File No: LTD/1737

September 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Quinoline, 5,6,7,8-tetrahydro-8-(1-methylpropyl)-

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

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1737	Givaudan Australia Pty Ltd	Quinoline, 5,6,7,8-tetrahydro-8-(1-methylpropyl)-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Sensitisation, Skin (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

Xi; R38: Irritating to the skin
Xi; R43: May cause sensitisation by skin contact

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

Hazard classification	*Hazard statement*
Acute Category 2	H401 - Toxic to aquatic life
Chronic Category 2	H 411 - Toxic to aquatic life with long lasting effects

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

When used at ≤ 0.03% in fine fragrances, ≤ 0.006% in other cosmetic products and ≤ 0.00075% in household products, the notified chemical is not considered to pose an unreasonable risk to public health.

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

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

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

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

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 during reformulation processes:
  - Avoid contact with skin and eyes

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

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

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

Disposal

- The notified chemical should be disposed of to landfill.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

Emergency procedures

- Spills or accidental release of the notified polymers 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 0.03% in fine fragrances, 0.006% in other cosmetic products, or 0.00075% in household products;

or

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Givaudan Australia Pty Ltd (ABN: 87 000 470 280)
Unit 36, 5 Inglewood Place
BAULKHAM HILLS NSW 2153

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

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU (2012), USA (2013)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Bigaryl

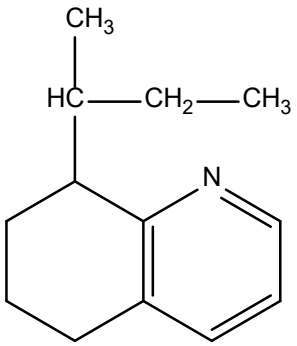
CAS NUMBER
1352745-21-1

CHEMICAL NAME
Quinoline, 5,6,7,8-tetrahydro-8-(1-methylpropyl)-

OTHER NAME(S)
GR-50-0572

MOLECULAR FORMULA
$C_{13}H_{19}N$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
189.3 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 99%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

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

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: liquid

Property	Value	Data Source/Justification
Freezing Point	< -50 °C	Measured
Boiling Point	276 °C at 101.3 kPa	Measured
Density	972 kg/m^3 at 20 °C	Measured
Vapour Pressure	6.1 × 10^{-4} kPa at 20 °C	Measured
Water Solubility	0.069 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at 25°C and pH 4, 7, and 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 4.0	Measured
Surface Tension	55.6 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.18	Measured
Dissociation Constant	pKa = 6.5	Measured
Particle Size	Not determined	Liquid
Flash Point	118 °C at 101.3 kPa	Measured
Flammability (contact with water)	Not expected to react significantly with water at 20 °C and will not emit flammable gases	Estimated based on chemical structure
Autoignition Temperature	338 °C	Measured
Explosive Properties	Predicted negative	Estimated based on chemical structure
Oxidising Properties	Predicted negative	Estimated based on chemical structure

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

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

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia as a component (≤ 0.15%) of compounded fragrances.

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

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

PORT OF ENTRY
Sydney (by sea or air), Perth (by air)

IDENTITY OF RECIPIENTS
Givaudan Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical as a component (≤ 0.15% concentration) of compounded fragrance products will be imported into Australia in glass, lacquer-lined containers of 1, 5, 10, 25, 100 and 190 kg size. The compounded fragrance products will be transported from the port of entry by road to the notifier's warehouse facilities for storage and then distributed to reformulation sites. The end-use products (≤ 0.03% notified chemical) will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance component in a variety of cosmetic and household products. The content in the final consumer products will vary, with the proposed usage concentrations of ≤ 0.03% for fine fragrances, ≤ 0.006% for other cosmetic products (including hair spray), ≤ 0.00015% for fabric care products and ≤ 0.00075% for other household products.

OPERATION DESCRIPTION
The procedures for incorporating the imported fragrance preparations (containing ≤ 0.15% notified chemical) into end-use products will likely vary depending on the nature of the cosmetic and personal care/household products being formulated, and may involve both automated and manual transfer steps. However, in general, it is expected that the reformulation processes will involve blending operations that will be highly automated and occur in enclosed environments, followed by automated filling of the reformulated products into containers of various sizes.

The finished products containing the notified chemical at ≤ 0.03% concentration may be used by consumers and professionals such as hairdressers, workers in beauty salons or cleaners. Depending on the nature of the product, these could be applied in a number of ways, such as by hand, using an applicator or sprayed.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and Warehouse Workers	unknown	unknown
Mixer (Plant Operators)	4	2
Drum Handling	4	2
Drum Cleaning/Washing	4	2
Maintenance	4	2
Quality Control	4	2
Packaging	4	2
End Users (Professionals)	1-8	200

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical, as a component of the imported fragrance preparations (≤ 0.15% concentration) or end-use products (≤ 0.03% concentration), only in the event of accidental rupture of containers.

During reformulation of the notified chemical into the final consumer products, dermal, ocular and inhalation exposure of workers (at ≤ 0.15% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of local and general ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses, face masks and impervious gloves.

