notified chemical is non-irritating to the eye.
TEST FACILITY	Pharmakon (1984)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test (July 17, 1992). EU B.6 Directive 92/69/EEC (December 29, 1992).
Species/Strain	Guinea pig/Dunkin-Hartley Albino
PRELIMINARY STUDY	Maximum Non-irritating Concentration intradermal: 30% topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
Vehicle	Intradermal: Alembicol D Topical: None
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using formalin.
INDUCTION PHASE	Induction Concentration intradermal: 40% topical: 100%
Signs of Irritation	All test and control animals displayed very slight irritation after intradermal and topical induction.
CHALLENGE PHASE	
1st challenge	topical: 100% and 50% (in Alembicol D)
Remarks - Method	10% sodium lauryl sulfate in petrolatum was applied 24 hours before topical induction.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*	
		24 h	*48 h*
Test Group	50%	0/10	0/10
	100%	0/10	0/10
Control Group	50%	0/5	0/5
	100%	0/5	0/5

Remarks - Results	No signs of irritation were observed after challenge in control and test animals.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Huntingdon (1996c)

B.6. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical (1% concentration)
METHOD	Shelanski 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 Thursday) for a total of 9 applications. Patches were removed by the applicants after 24 h and graded. Patches applied on Monday were graded 48 h after patch removal. Rest Period: 21 days Challenge Procedure: Patches was applied to virgin sites and removed by the applicants after 24 h. Sites were graded 24, 48 and 72 h post-patch removal.
Study Group	47 F, 7 M; age range 20 - 69 years
Vehicle	Alcohol SD-39C
Remarks - Method	Occluded. The test substance was spread on a 3.8 cm × 3.8 cm patch.
RESULTS	
Remarks - Results	50/54 subjects completed the study. Two subjects voluntarily withdrew before the study started. An additional two subjects voluntarily withdrew following the first-week induction reading. At the third-week induction reading, 3/49 subjects (one subject absent on the reading date) displayed slight erythema. No other adverse responses were noted during the induction period. Slight erythema was observed in 1/49 subjects 24 h after patch removal. This effect was resolved by the 48 h reading. No other adverse responses were noted at challenge.
CONCLUSION	The test substance was non-sensitising under the conditions of the test.
TEST FACILITY	Techni-Med (1983)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EU B.7 Directive 92/69/EEC (29 December, 1992).
Species/Strain	Rats/Sprague-Dawley (Crl: CD® BR VAF PLUS™)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Corn oil
Remarks - Method	No recovery period was included in the study; all animals sacrificed after 28 days of treatment. Urinalysis was not conducted.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M/5F	0	0/10
low dose	5M/5F	15	0/10
mid dose	5M/5F	150	0/10
high dose	5M/5F	250	0/10

Mortality and Time to Death
All animals survived the scheduled treatment period.

Clinical Observations
Paddling of the forelimbs was noted in 3/10 high-dose animals during Week 1 of treatment, and in 2/10 mid-dose animals during Week 2 of treatment. This effect was observed immediately after dosing and was short-lived. The authors consider this paddling to be associated with discomfort rather than toxicity.

Mid-high dose animals showed increased salivation after dosing (and associated wet fur) throughout the study. This may be linked to increased water consumption by high-dose rats compared to controls. Post-dose red/brown perioral staining was also seen sporadically in high-dose animals and one male mid-dose rat during Week 3 of treatment. The authors state that the salivation and its associated effects are commonly seen in rat studies using oral dosing on account of the unpleasant taste of the test substance. As such, these effects were not considered to be of toxicological importance.

Yellow-stained fur on the ventral surface of the body was observed on one high-dose female rat During Week 2 of treatment. Greasy fur was seen in all rats in this study and was most likely linked to the use of corn oil for dosing and therefore not toxicologically linked.

There were no significant differences in bodyweight and no treatment-related differences in food consumption between control and treated animals.

Laboratory Findings – Clinical Chemistry, Haematology
Haematology
The mean corpuscular volume of high-dose males and the mean corpuscular haemoglobin concentration of high-dose females were statistically higher than controls. However, these differences were not considered to be treatment-related as they were small and the values themselves were within the expected range.

Clinical Chemistry
High-dose male rats displayed significantly lower glucose levels and significantly high creatinine and sodium ion concentrations than controls. These differences however, were minor and hence not considered to be toxicologically relevant.

Female rats of all treatment groups displayed higher potassium ion concentrations than controls. As differences to controls were small and there was an absence of a dose-effect relationship between dose and potassium ion concentration, this effect was not considered to be treatment-related.

