LTD/1947

February 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Spiro[1,3-dioxolane-2,8'(5'*H*)-[2*H*-2,4a]methanonaphthalene], hexahydro-1',1',5',5'-tetramethyl-, (2'*S*,4'a*S*,8'a*S*)-

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:

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

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1947	Symrise Pty Ltd	Spiro[1,3-dioxolane-2,8'(5'H)-[2H-2,4a]methanonaphthalene], hexahydro-1',1',5',5'-tetramethyl-, (2'S,4'aS,8'aS)-	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.

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

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

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

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A 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 at high concentrations during reformulation processes:
  - Avoid contact with skin and eyes

- 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 at high concentrations during reformulation processes:
  - Coveralls
  - Impervious gloves

  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, 0.1% in cosmetic 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)
Symrise Pty Ltd (ABN: 67 000 880 946)
168 South Creek Road
DEE WHY NSW 2099

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: absorption/desorption, dissociation constant and flammability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Korea (2008)

2. IDENTITY OF CHEMICAL

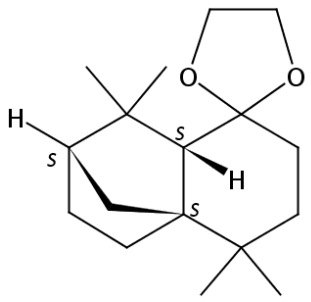
MARKETING NAME(S)
Ysamber K

CAS NUMBER
154171-77-4

CHEMICAL NAME
Spiro[1,3-dioxolane-2,8'(5'*H*)-[2*H*-2,4a]methanonaphthalene], hexahydro-1',1',5',5'-tetramethyl-, (2'*S*,4'a*S*,8'a*S*)-

MOLECULAR FORMULA
$C_{17}H_{28}O_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
264.41 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 95%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

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

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: colourless to light yellow viscous liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -80 °C	Measured
Boiling Point	> 280 °C at 102.8 kPa	Measured
Density	1,048 kg/m^3 at 20 °C	Measured
Vapour Pressure	5.8 x 10^{-4} kPa at 25 °C	Measured
Water Solubility	1.59 x 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ = 1,344 h at pH 7, 25 °C $t_{½}$ = 30 h at pH 4, 25 °C $t_{½}$ = 177 h at pH 9, 25 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow = 5.69 at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.68 (P_{OW} method) log K_{oc} = 3.46 (MCI method)	Estimated. KOCWIN v2.00, US EPA 2011
Dissociation Constant	Not determined	The notified chemical does not contain functional groups that are expected to dissociate under environmental conditions
Flash Point	146 °C at 101.3 kPa	Measured
Flammability	Not determined	Not expected to be highly flammable based on measured flash point
Autoignition Temperature	325 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidative 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 imported as a constituent of finished consumer products at concentrations ≤ 0.075% concentration. The notified chemical will also be introduced as a component of fragrance oil at a typical concentration of 0.5% (maximum concentration 53%) for local reformulation into finished consumer products. The notified chemical may also be introduced in the neat form.

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

TRANSPORTATION AND PACKAGING
The neat form of the notified chemical and fragrance oil containing the notified chemical will be introduced in 30 L and 216 L tight-head lacquered metal drums and plastic 30 L HDPE/EVOH canisters. Finished consumer products containing the notified chemical will be packaged in in typical consumer-sized containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient for use in cosmetic and household cleaning products. The typical use concentration of the notified chemical in consumer products will be ≤ 0.075% for fine fragrances, ≤ 0.007% for other cosmetic products and ≤ 0.016% in household cleaning products

OPERATION DESCRIPTION
The notified chemical will not be manufactured within Australia. At customer sites the notified chemical will be reformulated into either fragrance oil or finished end-use cosmetic/household products. The fragrance oil will be used for further reformulation of consumer products.

Reformulation
The procedures for reformulating the notified chemical or the fragrance oil containing the notified chemical (typically 0.5%, max. 53%) will likely vary depending on the nature of the cosmetic/household products, and may involve both automated and manual transfer steps. In general, it is expected that the reformulation processes will involve blending operations that will normally be automated and occur in an enclosed system, followed by automated packaging of the finished products.

Household products
Household cleaning products containing the notified chemical at ≤ 0.016% concentration may be used by consumers and professional cleaners. 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 followed by wiping. The household products may be diluted with water prior to application.