Exposure to the notified chemical in end-use products (at ≤ 0.03% concentration) may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hair dressers, workers in beauty salons) or in the cleaning industry. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of the household products and the rinse-off and leave-on cosmetic products (≤0.03% in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

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, 2010; Cadby *et al.*, 2002; SDA, 2005). 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, the default dermal absorption of 100% was assumed for calculation purposes (European Commission, 2003). For the inhalation exposure assessment (European Commission, 2003; SDA, 2005), an adult inhalation rate of 23 m^3/day (enHealth, 2004) was used and it was assumed that the bioavailability of the notified chemical via the inhalation route is 100%. An adult bodyweight of 60 kg 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	0.006	1	0.0078
Face cream	1540	0.006	1	0.0015
Hand cream	2160	0.006	1	0.0022
Fine fragrances	750	0.03	1	0.0038
Deodorant spray	1430	0.006	1	0.0014
Shampoo	10460	0.006	0.01	0.0001
Conditioner	3920	0.006	0.01	0.000039
Shower gel	18670	0.006	0.01	0.0002
Hand soap	20000	0.006	0.01	0.0002
Hair styling products	4000	0.006	0.1	0.0004
Total				0.0176

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

- 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	0.00075	0.95	10	27×10^{-6}
Fabric softener	90	0.00015	0.95	10	2×10^{-6}
Total					29×10^{-6}

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 Use C (g/cm^3)	Film Thickness (cm)	Time Scale Factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	0.00075	1980	0.01	0.01	0.007	2×10^{-7}
Dishwashing liquid	3	0.00075	1980	0.0093	0.01	0.03	20×10^{-7}
All-purpose cleaner	1	0.00075	1980	1	0.01	0.007	173×10^{-7}
Total							1.95×10^{-5}

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

- Cosmetic products (Inhalation exposure):

Product type	Frequency (use/day)	Amount (g/use)	C (%)	Inhalation rate (m^3/day)	Exposure duration (mins)	Airspace volume (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	2	10	0.00075	23	15	2	0.0003

Daily systemic exposure = (Frequency × Amount × C × Inhalation rate × Exposure duration × bioavailability via the inhalation route)/(body weight × Airspace volume)

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 0.0179 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 hair spray inhalation exposure assessment parameters, in particular assuming an airspace volume of 2 m^3, 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 (e.g. air fresheners).

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
Rabbit, skin irritation	irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 15 mg/kg bw/day NOAEL = 150 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration	non genotoxic

Toxicokinetics.

Based on the water solubility (0.069 g/L at 20^0C), partition coefficient (log K_{ow} = 4) and the low molecular weight (189.3 Da) of the notified chemical, passive diffusion across the gastrointestinal (GI) tract and dermal absorption could occur. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.

The notified chemical was found to have low acute oral toxicity in studies in rats.

No acute inhalation or dermal toxicity data were provided for the notified chemical

Irritation

In studies conducted in rabbits, the notified chemical was found to be irritating to the skin and slightly irritating to the eyes.

In the skin irritation study, well-defined erythema and very slight oedema was noted for all animals at the 24, 48 and 72 hour observations. Moderate desquamation and glossy skin were noted for all test animals at the 7 day observation. All treated skin sites appeared normal at the 14 day observation. The irritation scores did not warrant classification of the chemical as a skin irritant according to the *GHS*, as adopted for industrial chemicals in Australia, but did warrant classification of the chemical according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

In the eye irritation study, minimal to moderate conjunctival irritation was observed up to the 48-hour observation period. Iridial inflammation was also observed at the 1-hour observation. All signs of irritation were resolved at the 72-hour observation period.

Sensitisation
In a LLNA study in mice, the notified chemical was found to be a skin sensitiser. The EC_3 value was calculated to be 6.1%.

Repeated dose toxicity.
A 28 day repeat dose study by oral gavage was conducted in rats with the notified chemical at dose levels of 15, 50 and 150 mg/kg bw/day. Based on the results of this study, the No Observed Adverse Effect level was established at 150 mg/kg bw/day as the observed changes noted in the mid- and high-dose groups were either completely reversible or showed definitive trends towards reversibility. Furthermore, the changes were considered to be largely stress related rather than changes of systemic toxicity.

Mutagenicity/Genotoxicity.
The notified chemical was found to be non-mutagenic in a bacterial reverse mutation assay and was not clastogenic in an *in vitro* mammalian chromosome aberration test.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Sensitisation, Skin (Category 1B)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):
R38: Irritating to the skin
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation
Exposure of workers to the notified chemical (at ≤ 0.15% concentration) may occur during blending operations. The notified chemical has the potential to cause skin irritation and slight eye irritation effects and is considered to be a skin sensitiser. Given the low proposed introduced concentration irritation effects are not expected. The risk of sensitisation is also expected to be limited. Therefore, 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 could potentially handle the notified chemical at ≤ 0.03% concentration, similar to public use. Therefore the risk to workers who regularly use the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the general 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 (at ≤ 0.03% concentration) through the use of the cosmetic and household products.

Local effects
The notified chemical is irritating to the eyes and skin. However at the low proposed end use concentrations in cosmetic and household products irritation effects are not expected.