Effects in Organs
Significantly higher liver weights were observed in high-dose animals and mid-dose females. Livers from 4/10 high-dose animals appeared enlarged; no livers from the control group appeared enlarged. The livers from latter group however, had weights within the expected range and appeared microscopically normal. As such, only the higher liver weights of high-dose animals were considered to be treatment-related. Hepatocyte enlargement was seen in high-dose animals only and most likely accounts for the liver enlargements and higher liver weights observed in these animals. This is most likely a resultant adaptation to the metabolism of the test substance.

High-dose males and 3/5 mid-dose males displayed pale kidneys, whilst controls did not display this. Therefore, this effect was considered treatment-related. This may be due to the increased incidence of minimal basophilic cortical tubules in high-dose male rats. The authors of this report however, note that this is a commonly observed background finding and not adverse.

All treated male rats displayed eosinophilic inclusions in the proximal convoluted tubular epithelium. This is symptomatic of light hydrocarbon nephropathy syndrome, which is specific to male rats and occurs in response to various hydrocarbon compounds (Alden, 1986). This pathology however, remains to have little relevance to humans (Bus, 2015).

Remarks – Results
Treatment with the test substance resulted in effects on the liver and on the kidneys of male rats. Effects noted in the kidney (eosinophilic inclusions in the proximal convoluted tubular epithelium) and liver (hepatocellular hypertrophy in males and females) were regarded as specific to rats or an adaptive response.

CONCLUSION	The No Observed (Adverse) Effect Level (NO(A)EL) was established as 250 mg/kg bw/day in this study, as treatment related changes were not considered to represent serious damage to health.
TEST FACILITY	Huntingdon (1996d)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1538, 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: 31.25 – 500.00 µg/plate (TA100, TA1535, TA98, TA1537); 312.5 – 5000.0 µg/plate (WP2uvrA, TA1538) b) Without metabolic activation: 31.25 – 500.00 µg/plate (TA100, TA1535,TA98, TA1537); 312.5 – 5000.0 µg/plate (WP2uvrA, TA1538)
Vehicle	DMSO
Remarks - Method	TA102 was not tested.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 500	> 5000* / > 500^{+}	> 5000	Negative
Test 2	-	> 5000* / ≥ 500^{+}	> 5000	Negative
Present				
Test 1	≥ 500	> 5000* / > 500^{+}	> 5000	Negative
Test 2	-	> 5000* / ≥ 500^{+}	> 5000	Negative

* For test strains WP2uvrA, TA1538
$^{+}$ For test strains TA100, TA1535,TA98, TA1537

Remarks - Results	In the preliminary test, the test substance was not toxic to WP2uvrA and TA1538 bacterial strains, but was toxic to the remaining strains at doses of 500 µg/plate and/or 5000 µg/plate. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, in the presence or absence of metabolic activation. Positive controls performed as expected, confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Huntingdon (1990a)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 473 In vitro Mammalian Chromosome Aberration Test
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver.
Vehicle	DMSO

Remarks - Method

The highest concentration chosen for testing was 250 µg/mL, as this was the limit of solubility for the test substance (in DMSO) within the tissue culture medium. No preliminary test for cytotoxicity was conducted.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.5, 1.0, 2.0, 3.9, 7.8*, 15.6, 31.3*, 62.5*, 125, 250	18 h	18 h
Test 2a	3.9, 7.8*, 15.6, 31.3*, 50*, 62.5, 75, 100, 125	18 h	18 h
Test 2b	0.5, 1.0, 2.0, 3.9, 7.8*, 15.6*, 31.3, 62.5*, 125, 250	32h	32 h
Present			
Test 1	0.5, 1.0, 2.0, 3.9, 7.8, 15.6*, 31.3, 62.5*, 125*, 250	3 h	18 h
Test 2a	7.8, 15.6*, 31.3, 62.5*, 125*, 150, 175, 200, 225, 250	3 h	18 h
Test 2b	7.8*, 15.6, 31.3*, 62.5*, 125, 250	3 h	32 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	Test Substance Concentration (µg/mL) Resulting in: *Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 62.5	≥ 125	Negative
Test 2a	≥ 50	≥ 125	Negative
Test 2b	≥ 62.5	≥ 125	Negative
Present			
Test 1	≥ 125	≥ 125	Negative
Test 2a	≥ 125	≥ 125	Negative
Test 2b	≥ 62.5	≥ 125	Negative

Remarks - Results

In both Test 1 and 2, no biologically significant increase in the frequency of chromosomal aberrations was observed at any harvest time and test substance concentration, in the absence of metabolic activation.