Cosmetic products
The finished cosmetic products containing the notified chemical at ≤ 0.075% concentration will be used by consumers and beauticians. 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 workers	Unknown	Unknown
Mixer	4	2
Drum handling	4	2
Drum cleaning/washing	4	2
Equipment maintenance	4	2
Quality control worker	0.5	2
Packager	4	2
Professional cleaners, hair dressers, beauticians	1-8	200

EXPOSURE DETAILS

Transport and storage workers may come into contact with the notified chemical, either in neat form or at various concentrations (in intermediate fragrance oil and finished consumer products), only in the event of accidental rupture of packaging.

At reformulation sites, dermal, ocular and inhalation exposure of workers to the notified chemical (at up to 100% concentration) may occur when handling the notified chemical or products containing it. Exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems and personal protective equipment (PPE) such as coveralls, safety glasses and impervious gloves.

Dermal, ocular and inhalation exposure to the notified chemical in the finished end-use products (at ≤ 0.075% concentration) may occur where workers provide services involving the application of products to clients (e.g. hair dressers, beauty salon workers), or in the cleaning industry. Such workers 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 a wide range of cosmetic and household products (at ≤ 0.075% concentration 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.

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%, with the reminder ending up, as intended, on the hair. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

- Cosmetic products (Dermal exposure):

Product type	Amount (mg/day)	C (%)	RF (unitless)	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	0.007	1.000	0.00855
Face cream	1540	0.007	1.000	0.00168
Hand cream	2160	0.007	1.000	0.00236
Fine fragrances	750	0.075	1.000	0.00879
Deodorant spray	1500	0.007	1.000	0.00164
Shampoo	10460	0.007	0.010	0.00011
Conditioner	3920	0.007	0.010	0.00004
Shower gel	18670	0.007	0.010	0.00020
Hand soap	20000	0.007	0.010	0.00022
Hair styling products	4000	0.007	0.100	0.00044
Total				**0.02405**

C = concentration (%); RF = retention factor.
Daily systemic exposure = (Amount x C x RF x DA)/BW

- Household products (Indirect dermal exposure - from wearing clothes):

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	0.016	0.95	10	0.00055
Fabric softener	90	0.016	0.95	10	0.00021
Total					**0.00076**

Daily systemic exposure = (Amount x C x PR x PT x DA)/BW

- Household products (Direct dermal exposure):

Product type	Frequency (use/day)	C (%)	Contact Area (cm^2)	Product Use (g/cm^3)	Film Thickness (cm)	Time Scale Factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	0.016	1980	0.01	0.01	0.007	0.00000
Dishwashing liquid	3	0.016	1980	0.009	0.01	0.03	0.00004
All-purpose cleaner	1	0.016	1980	1	0.01	0.007	0.00035
Total							**0.00039**

Daily systemic exposure = (Frequency x C x Contact area x Product Use Concentration x Film Thickness on skin x Time Scale Factor x DA)/BW

Aerosol products (Inhalation exposure)

Product type	Amount (g/day)	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	9.89	0.007	20	1	20	50	1	10	0.0002

Daily systemic exposure = [(Amount × C × Inhalation Rate × Fraction Inhaled × 0.1) / BW × 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 tables that contain the notified chemical. This would result in a combined internal dose of 0.0254 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products may occur. However, it is considered that 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 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	moderately irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL 50 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – chromosome aberration test	non genotoxic

Toxicokinetics

Dermal absorption is expected to be limited given the low water solubility (1.59 x 10^{-3} g/L) and high lipophilicity (log Pow = 5.69) of the notified chemical, limiting penetration of the hydrophilic epidermis. Given the low molecular weight (264.41 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 moderately irritating to skin and slightly irritating to eyes.

In the skin irritation study, very slight to well-defined erythema and very slight to slight oedema was observed in all animals. Overall, signs of irritation were resolved at the 5 day observation period. However, fissures and/or scab formation was present in 2/3 of the tested animals from day 4 to day 14. At the end of the observation period (day 15), only slight erythema was observed in one animal.

In the eye irritation study, slight to moderate conjunctival irritation was observed in all animals that was fully resolved at the 72 hour observation period.

In the guinea pig maximisation test, the notified chemical did not show evidence of skin sensitisation when tested up to 100% concentration.