The notified chemical is considered to have the potential to cause skin sensitisation. Methods for the quantitative risk assessment of dermal sensitisation have been proposed and been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). Using a fine fragrance (containing 0.03 % notified chemical) as an example product that may contain the notified chemical, as a worst case scenario, the Consumer Exposure Level (CEL) is estimated to be 1.13 μg/cm^2 (Cadby *et al.*, 2002).

When tested in an LLNA study, the notified chemical was a skin sensitiser with an EC_3 value of 6.1%. Consideration of the study details and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 4.94 μg/cm^2. In this instance, the factors employed included an interspecies factor (3.16), intraspecies factor (10), a matrix factor (3.16) and a use and time factor (3.16), giving an overall safety factor of ~300.

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of fine fragrances (a worst case example of a leave-on cosmetic product) at ≤ 0.03% concentration is not considered to be unreasonable. Based on the significantly lower expected exposure level from other leave on and rinse-off cosmetic products (containing ≤ 0.006% notified chemical), and household products (≤ 0.00075% notified chemical), by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on the aggregate exposure has not been conducted.

Systemic effects
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 0.018 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 150 mg/kg bw/day, which was established in a 28-day repeated dose toxicity study on the notified chemical. A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences. Using the abovementioned NOAEL, a MoE of 8,343 was estimated, which is considered to be acceptable.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 0.03% in fine fragrances, ≤ 0.006% in other cosmetic products and ≤ 0.00075% 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

As a component of fragrance, the notified chemical will be imported in Australia for further reformulation into a variety of cosmetic and household products. No significant release of the notified chemical to the environment from transportation and storage is expected. Accidental spills of the notified chemical are expected to be contained and the spilled chemical likely to be adsorbed on an inert support and disposed of to landfill.

It is expected that most reformulation sites have closed, automated mixing and dosing equipment. Release from reformulation is estimated to be up to 1.5% of the import volume, which is mainly from cleaning the blending equipment. The disposal route for these waste waters may include disposal to on-site waste water treatment plants and/or the sewer system. The quantity of notified chemical remaining in the emptied import containers may be up to 1 % of the import volume. The disposal route for container rinsate may include on-site waste water treatment plants and/or the sewer system.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used in household, laundry, and personal cleaning products. It is anticipated that the entire products will be eventually washed into the sewer system after use. Therefore, majority of the imported notified chemical (> 98%) is therefore expected to be disposed to sewers.

RELEASE OF CHEMICAL FROM DISPOSAL

It is anticipated that up to 1% of the notified chemical may be lost as residues in consumer containers, which are primarily sent to landfill.

7.1.2. Environmental Fate

The notified chemical is not readily or inherently biodegradable. For the details of the environmental fate studies please refer to Appendix C. It may have potential for bioaccumulation expected from the high partition coefficient log P_{OW} of 4.0. However, this may also be minimised by the potential cationic functional groups. The notified chemical has ecotoxicity endpoints of > 1 mg/L to fish, daphnids and algae, which does not meet the toxicity for PBT consideration. Therefore, it is not considered to have a PBT concern.

SimpleTreat model (EC, 2003) predicted that 1% of the notified chemical in STPs may be volatile to air. This is not considered to be a concern considering the low proportion. The overall half-life of the notified chemical in the air has been predicted to be 10.3 hours using EPISuite v. 4.10 (AopWin v1.92; US EPA, 2011). On this basis, the notified chemical is not expected to be persistent in the atmospheric compartment.

A small amount of the notified chemical may be sent to landfill with the empty containers. Most of the notified chemical is expected to be released into sewer systems after use or with wash water. In the sewage treatment plants (STPs), a proportion of the notified chemical is expected to be removed by adsorption to sludge due to the log K_{OC} of 3.18. A significant amount of the notified chemical is expected to remain in STP effluent and released into the public surface water. The sludge may be disposed of to landfill, where the notified chemical is not expected to leach. In landfill or water, the notified chemical will eventually undergo biotic or abiotic degradation to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated assuming 100% of release of the notified chemical into the sewer systems nationwide and no removal from 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	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	100%	
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 4.0 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 = 3 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 4.9 mg/L	Toxic to daphnids
Algal Toxicity	96 h EC50 = 3.1 mg/L	Toxic to algae
Inhibition of Bacterial Respiration	3 h IC50 = 80 mg/L	Not significantly inhibitory to bacteria respiration

The notified chemical is considered to be toxic to aquatic organisms based on the above endpoints for fish, daphnids and alga. The classification under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) has been made based on these endpoints. The notified chemical is formally classified as 'Acute Category 2; Toxic to aquatic life' under the GHS. Based on the acute toxicity data and the lack of ready biodegradability, the notified chemical is formally classified as 'Chronic Category 2; Toxic to aquatic life with long lasting effects' under the GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) was calculated using the endpoint of the most sensitive species (fish LC50 = 3 mg/L). A safety factor of 100 was used since endpoints for three trophic levels were available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish)	3	mg/L
Assessment Factor	100	
PNEC:	30	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.61	30	0.020
Q - Ocean:	0.06	30	0.002

The risk quotient (Q = PEC/PNEC) was calculated to be < 1 for the ocean and fresh waters. This suggests that the risk from the use of the notified chemical is not a concern to the aquatic organisms.