In Test 2a in the presence of metabolic activation there was a significant increase in the frequency of chromosomal aberrations compared to the negative control. Though significant, this increase lies within historical reference values and may have been enhanced by a relatively low frequency of aberrant cells in the negative control (as compared to historical solvent control values). As such, the authors conclude that this increase is not related to the test substance. No other increases were noted in test 1 and 2 in the presence of metabolic activation, at any harvest time.

The positive controls performed as expected, confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

Huntingdon (1996e)

B.10. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Official Journal of the European Community No. L251 B12: Other effects, Mutagenicity, Micronucleus Test (19 September, 1984).

Species/Strain: Mouse/Swiss (SPF/CD-1)
Route of Administration: Oral – gavage

Vehicle	1% aqueous methylcellulose
Remarks - Method	No deviations from the study plan were noted. A preliminary toxicity study was carried out using 32 male and 32 female mice dosed with the test substance at 400 - 6400 mg/kg bw. Signs of toxicity after the first 24 hours included piloerection, ptosis, lethargy and loss of righting reflex. The duration and severity of these symptoms was dose-dependent. Mortality occurred from 21 hours after treatment onwards, at doses of 1600, 4000, 6250 and 6400 mg/kg bw. However, no mortality was seen at doses of 3200 and 5000 mg/kg bw. Based on this data, 5000 mg/kg bw was chosen dose for the main test.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5M/5F	0	24
	5M/5F	0	48
	5M/5F	0	72
II	5M/5F	5000	24
III	5M/5F	5000	48
IV	5M/5F	5000	72
V (positive control,M)	5M/5F	12	24

M=mitomycin C dissolved in sterile 0.9% saline.

RESULTS

Doses Producing Toxicity	One female and three male animals died following treatment with the test substance. These animals were replaced with animals from the additional/satellite group. Clinical signs of toxicity observed in treated animals included lethargy, piloerection, hunched posture, and coma. No clinical signs of toxicity were observed in the vehicle or positive control animals during the test period.
Genotoxic Effects	The test substance induced no statistically significant increases in micronucleated, polychromatic erythrocytes (PCEs) at any of the sacrifice times.
Remarks - Results	Post-mortem examination revealed that the premature deaths observed in this study were not due to incorrect dosing (post mortem report not provided). Significant reductions in the PCE/NCE ratio (cytotoxicity) were observed with test substance treatment at the 48 and 72 hour sacrifice times. This result may indicate that the test substance caused bone marrow cell toxicity. The positive control performed as expected, confirming the validity of the test system.

CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* mouse micronucleus test.
TEST FACILITY	Huntingdon (1990b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated Sewage Sludge
Exposure Period	28 days
Auxiliary Solvent	The test substance was dissolved in chloroform and aliquots of stock solution were placed on individual pieces of Whatman GFA glass filter paper which were placed in solvent and allowed to evaporate
Analytical Monitoring	Measurement of BOD with a close system oxygen consumption measuring apparatus. Biodegradability of the test and reference substance was calculated based on their theoretical oxygen demand (TOD).
Remarks - Method	The tests were conducted in duplicate at each data point.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	4	7	68
14	1	14	76
21	10	21	78
28	11	28	80

Remarks - Results	All validity criteria were satisfied. The percentage degradation of the reference compound was 68% by 7 days. Therefore, the tests indicate the suitability of the inoculum. Cultures containing the notified chemical and standard substances combined showed an oxygen depletion value 10% greater than that anticipated on the basis of results from separate cultures at Day 14. Therefore, the notified chemical did not show inhibitory effect on sewage bacteria under the conditions of this test. The degree of degradation of the test substance after 28 days was 11%.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Huntingdon (1996f)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test. EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - continuous flow conditions.
Species	Rainbow trout
Exposure Period	96 h
Auxiliary Solvent	10% Tween 80 dimethylformamide (100 µl auxiliary solvent/L)
Water Hardness	161 ± 9 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography
Remarks – Method	The test was conducted in accordance with the test guideline above, with

no significant deviation in protocol reported.

The notified chemical was dissolved in 10% Tween 80 to give an initial stock solution of 100 mg/ml. Serial dilutions of this stock solution were prepared with the auxiliary solvent in order to produce a stock series of 4.6, 10, 22, 46 and 100 mg/ml solutions which were dispensed automatically by the dosing apparatus into the flowing test water.