Repeated dose toxicity

In a 28-day repeated dose oral toxicity study in rats the No Observed Effect Level (NOEL) for the notified chemical was established as 50 mg/kg bw/day based on test substance related effects on red blood cell parameters. At the high dose level of 1250 mg/kg bw/day, a significant decrease in the erythrocyte, haematocrit and haemoglobin values was observed in both sexes. At the mid dose level of 250 mg/kg bw/day, only some blood cell parameters were slightly affected, particularly in the females. However, at the end of the post-treatment recovery period, the red blood picture showed clear signs of recovery. Other effects noted in the kidney (hyaline droplet accumulation in the epithelium of proximal tubes in males) and liver (heptacellular hypertrophy in males and females) in all dose groups were regarded as specific to rats or an adaptive response.

Mutagenicity/Genotoxicity

The notified chemical tested negative in both a bacterial reverse mutation assay and an *in vitro* chromosomal aberration assay using Chinese hamster V79 cells. 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 moderate skin irritant and a slight eye irritant.

Reformulation

During reformulation, workers may be at risk of skin and eye irritation effects when handling the notified chemical at high concentrations. 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 0.075% 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 ≤ 0.075% 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 moderately irritating to skin and slightly irritating to eyes. Given the low proposed use concentration (≤ 0.075%) 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 0.0254 mg/kg bw/day (see Section 6.1.2). Using a NOEL of 50 mg/kg bw/day derived from a 28 day repeated dose oral toxicity study, the margin of exposure was estimated to be 1,967. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences. Based on the potential systemic exposure from the notified chemical in cosmetics and household products, an MOE value greater than or equal to 100 is also expected where the notified chemical is present at ≤ 0.1% concentration.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 0.075% in fine fragrances, ≤ 0.007% in other cosmetics and ≤ 0.016% 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 neat or as a component of fragrance formulations, for reformulation into finished cosmetic formulations and household products. There is unlikely to be any significant release of the notified chemical to the environment from transport and storage, except in the case of accidental spills and leaks. In the event of spills, the products containing the notified chemical is expected to be collected with adsorbents, and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve blending operations that will be highly automated, and is expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. The process will be followed by automated filling of the formulated

products into containers of various sizes suitable for retail and end-use. Wastes containing the notified chemical generated during reformulation include equipment wash water, residues in empty import containers and spilt materials. It is estimated by the notifier that up to 1% of the import volume of the notified chemical (or up to 10 kg) may be released from reformulation processes. These will be collected and released to on-site waste water treatment processes, or released to sewers in a worst case scenario. Empty import containers are expected to be recycled or disposed of to landfill.

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.

RELEASE OF CHEMICAL FROM DISPOSAL
A small proportion of the notified chemical may remain in end-use containers once the consumer products are used up. Wastes and residues 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
For the details of the environmental fate studies please refer to Appendix C.

The majority of the notified chemical is expected to be released to sewers across Australia. The notified chemical is not readily biodegradable (59.1% in 28 days). However, it exhibits relatively high biodegradation with a relatively long lag phase of 7 days. The notified chemical contains hydrolysable functionalities that are expected to hydrolyse slowly ($t_{½}$ = 56 days) in the environment at pH 7, however, hydrolysis occurs rapidly under acidic or basic conditions ($t_{½}$ < 10 days).

Volatilization of the notified chemical is not rapid but may be significant based on Henry's Law constant (9.41×10^{-4} atm-m^3/mole) (US EPA 2011). The half-life of the notified chemical in air is calculated to be 5.03 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 sewage treatment plants (STPs) the notified chemical is expected to be efficiently removed (based on its adsorption and partition coefficients) from influent by adsorption to sludge and only a small portion may be released to surface waters. A proportion of the 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 = 3.46-3.68). The notified chemical has the potential to bioaccumulate based on its high octanol-water partition coefficient value (log Pow = 5.69) and lack of ready biodegradability. However, the notified chemical is not expected to be significantly released to surface waters and is not harmful to aquatic life up to the limit of its water solubility. In surface waters, soils and landfill, the notified chemical is expected to eventually degrade through both biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)
The predicted environmental concentration (PEC) has been calculated to assume a worst case scenario, with 100% release of the notified chemical into sewer systems nationwide and no removal within sewage treatment plants (STPs).