On the basis of the calculated PEC/PNEC and the assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

PBT Potential of the Notified Chemical
The notified chemical is expected to be persistent. It has potential to be bioaccumulative based on the log P_{OW} of 4.0. Considering the presence of potential cationic functional groups, the notified chemical may not be bioaccumulative. In addition, the notified chemical has ecotoxicity of > 1 mg/L for fish, daphnids and algae, indicating it does not meet the toxicity criteria to be considered a persistent, bioaccumulative and toxic (PBT) chemical. Therefore, the notified chemical is not considered to have a PBT potential.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -50 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks No freezing was observed down to a temperature of -50.0 °C.
Test Facility Givaudan (2011a)

Boiling Point 276 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks A Buchi 540 boiling point device was used.
Test Facility Givaudan (2011b)

Density 972 ± 1 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 The oscillating densitometer method was used.
Test Facility Givaudan (2013)

Vapour Pressure 6.1×10^{-4} kPa at 20 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks The gas saturation method was used.
Test Facility Givaudan (2012a)

Water Solubility 0.069 g/L at 20 °C

Method OECD TG 105 Water Solubility
Remarks Flask Method was used. High performance liquid chromatography (HPLC) was used for concentration determination.
Test Facility Givaudan (2012b)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at 25 °C and pH 4, 7, and 9

Method OECD TG 111 Hydrolysis as a Function of pH

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

Remarks Less than 10% hydrolysis occurred at 50°C and pH values of 4, 7 and 9 after 120 h. This corresponds to a half-life time of more than one year at 25°C.
Test Facility Givaudan (2014a)

Partition Coefficient (n-octanol/water) log Pow = 4.0

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method. The column temperature was 35°C.
Test Facility Givaudan (2011d)

Surface Tension 55.6 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	Concentration: 62 mg/L
Test Facility	Givaudan (2012b)

Adsorption/Desorption – screening test log K_{oc} = 3.18

Method	OECD TG 121 Estimation of the Adsorption Coefficient (*Koc)* on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC)
Remarks	HPLC method. The column temperature was 35°C. The adsorption coefficient of the notified chemical was determined as log K_{OC} = 3.18.
Test Facility	Givaudan (2011e)

Dissociation Constant pKa = 6.5

Method	OECD TG 112 Dissociation Constants in Water.
Remarks	The dissociation constant (pKa) of the acid corresponding to the notified chemical was determined for 7 pH values, ranging from pH 3.0 to pH 7.0. The compound was at least 10% and less than 90% ionised in this pH range. The average result of pKa was 6.5 with a standard deviation of 0.13.
Test Facility	Givaudan (2014b)

Flash Point 118 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	The Pensky-Martens method was used.
Test Facility	Givaudan (2011c)

Autoignition Temperature 338 ± 5 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Determined by heating aliquots of the test substance in a flask and observing for any ignition.
Test Facility	Harlan (2012a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain: Rat/ RCCHan:WIST

Vehicle: 300 mg/kg dose level: Arachis oil BP
2000 mg/kg dose level: None; test substance administered as supplied

Remarks - Method: No protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3 F	300	0/3
II	3 F	2000	1/3
II	3 F	2000	1/3

LD50: > 2000 mg/kg bw

Signs of Toxicity: One animal in group III was humanely killed one day after dosing. One animal in group II was found dead on day five after dosing. No other unscheduled deaths occurred.

No signs of systemic toxicity were noted during the observation period in the 300 mg/kg group.

Clinical signs that were observed in the treated animals in the 2000 mg/kg groups included hunched posture, red/brown staining around the snout, ataxia and piloerection. Additional signs of toxicity observed in the animal that was killed one day after dosing included lethargy, occasional body tremors, dehydration, prostration, pallor of the extremities, emaciation, ptosis, decreased respiratory rate, laboured respiration and hypothermia.

Surviving animals appeared normal one or two days after dosing.

Effects in Organs: Abnormalities observed at necropsy in the animal killed one day after dosing included epithelial sloughing of the gastric mucosa and reddened non-glandular epithelium of the stomach. In the animal found dead on day five, abnormalities noted at necropsy additionally included dark liver, dark kidneys, and haemorrhage of the gastric mucosa and non-glandular epithelium of the stomach.

No adverse macroscopic findings were recorded at necroscopy for animals surviving to the end of the study.

Remarks - Results: Surviving animals showed expected bodyweight gains, aside from one animal in group II which showed bodyweight loss in the first week but expected bodyweight gain in the second week.