RESULTS

Concentration mg/L		*Number of Fish*	***Mortality***			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0
Solvent control	Solvent control	7	0	0	0	0
0.46	0.34	7	0	0	0	0
1.0	0.88	7	0	0	0	0
2.2	2.0	7	0	0	0	0
4.6	4.1	7	0	0	0	0
10	8.0	7	1	7	7	7

LC50	5.7 mg/L at 96 hours.
NOEC	0.88 mg/L at 96 hours.
Remarks – Results	At the lowest test level the mean measured concentration fell below 75% at 72 hrs due to unknown reasons, all other levels remained more than 78% of the nominal throughout the duration of the study. This did not affect the validity or integrity of the test. All other validity criteria for the test were satisfied. The 96 h LC50 for fish was determined to be 5.7 mg/L (95% CI 4.1–8.0 mg/L) based on nominal concentrations.

CONCLUSION The notified chemical is toxic to fish.

TEST FACILITY Huntingdon (1996g)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	10% Tween 80 dimethylformamide (50 µl auxiliary solvent/L)
Water Hardness	Not reported
Analytical Monitoring	Gas Chromatography
Remarks - Method	The notified chemical was dissolved in 10% Tween 80 dimethylformamide to give an initial stock solution of 200 mg/ml. Serial dilutions were prepared with the auxiliary solvent and aliquots were added to test water to achieve the desired series of exposure levels.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
Control	Control	20	0	0
Solvent control	Solvent control	20	0	0
0.10	0.12	20	0	0
0.22	0.23	20	0	0
0.46	0.52	20	0	0
1.0	0.98	20	0	0

2.0	2.3	20	0	25
4.6	4.2	20	20	100
10	8.5	20	75	100

LC50	2.7 mg/L at 48 hours
NOEC (or LOEC)	0.87 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. Measured concentrations of the test substance were within 20% difference of the nominal concentrations. The 48 h EC50 for daphnids was determined to be 2.7 mg/L (95% CI 2.3 – 4.2 mg/L).

CONCLUSION The notified chemical is toxic to aquatic invertebrates.

TEST FACILITY Huntingdon (1996h)

C.2.3. 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	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range	Nominal: 10 mg/L Actual: 9.04 mg/L at 0 hrs (90% of the nominal concentration) Actual: ND mg/L (<2% of the nominal concentration) at 72 hrs
Auxiliary Solvent	10% Tween 80-dimethylformamide (100 µl auxiliary solvent/L)
Water Hardness	Not reported
Analytical Monitoring	Gas Chromatography
Remarks - Method	The notified chemical was dissolved in the auxiliary solvent 10% Tween 80 to give a stock solution of 100 mg/ml. A 100 µl of this stock solution was added to 1 litre of algal pre-culture to give the final test concentration of 10 mg/L. 10 mg/L was the highest test concentration that could be prepared due to the limited solubility of the notified chemical in the medium and the maximum amount of solvent permitted under the conditions of this study.

RESULTS

Biomass		*Growth*	
EC50 *mg/L at 0-72 h*	*NOEC* *mg/L*	*EC50* *mg/L at 72 h*	*NOEC* *mg/L*
> 9.0		> 9.0	9.0

Remarks - Results	The test was conducted in accordance with the test guideline, with no significant deviation in protocol reported. There were substantial losses of test compound in both inoculated and non-inoculated test vessels possibly due to the volatile nature of the test compound. Measured concentrations were less than 2% of the nominal concentration by the end of the test. The 72 h EL50 for algae was determined to be > 9.0 mg/L based on initial measured concentrations.

CONCLUSION The notified chemical is not harmful to algae up to its solubility limit in test medium.

TEST FACILITY Huntingdon (1996i)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	The 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: 10 mg/L Actual: unknown
Remarks – Method	The notified chemical was dispersed in 10% Tween 80 dimethylformamide to give a preliminary stock solution of 100 mg/ml. A 50 µl of this stock solution was added to a final culture volume of 500 ml to give a test concentration of 10 mg/l. 10 mg/L was the highest test concentration that could be prepared due to the limited solubility of the notified chemical in the medium and the maximum amount of solvent permitted under the conditions of this study. Actual concentrations were not reported in this test. All validation criteria were fulfilled. 3,5-Dichlorophenol was used as the reference control. The respiration rate was determined electrochemically during the test after 3 hours of exposure.
RESULTS	
EC50	> 10 mg/L
Remarks – Results	The 3 h EC50 were determined to be >10 mg/L based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial respiration up to its solubility limit in test medium.
TEST FACILITY	Huntingdon (1996j)

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