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/m2/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/m3). 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 the studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 2.9 mg/L	Not harmful to fish up to water solubility limit
Daphnia Toxicity	48 h EC50 = 3.5 mg/L	Not harmful to aquatic invertebrates up to water solubility limit
Algal Toxicity	48 h EC50 = 110.2 mg/L NOEC = 6.3 mg/L	Not harmful to algae
Growth inhibition test with *Pseudomonas putida*[¥]	16 h EC50 > 800 mg/L	Not inhibitory to microbial growth

[¥] German Standard Method DIN 38412 L8

Based on the above ecotoxicological endpoints, the notified chemical is not expected to be harmful to aquatic life up to the limit of its water solubility. Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated from the most sensitive endpoint for fish. An assessment factor of 100 was used given acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish)	2.9	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	29	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.61	29	**0.021**
Q - Ocean	0.06	29	**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. Although the notified chemical is not readily biodegradable and has the potential for bioaccumulation, it is not expected to be harmful to aquatic life up to the limit of its water solubility. 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

Melting Point < -80°C

Method OECD TG 102 Freezing Point/Freezing Range.
EEC Guideline 92/69/EWG A.1 Determination of the Melting/Freezing Temperature.
Remarks Samples of the notified chemical were incubated at -20 °C and -80 °C. At -20 °C, the notified chemical was found in a highly viscous, liquid state. At -80 °C, the notified chemical was found in a nearly immovable state. A freezing point for the notified chemical was therefore not determinable.
Test Facility IBR (1993a)

Boiling Point > 280 °C at 102.8 kPa

Method OECD TG 103 Boiling Point.
Remarks A boiling point for the notified chemical was not determinable at temperatures below 280 °C under atmospheric pressure.
Test Facility IBR (1994a)

Density 1,048 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Determined using the pycnometer method.
Test Facility IBR (1994b)

Vapour Pressure

5.8×10^{-4} kPa at 25 °C

Method OECD TG 104 Vapour Pressure Curve.
EEC directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.4 "Vapour pressure".
Remarks Determined using the static technique.
Test Facility NOTOX B.V. (1994)

Water Solubility 1.59×10^{-3} g/L at 20 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Determined using the column elution method.
Test Facility IBR (1994c)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$ (hours)*
4	25	30
7	25	1344
9	25	177

Remarks Two different reaction mechanisms were assumed under basic and neutral-acidic conditions due to difference in activation energy values.
Test Facility IBR (1994d)

Partition Coefficient (n-octanol/water) log Pow = 5.69 at 20 °C

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

	EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Determined using the flask method
Test Facility	IBR (1994e)

Flash Point 146 °C at 101.3 kPa

Method	EEC Guideline 92/69/EWG A.9 - Determination of the flash point (29.12.92)
Remarks	Determined using a Pensky-Martens flash point tester.
Test Facility	IBR (1993b)

Autoignition Temperature 325 °C

Method	EEC-Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.15 "Autoignition temperature (liquids and gases)"
Test Facility	NOTOX B.V. (1993)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity – Limit Test (1987). EC Council Regulation No 440/2008 B.1 Acute Toxicity (Oral) – Limit Test.
Species/Strain	Rat/Bor:WISW (SPF Cpb)
Vehicle	None
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	None
Remarks - Results	No clinical signs or mortality was observed during the study period. Animal body weights were within normal range for their strain and relevant age. No macroscopic abnormalities were recorded during post mortem examination.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	IBR (1993c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test (February 24, 1987). EEC directive 84/449/EEC (September 19, 1984).
Species/Strain	Rat/Bor:WISW (SPF, Cpb)
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Very slight erythema was presented in two male rats on day 2 only and well-defined erythema was presented in one male rat on days 1 and 2.
Signs of Toxicity - Systemic	None
Effects in Organs	None
Remarks - Results	No clinical signs or mortality was observed during the study period. Mean animal body weights were within normal range for their strain and relevant age. No treatment-related macroscopic changes were recorded during post mortem examination.