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

TEST FACILITY: Harlan (2012b)

B.2. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Males
Vehicle	Test substance administered as supplied
Observation Period	14 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	2	2	2	2	< 7 days	0
Oedema	1	1	1	2	< 7 days	0

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

Remarks - Results	Very slight erythema was noted in 2/3 animals immediately after patch removal. Well-defined erythema and slight oedema were noted in 1/3 animals at the one hour observation, and very slight erythema and oedema were noted for the remaining two animals. Well-defined erythema and very slight oedema was noted for all animals at the 24, 48 and 72 hour observations. Moderate desquamation and glossy skin were noted for all test animals at the 7 day observation. All treated skin sites appeared normal at the 14 day observation.
CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	Harlan (2012c)

B.3. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 Males
Observation Period	3 days
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	1	1	2	< 72 h	0
Conjunctiva: chemosis	0.3	0.7	0.7	2	< 72 h	0
Conjunctiva: discharge	0.3	0.3	0	2	< 48 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 h	0

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

Remarks - Results	Moderate conjunctival irritation was recorded in all treated eyes and iridial inflammation in 2/3 animals one hour after treatment. No corneal effects were noted during the study. Minimal conjunctival irritation was noted in all treated eyes at 48 hours. Al treated eyes appeared normal at 72 hours.
CONCLUSION	The notified chemical is considered to be slightly irritating to the eye under the conditions of the test.
TEST FACILITY	Harlan (2012d)

B.4. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	Acetone/olive oil [4:1 (v/v)]
Remarks - Method	No significant protocol deviations. Topical application was made at the dorsum of each ear. Concurrent positive and vehicle control studies were run. Total of 25 females: 5 females/group (3 test group,1 vehicle control group, 1 positive control group) ^{3}H-methyl thymide (^{3}HTdR) used to visualise the lymph node cells.

RESULTS

Concentration (% w/w)	*Proliferative response (mean DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	479.3 (± 79.3)	1.00
5	1295.7 (± 258.3)	2.70
10	1940.1 (± 503.8)	4.05
25	5208.3 (± 925.6)	10.87
Positive Control (25% α- Hexyl cinnamaldehyde)	5160.1 (± 2268.0)	10.77

Remarks - Results	No mortalities and no signs of systemic toxicity were noted in the test or control animals. No signs of local skin irritation were observed No statistically significant increase in ear weight or thickness was observed in the test substance groups. A statistically significant increase in ear weight was observed in the positive control group. The results show that the test substance elicited stimulation indices > 3. A statistically significant increase was observed in comparison to the vehicle control group and a clear dose-response was obtained. The estimated concentration of notified chemical to produce a stimulation index (S.I.) of 3 was 6.1% (w/w). Two outliers (exhibited lower DPM values compared to rest of group) were identified in the vehicle control group and in the high dose group. As the exclusion of the outliers did not have an influence on the overall test result, the values in question were not excluded from the calculation.

	The positive controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Harlan (2012c)

B.5. Repeat dose toxicity

TEST SUBSTANCE	Notified Chemical
METHOD	EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rats/RccHan™: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	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0/10
low dose	5 per sex	15	0/10
mid dose	5 per sex	50	0/10
high dose	5 per sex	150	0/10
control recovery	5 per sex	0	0/10
high dose recovery	5 per sex	150	0/10

Mortality and Time to Death

There were no unscheduled deaths during the study.

Clinical Observations

Analysis of clinical appearance, functional observations, and food and water consumption did not reveal any toxicologically significant abnormalities between the treated and control groups. A transient reduction of mean daily food consumption was noted in male rats (week 1) however females were unaffected.

Within the high dose group, differences in mean body weights and mean body weight gain were noted in males (remaining males and all female rats were unaffected). Salivation was noted in the high dose during the daily observations in males (day 20) and females (day 22) which continued until day 1 of the recovery period. This was considered to be related to the taste of the dose formulation rather than a systemic effect.

Within the mid- and high-dose groups, a reduction in mean forelimb grip strength was noted in males which was considered to be the result of the lower mean body weights. While this is related to exposure to the notified chemical, it was not considered to be an indication of neurotoxicity. The mean, hind limb grip strength of these males was normal. All remaining animals were unaffected. A reduction of the mean locomotor activity was also noted in males within these two dose groups. Males in the low-dose group and females at all dose levels were unaffected.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Differences to the haematological and clinical biochemistry control values were noted, with most observed in males of the high-dose group. The majority of values remained within the range of historical control values.

Males within the high-dose group showed an elevated red cell count and reduced mean absolute and relative reticulocyte counts. Females within this group also showed an elevated red blood cell count, but reductions in the mean absolute and relative reticulocyte counts were not observed at the end of the treatment period. Males and females showed similar patterns of reticulocyte maturity indices.

Males and females in the high-dose group showed exhibited slightly lower cholinesterase levels, reduced triglycerides and reduced phospholipid levels. These finding were considered to be an indication of slightly impaired fat metabolism, and were partly reversible after the recovery period. Significant reduction of albumin and globulin in males, and albumin in females reduced the total protein of animals in the high-dose group.
These were considered to be indications of a slightly hepatic insufficiency and were largely reversible after the recovery period.

Other differences in clinical biochemistry parameters were considered to be secondary to a generalized stress reaction rather than a system effect as values were considered to be within the ranges of the historical control values, unrelated to dose levels or contrary to changes commonly associated with a toxic response.

Differences in urinalysis values were also considered to be unrelated to the notified chemical as the values were either within the ranges of the historical control values or unrelated to dose.

Effects in Organs

Most differences in the mean absolute organ weights noted in the high-dose group males treated were considered to be associated with the marked differences in terminal body weights.

Within the high dose group, males and females showed reduced mean absolute pituitary weights, mean absolute thymus weights, mean absolute adrenal weights and mean thymus-to-body weight ratios were reduced in males and females. Similar increases in the mean kidney-to-body weight ratios were noted in males and females. The various organ-to-brain weight ratios showed a similar pattern: the pituitary-to-brain weight ratio was reduced, the thymus-to-brain and adrenal-to-brain weight ratios were reduced in males and females. Females in this group showed increased mean absolute kidney weights, whereas ovaries and uterus/oviducts were reduced. The mean pituitary-to-body weight ratio noted in females was also reduced and the mean adrenal-to-body weight ratio was lower in females. The kidney-to-brain weight ratio was reduced when compared with controls.

The prostate gland, seminal vesicles and thymus were reduced in size in males, and the spleen and thymus were reduced in size in females. Lymphoid depletion in the mesenteric lymph nodes, spleen and thymus, and reduced secretion in the prostate gland, coagulating glands and seminal vesicles were observed.

The macroscopic and microscopic changes observed in the high-dose group were considered secondary to the stress condition due to the body weight reduction and not directly related to the toxic effect of the notified chemical. These changes were completely reversible after the 14-day recovery period. Males in the high-dose recovery group also exhibited a higher incidence and severity of increased hemopoiesis in the spleen. This was attributed to physiological activation of the spleen in response to an increased demand of erythrocytes rather than an adverse effect of the notified chemical.

Within the mid-dose group, males showed reduced mean absolute thymus weights. The mean absolute organ weights of the females showed only reduced adrenal weights. The absolute organ weights of males and females in the low-dose group were unaffected by treatment with the notified chemical.

Remarks – Results

Based on the results of this study, 15 mg/kg bw/day was established as the no observed effect level (NOEL), whereas 150 mg/kg bw/day was considered to be the no observed adverse effect level (NOAEL) as the observed changes were either completely reversible or showed definitive trends towards reversibility and considered to be largely stress related changes rather than changes of systemic toxicity.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 150 mg/kg bw/day in this study, based on reversibility of effects observed at the highest dose level.

TEST FACILITY Harlan (2012f)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure (Test 1)/Pre incubation procedure (Test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.
Concentration Range in Main Test	Test 1 a) With metabolic activation: 3-5000 µg/plate b) Without metabolic activation: 3-5000 µg/plate Test 2 a) With metabolic activation: 1-2500 µg/plate b) Without metabolic activation: *S. typhimurium:* 0.3-1000 µg/plate *E. coli:* 1-2500 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	No significant protocol deviations. Negative (untreated and solvent) and positive controls were used in parallel with the test material. Positive controls: i) without S9: sodium azide (TA1535, TA100), 4-nitro-o-phenylene-diamine (TA1537, TA98), methyl methane sulfonate (WP2 *uvrA*); ii) with S9: 2-aminoanthracene (TA1535, TA1537, TA98, TA100, WPS *uvrA*). The preliminary toxicity test (test 1; concentration range: 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate) was performed for all strains using the same experimental conditions as the plate incorporation test. Based on the toxic effects observed, 8 concentrations were tested (pre-incubation procedure – test 2) with the maximum concentration as 2500 µg/plate.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 100	≥ 100	≥ 1000	Negative
Test 2		≥ 33	≥ 1000	Negative
Present				
Test 1	≥ 333	≥ 333	≥ 1000	Negative
Test 2		≥ 100	≥ 1000	Negative

Remarks - Results	The test substance precipitated in the overlay agar in the test tubes from 1000 to 5000 µg/plate (plate incorporation procedure) and 1000 and 2500 µg/plate (pre-incubation procedure). Precipitation of the test substance in the overlay agar on the incubated plates was observed from 1000 to 5000 µg/plate (plate incorporation procedure) and 1000 to 2500 µg/plate (pre-incubation procedure). The undissolved particles had no influence on the data recording. No substantial increase in revertant colony numbers was observed following treatment with the notified chemical at any dose level with or

without metabolic activation.

No clear dose-response relationship was observed between higher mutation rates with increasing.

The notified chemical caused a reduced growth of the background bacterial lawn, with and without metabolic activations in both procedures.

Distinct toxic effects (reduction in the number of revertants) occurred with and without metabolic activation in the plate incorporation (test 1) and pre-incubation (test 2) procedures.

Positive controls gave satisfactory responses confirming the validity of the test system.

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

TEST FACILITY Harlan (2012g)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

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

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.
Vehicle	Dimethyl sulphoxide
Remarks - Method	No significant protocol deviations.