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

TEST FACILITY IBR (1993d)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion (May 12, 1981).
EU B.4 EEC directive 84/449/EEC (September 19, 1984).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	15 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.33	1.33	1.33	2.00	15 days	1.00
Oedema	1.33	1.33	1.33	2.00	< 5 days	0.00

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

Remarks - Results Thirty minutes after treatment with the test substance, well-defined erythema was noted in all animals which persisted until the 24-hour reading. The erythema was downgraded to a very slight erythema by the 48-hour reading. This effect was reversible and no longer evident 5 days after treatment. However, a very slight erythema returned in one animal 15 days after treatment. Very slight to slight oedema was observed in all animals which fully resolved in all animals by Day 5. Fissuration and scab formation was observed in 2 animals from Day 4 until Day 14.

No signs of systemic toxicity were observed in the animals during the study. No mortality occurred.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY IBR (1993e)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion (February 24, 1987).
EU B.5 Commission Directive 84/449/EEC (September 19, 1984).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	72 hours
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			

Conjunctiva: redness	1.0	1.0	1.0	2.0	< 72 h	0.0
Conjunctiva: chemosis	0.7	0.7	0.7	2.0	< 72 h	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

All animals displayed slight to moderate conjunctival swelling/secretion and redness up to the 48 hour reading. These findings were reversible within 48-72 hours post-treatment. No changes to the cornea or iris were observed.

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

IBR (1993f)

B.5. Skin sensitisation

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 406 Skin Sensitisation - Guinea Pig Maximisation Test (May 12, 1981)

Species/Strain/Substrain

Guinea pig/White Pirbirght/Bor:DHPW(SPF)

PRELIMINARY STUDY

Maximum Non-irritating Concentration
intradermal: 5% (maximum concentration applied)
topical: 100%

MAIN STUDY

Number of Animals

Test Group: 20 Control Group: 20

Vehicle

Intradermal: peanut oil
Topical: none

Positive control

Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using 2,4 dinitrochlorobenzene and benzocaine.

INDUCTION PHASE

Induction Concentration
intradermal: 5%
topical: 100%

Signs of Irritation

None

CHALLENGE PHASE

1st challenge

topical

Remarks - Method

10% sodium lauryl sulfate in vaseline was applied 24 hours before topical induction

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: Challenge 1*	
		24 h	*48 h*
Test Group	100%	0	0
Control Group	100%	0	0

Remarks - Results

No signs of irritation were noted during induction or challenge.

CONCLUSION

There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY

IBR (1993g)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (May 12, 1981).
Species/Strain	Rats/Wistar (Han:WIST)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Corn oil
Remarks - Method	Haematology and clinical chemistry values presented heterogeneity of variance. As such, these dose group values were compared relative to control by the "Mann-Whitney U-test" instead of the "Dunnett test" (two-tailed).

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M/5F	0	0/10
low dose	5M/5F	50	0/10
mid dose	5M/5F	250	0/10
high dose	5M/5F	1250	0/10
control recovery	5M/5F	0	0/10
high dose recovery	5M/5F	1250	0/10

Mortality and Time to Death

All animals survived the scheduled treatment or recovery periods.

Clinical Observations

Abdominal distension was noted in 3/5 high-dose females during week 4 of treatment. This may be due to significant increases in the size/weight of liver and spleen in the high-dose female group. Two out of the three high-dose females displaying abdominal distention also displayed a rough coat (an indication of pain). During the first week of post-treatment observation, these females continued to display abdominal distention and rough coat. Two high-dose males also displayed a rough coat during treatment. Vocalisation, another indicator of animal distress/pain, was noted in one high-dose male and one high-dose female. Decreased skin tugor, a sign of dehydration, was seen in one mid-dose and two high-dose males.

There were no test substance related effects on food consumption during the treatment and post-treatment observation period. Female rats did not show any significant differences in body weight gain throughout the study. High-dose males displayed significantly decreased weight gain from weeks 0-2 and 0-4 of treatment. During the post-treatment period, non-significant increases in body weight occurred in the high-dose males. These increases indicate a compensatory effect in response to reduced weight gain during the treatment period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

There were clear treatment-related effects on red blood cell parameters. Animals in the high and mid-dose cohorts presented significant decreases in red blood cells, haemoglobin concentrations and haematocrit. These animals also showed significant increases in thrombin. During the post-treatment period, the high-dose animals presented significantly increased haematocrit and mean corpuscular volume. This indicates a compensatory effect in response to reduced red blood cell parameters during the treatment period, reinforcing the notion that the treatment was responsible for these effects on red blood cell parameters. There were no differences in the differential white blood cell count during and after treatment, in comparison with controls.

Clinical Chemistry

Bilirubin levels were significantly increased in high-dose animals. This correlates with the significantly decreased red blood cell parameters discussed previously and as such, indicates that this result is treatment-related. Significantly increased triglyceride and cholesterol levels were found in high-dose animals. High-dose

males also displayed significant increases in creatine levels, indicating potential renal dysfunction. High-dose animals displayed significantly elevated glucose levels. High-dose males continued to show this result after treatment. The authors note that these values were in the range of reference data and therefore may not be treatment related.

Urea nitrogen levels were significantly decreased in high-dose females, indicating liver dysfunction. However, these values were noted to be within the limits of reference data and corresponding liver histopathology data only showed moderate hepatocellular hypertrophy. This effect was also seen in male rats, despite the absence of changes in urea nitrogen values. This may exclude a treatment-related link with urea nitrogen levels.

High and mid-dose animals showed significantly increased calcium levels and significantly decreased uric acid and alkaline phosphatase levels. However, these values were within the range of reference data and hence not considered biologically relevant. Similarly, though albumin levels were significantly increased in high-dose males, values were not of biological importance as they were also within reference values.

Gamma glutamyl transferase was significantly increased in all treatment groups. However, differences were slight in comparison to controls and not indicative of toxic effects. Significant decreases in iron levels were seen in high-dose females during and after treatment. The authors however, do not think this was linked to changes in red blood cell parameters because high-dose males, who demonstrated the same red blood cell parameter patterns before and after treatment, did not show any notable changes in iron levels.

Alanine aminotransferase, phosphorus, potassium and sodium/potassium ratio values changed significantly at various doses during the treatment period. These changes were slight and not dose-related. Sodium and albumin levels changed significantly in high-dose female rats during the post treatment period. These levels were close to their mean reference values and therefore discounted as being biologically relevant.

Urinalysis

Increased leukocyte levels (indicative of infection) were detected in all treated males and females, compared to controls. However, no dose relation was present. Furthermore, histopathological findings conducive to increased leukocyte levels, such as infection etc, were not seen in the kidneys or bladder.

No other notable changes were found in the urine of treated animals, compared to controls, during and after the treatment period.

Effects in Organs

Many rats in the high-dose groups displayed enlarged spleens at the end of treatment. The respective spleen weights of these animals were proportionate to their increased size. Correspondingly, haematopoiesis was relatively high in the spleen of high-dose animals. This, along with notable elevations in haematopoiesis also seen within bone marrow, liver and adrenal glands in mid-high dose animals during treatment, may interpreted as an adaptive response to the significant decreases in red blood cells, haemoglobin concentrations and haematocrit in these animals, as noted earlier. After the recovery period, no animals presented enlarged spleens and extramedullary haematopoiesis was not observed. Overall these macroscopic and histological changes, and their reversibility, indicate that they are treatment-related.

Mid-high dose males presented kidneys with yellow-light discolouration and high-dose males showed increased kidney weight. Dose-specific renal hyaline droplet accumulation was noted in high-dose males during the treatment and was seen to decrease after treatment had ceased. These kidney effects, in addition to the increases in creatine noted earlier, are characteristic of alpha$_{2\mu}$ globulin nephropathy, a lesion that occurs in male rats following the administration of various xenobiotics. (Alden, 1983; Boorman 1990; Greaves 1990). This pathology however, remains to have little relevance to humans (Bus, 2015).

Two high-dose female rats displayed grey/green kidney discolouration but this was found to have no histological relevance.

Liver weights were significantly increased in high-dose females and notably increased in high-dose males at the end of treatment. Liver weights remained increased in these animals following the recovery period, but these increases were not as high as that following treatment. Histological examination revealed liver hypertrophy in all treatment groups, with a dose-related increase in severity. High-dose animals were also found that have increased liver haematopoiesis after the treatment period only. The authors deduce that these changes may be caused by liver adaptation to the administration of xenobiotics and significantly increased triglyceride and

cholesterol levels. This hypertrophic effect was drastically minimised at the end of the recovery period further indicating that the treatment caused this effect.

Remarks – Results

Treatment with the test substance resulted in effects on the red blood picture, on the liver and on the kidneys of male rats.