Vehicle and positive controls (ethylmethane sulfonate without S9 and cyclophosphamide with S9) were used in parallel with the test material.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1A	12.3, 21.5, 37.6, 65.9*, 115.3*, 201.7*, 353.0, 617.8, 1081.1, 1892.0	4 h	22 h
Test 1B	3.1, 6.3, 12.5, 25.0, 50.0*, 75.0*, 100.0*, 125.0, 150.0, 175.0, 200.0, 300.0	4 h	22 h
Test 2	1.0, 1.7, 3.0, 5.2, 9.1, 16.0, 28.0*, 49.0*, 85.7*, 150.0	22 h	22 h
Present			
Test 1	12.3, 21.5, 37.6*, 65.9, 115.3, 201.7*, 353.0*, 617.8, 1081.1, 1892.0	4 h	22 h
Test 2	50.0*, 100.0*, 200.0, 250.0*, 300.0*, 350.0, 375.0, 400.0, 425.0, 450.0, 500.0, 600.0	4 h	22 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 1A	≥ 353.0	> 1892.0	Negative
Test 1B	≥ 150.0	> 300.0	Negative
Test 2	≥ 150.0	> 150.0	Negative
Present			

Test 1A	≥ 617.8	> 1892.0	Negative
Test 2	≥ 350.0	> 600.0	Negative

Remarks - Results	Phase separation was reported at the following concentrations: ≥ 65.9 µg/mL and ≥ 115.3 µg/mL in Test 1A (with and without metabolic activation, respectively), ≥ 100.0 µg/mL in Test 1B (without metabolic activation), and ≥ 200.0 µg/ml in Test 2 (with metabolic activation). No visible precipitation of the notified chemical was observed. No relevant influence on osmolarity or pH value was observed. No statistically significant increase in the number of cells with aberrations was observed at any concentration, with and without metabolic activation. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Harlan (2012h)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD Guideline for the Testing of Chemicals No. 301F, Paris, 1992
Inoculum	Activated sludge from a biological waste water plant
Exposure Period	28 days
Auxiliary Solvent	Not applied
Analytical Monitoring	The biological oxygen demand (BOD) was determined and expressed as a percentage of the theoretical oxygen demand (ThOD) presenting the degree of biodegradation
Remarks - Method	The test was conducted following the test guidelines and good laboratory practice (GLP). The test concentration was 30 mg/L of the notified chemical.

RESULTS

Notified chemical		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	-1	5	76
28	1	28	100

Remarks - Results	A toxicity control was not performed. The notified chemical undergoes no biodegradation after 28 days in the test conditions. According to the inherent biodegradation study summarised below, inhibition of the notified chemical to the intrinsic respiration of the inoculum cannot be excluded. The test results suggest that the notified chemical is not readily biodegradable. This may also due to the toxicity of the notified chemical to the inoculum.
CONCLUSION	The notified chemical may be not readily biodegradable based on the test outcome
TEST FACILITY	Givaudan (2012d)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD Guideline for the Testing of Chemicals No. 302C. Paris, 1992.
Inoculum	Activated sludge from a biological waste water plant
Exposure Period	28 days
Auxiliary Solvent	Not applied
Analytical Monitoring	The biological oxygen demand (BOD) was determined and expressed as a percentage of the theoretical oxygen demand (ThOD) presenting the degree of biodegradation
Remarks – Method	The test was conducted following the test guidelines and good laboratory practice (GLP). The test concentration used was 30 mg/L of the notified chemical.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	-19	5	73
28	-22	28	94

Remarks – Results	All the test validity criteria were met.

At the tested concentration the notified chemical showed significant inhibition to the intrinsic respiration of the inoculum (respiration reduced by more than 20%). Therefore, it cannot be excluded that the notified chemical inhibits the activity of the aquatic micro-organisms at the test concentration.
The notified chemical undergoes no biodegradation after 28 days. The notified chemical should be regarded as not inherently biodegradable according to the test outcome.

CONCLUSION The notified chemical may not be inherently biodegradable

TEST FACILITY Givaudan (2012e)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test - Semi-static.
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - Semi-static.
OPPTS Guideline No. 850.1075 (Public draft, 1996).

Species	Zebra fish (*Danio rerio)*
Exposure Period	96 hours
Auxiliary Solvent	Not applied
Water Hardness	125 mg $CaCO_3$/L
Analytical Monitoring	The test concentrations were analysed with high performance liquid chromatography and UV detection (HPLV/UV) using external calibration
Remarks – Method	The test was conducted following the test guidelines and good laboratory practice (GLP). Due to the low water solubility of the test item, a dispersion of the test item with the loading rate of 100 mg/L was continuously stirred at room temperature in the dark over 3 hours supported by 15 minutes ultrasonication. The dispersion was further filtered and diluted to the extents of 1: 5, 1:10, 1:20, 1:40, 1:80 and 1:160. The diluted preparations were used as test media and renewed on a daily basis. Additionally, a control was tested in parallel. The LC50 and the 95% confidence limits were determined as geometric mean values of the two consecutive test concentrations with 0 and 100% mortality.

RESULTS

Concentration		*Number of Fish*	*Number of Mortality*				
Dilution ratio	*Actual (mg/L)*		*3h*	*24 h*	*48 h*	*72 h*	*96 h*
1:160	0.32	7	0	0	0	0	0
1:80	0.64	7	0	0	0	0	0
1:40	1.1	7	0	0	0	0	0
1:20	2.2	7	0	0	0	0	0
1:10	4.2	7	0	0*	0*	0*	7
1:5	10.2	7	0*	7	7	7	7

* Abnormalities including apathy, fish at the bottom, tumbling, and fish lying on side or back on the bottom were observed.