At the high dose level of 1250 mg/kg bw/day a significant decrease in the erythrocyte, haematocrit and haemoglobin values was observed in both sexes. At the mid dose level of 250 mg/kg bw/day only some blood cell parameters were slightly affected, particularly in the females. However, at the end of the post-treatment recovery period, the red blood picture showed clear signs of recovery.

Effects noted in the kidney (hyaline droplet accumulation in the epithelium of proximal tubes in males) and liver (heptacellular hypertrophy in males and females) in all dose groups were regarded as specific to rats or an adaptive response.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 50 mg/kg bw/day in this study, based on treatment-related effects on red blood cell parameters.

TEST FACILITY IBR (1994f)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1983).
EU B.13/14 Directive 84/449/EEC
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 8 - 5000 µg/plate b) Without metabolic activation: 8 - 5000 µg/plate
Vehicle	Ethanol
Remarks - Method	TA102 and E.coli WP2 strains were not tested. Cytotoxicity in the presence of metabolic activation was not assessed in the preliminary test.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 10000	> 5000	≥ 1000	Negative
Present				
Test 1	-	> 5000	≥ 1000	Negative

Remarks - Results No substantial increase in revertant colony numbers of any of the four 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 IBR (1993h)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test (1983). EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Chinese hamster
Cell Type/Cell Line	Lung/V79
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver.
Vehicle	Notified chemical prepared in absolute ethanol and subsequently diluted in MEM supplemented with 2mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% non-essential amino acids.
Remarks – Method	- The dose of positive control without S-9 mix (methyl methane sulfonate) was increased from 0.2 to 0.4 mM in Test I because the frequency of chromosomal aberration at 0.2 mM was low in some experiments. - The dose of positive control with S-9 mix (cyclophosphamide) was decreased from 6.25 to 3.12 µg/ml because the frequency of chromosomal aberration at 6.25 µg/ml was too high in Test I.

Metabolic Activation	*Test Substance Concentration (mg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.01, 0.05, 0.1* 0.5*, 1*, 5	5 h	18 h
Test 2	0.01, 0.05, 0.1*, 0.5*, 1*, 5	5 h	24 h
Present			
Test 1	0.1, 0.5*, 1*, 5*	5 h	18 h
Test 2	0.1*, 0.5*, 1*, 5	5 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (mg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 5	> 1	> 1	Negative
Test 2	≥ 5	> 1	> 1	Negative
Present				
Test 1	≥ 5	> 5	> 5	Negative
Test 2	> 1	> 1	> 1	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, both in the presence and absence of metabolic activation. No biologically relevant increase in the frequencies of polyploidy cells was found after treatment with the test item, compared to controls. The positive controls performed as expected, confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.
TEST FACILITY	IBR (1993i)

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 sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Oxygen Demand (ThOD)
Remarks - Method	Due to the limited solubility, the notified chemical was added directly into the test water at 1 mg/L and stirred over night. The test was conducted in duplicate.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	3.6	5	80.8
14	50.2	14	89.9
21	59.1	21	101.8
28	59.1	28	92.8

Remarks – Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound was 80.8% by day 5 and 89.9% by day 14. Therefore, the tests indicated the suitability of the inoculum. The relative biodegradation of the notified chemical was found to be 50.2% after 14 days. The degree of degradation of the test substance after 28 days was 59.1%. The pass levels were not achieved within 10-d window within 28-day period.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	IBR (1993j)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – flow-through conditions. EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - flow-through conditions.
Species	*Brachydanio rerio* (Zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	Tween 80 (Polyoxyethylene-sorbitan monooleate)
Water Hardness	249 mg CaCO3/L (14 °dH)
Analytical Monitoring	Gas Chromatography
Remarks – Method	The actual test concentrations were prepared by dilution the stock solution of the notified chemical with medium containing 100 mg/L Tween 80. For each concentration level, 10 l of stock solution was prepared daily. The test medium was replaced by fresh test solution within a period of 24 h.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*2-4 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control		10	0	0	0	0	0
Control solvent		10	0	0	0	0	0
1.1		10	0	0	0	0	0
2.0	2.36	10	0	0	0	0	0
3.5		10	0	0	0	0	0
6.3	9.24*	10	0	10	70	80	90
11.2		10	0	100	100	100	100
20.0		10	0	100	100	100	100