LC50	3.0 mg/L (95% confidence interval 2.2 mg/L – 4.2 mg/L) at 96 hours
NOEC	2.2 mg/L at 96 hours.
Remarks – Results	All the test validity criteria were met. At the end of the first and last test periods (Day 1 and Day 4), 82 to 119% of the initially measured concentrations were found in the old medium samples. All fish survived until the end of the test and no visible abnormalities were observed in the test fish at the mean measured test concentrations up to and including 2.2 mg/L. For calculation of the LC50 value, the sacrificed fish

due to the moribund condition were taken into account as dead fish.

CONCLUSION The notified chemical is toxic to fish

TEST FACILITY Harlan (2012i)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

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

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not applied
Water Hardness	150 mg $CaCO_3$/L
Analytical Monitoring	The test concentrations were analysed with high performance liquid chromatography and UV detection (HPLV/UV) using external calibration
Remarks - Method	The test was conducted following the test guidelines and good laboratory practice (GLP). A dispersion of the test item with the loading rate of 100 mg/L was prepared at room temperature in the dark over 3 hours supported by 15 minutes ultrasonication. The dispersion was filtered and further diluted at 1: 100, 1: 46, 1: 22, 1: 10, 1: 4.6 and 1: 2.2, which were used as test media. The test was performed in a closed system to avoid any loss of test item due to volatilisation. In order to avoid photolytic degradation of the test item, the test was performed as far as possible in the dark, in the event that the notified chemical may be susceptible to aqueous photolysis.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised (adverse effects*)*	
Dilution ratio	*Actual (mg/L)*		*24 h*	*48 h*
Control	0	20	1	0
1: 100	0.39	20	1	3
1: 46	n.a.	20	0 (1)	1
1: 22	n.a.	20	0 (1)	0 (1)
1: 10	3.2	20	0 (20)	0 (20)
1: 4.6	7.5	20	0 (20)	20
1: 2.2	13.0	20	20	20

* Adverse effects include daphnids trapped at the water surface, antennae sticking together, and reduced swimming activity.

LC50	4.9 mg/L (95% confidence limits 3.2 mg/L – 7.5 mg/L)) at 48 hours
NOEC	Not established
Remarks - Results	All the test validity criteria were met. The analysed concentrations demonstrated between 87% and 100% recovery over the 24 hour renewal period. The test outcomes were expressed using the mean measured concentrations. The NOEC was not established given the top concentration without effects observed was not available.

CONCLUSION The notified chemical is toxic to daphnids

TEST FACILITY Harlan (2012j)

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	Green Algae (*Pseudokirchneriella subcapitata*)

Exposure Period	96 hours
Concentration Range	Nominal: dilution of 100 mg/L loading rate to 1: 220, 1: 100, 1: 46, 1: 22 and 1: 10 mg/L; Actual: 0.20, 0.38, 0.71, 1.5, and 3.3 mg/L.
Auxiliary Solvent	Not applied
Water Hardness	15 mg $CaCO_3$/L
Analytical Monitoring	The test concentrations were analysed with high performance liquid chromatography and UV detection (HPLV/UV) using external calibration
Remarks - Method	The test was conducted following the test guidelines and good laboratory practice (GLP). A dispersion of the test item with the loading rate of 100 mg/L was prepared at room temperature in the dark over 3 hours supported by 15 minutes ultrasonication. The dispersion was filtered and further diluted at 5 levels, which were used as test media. The test was performed in a closed system to avoid any loss of test item due to volatilisation. A parallel stability study was conducted. The algal biomass in the samples was determined by fluorescence measurement.

RESULTS

Biomass		*Growth*	
E_bC50 (mg/L at 96 h)	*NOEC (mg/L)*	*E_rC50 (mg/L at 96 h)*	*NOEC (mg/L)*
1.3 (95% confidence limits 1.1-1.4)	0.38	3.1 (95% confidence limits 3.0-3.3)	0.38

Remarks - Results	All the test validity criteria were met. During the test period of 96 hours, a decrease of test item concentration in the test media (incubated with algae under light) occurred. At the end of the test, 69 to 104% of the initial measured concentrations were found, and from 83 to 102 % of the starting concentration when compared against the mean measured concentration of the test item. The stability test showed that algal uptake and interaction may be responsible for up to 8 to 25% of the degradation and/or uptake of the test item over a 72 and 96 hour exposure period, respectively. At the end of the test, the shape and size of the algal cells were visually inspected, and, no adverse effect was observed. The biological results were related to the mean measured test item concentrations calculated as the geometric means of the concentrations measured at the start of the test as well as at the end of the test (96 hours).

CONCLUSION The notified chemical is toxic to algae

TEST FACILITY Harlan (2012k)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 31.6, and 100 mg/L
Remarks – Method	The test was conducted following the test guidelines and good laboratory practice (GLP).

RESULTS

IC50	80 mg/L
Remarks – Results	All test validity criteria were met. The study reported that as the first reference test did not meet the criterion of EC50 = 5 – 30 mg/L, a second test was conducted. The second one met the validity criterion and was reported here. A significant inhibition of the respiration was observed at

	the top test level 100 mg/L only. The 3 h IC50 was determined to be 80 mg/L. The NOEC was not determined.
CONCLUSION	The notified chemical is not expected to significantly inhibit respiration to sludge bacteria at concentrations < 80 mg/L
TEST FACILITY	Givaudan (2012f)

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