*Mean value

LC50	2.9 mg/L at 96 hours.
NOEC	2.0 mg/L.
Remarks – Results	A decrease to 57% of the nominal concentrations was observed in the test vessels within a typical 24 h renewal period. The LC50 value was based on the corrected actual concentrations.
CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility in water
TEST FACILITY	IBR (1993k)

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 Method. EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia – Static Method.
Species	*Daphnia magna Strauss*
Exposure Period	48 hours *[acute study]*
Auxiliary Solvent	Tween 80 (Polyoxyethylene-sorbitan mono oleate)
Water Hardness	14.5 °dH (258.8 mg/L $CaCO_3$)
Analytical Monitoring	Gas Chromatography
Remarks - Method	The actual test concentrations were prepared by dilution the stock solution of the notified chemical (20 mg/L) with medium containing 100 mg/L Tween 80. All tested concentrations of the notified chemical were above its water solubility of 1.59 mg/L.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control 1	Control 1	20	0	0
Solvent control 2	Solvent control 2	20	0	0
0.10	ND[§]	20	0	0
0.18	ND	20	0	0
0.31	ND	20	0	0
0.56	0.73	20	0	0
1.0	ND	20	0	0
1.8	ND	20	0	5
3.2	ND	20	5	25
5.6	6.36	20	0	100
10.0	10.53	20	70	100

[§]ND=not determined

LC50	3.5 mg/L at 48 hours
NOEC	1.0 mg/L

Remarks - Results	All validity criteria for the test were satisfied. The immobilisation rate in the control groups did not exceed 10% at any stage of the test. In comparison to the control group, no obvious abnormal effects were at or below a concentration of 1.0 mg/L. The actual concentrations at three representative concentration levels were measured at the start and end of the 48 h test period. The 48 h EC50 value was 3.5 mg/L.
CONCLUSION	The notified chemical is not harmful to invertebrates up to the limit of its solubility in water
TEST FACILITY	IBR (1993l)

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	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 2.0, 6.3, 20.0, 63.3, and 200 mg/L Actual: 1.23 (2.0-nominal), 8.20 (20-nominal) and 40.34 (63.3 nominal) mg/L at 72 h
Auxiliary Solvent	Tween 80 (Polyoxyethylene-sorbitan mono oleate)
Water Hardness	Not reported
Analytical Monitoring	Gas Chromatography
Remarks - Method	For the concentration above 2.0 mg/L of the notified chemical, the test solution was prepared by adding the required amounts of the notified chemical and Tween 80 to the final volume of test medium. All tested concentrations of the notified chemical were above its water solubility of 1.59 mg/L.

RESULTS

Biomass		*Growth*	
EC50 *mg/L at 0-72 h*	*NOEC* *mg/L*	*EC50* *mg/L at 72 h*	*NOEC* *mg/L*
5.88		110.2 (88.2)*	6.3 (5.0)*

*NOEC values were calculated based on the corrected actual concentrations.

Remarks – Results	The final concentrations of the notified chemical corresponded to 50-70% of the initial concentration which was attributed to potential volatilization loss. The EC values were calculated by regression analysis after log transformations of the nominal concentration values.
CONCLUSION	The notified chemical is not harmful to algae up to the limit of its solubility in water.
TEST FACILITY	IBR (1993m)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	Modified OECD TG 209 Activated Sludge, Respiration Inhibition Test. German Standard Method DIN 38412 L8. Growth inhibition test with *Pseudomonas putida*.
Inoculum	*Pseudomonas putida* strain MIGULA
Exposure Period	16 hours
Concentration Range	Nominal: 100, 200, 400, 600, 800 mg/L
Remarks – Method	This test assessed the inhibitory effects of the notified chemical on the growth of bacteria in suspension following DIN 38412 L8 guidelines... Stock emulsions containing 1,000 mg/L of the notified chemical in the presence of the emulsifier Tween 80 (100 mg/L) were prepared. Following ultrasonic treatment for 15 minutes and stirring overnight, the emulsions were allowed to separate. After six hours the aqueous phase was collected.
RESULTS	
EC50	> 800 mg/L at 16 h
NOEC	
Remarks – Results	All validity criteria were met. The notified chemical did not exhibit inhibitory effects on the growth of *P. Putida* at the highest nominal concentration tested in this study.

CONCLUSION	The notified chemical is not inhibitory to microbial growth
TEST FACILITY	IBR (1993n)

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