File No.: STD/1714

STD/1715

September 2020

AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

1,3-Cyclohexanediamine, 4-methyl- (STD/1714)
1,3-Cyclohexanediamine, 2-methyl- (STD/1715)

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals Act 2019 (the IC Act) and Industrial Chemicals (General) Rules 2019 (the IC Rules) by following the Industrial Chemicals (Consequential Amendments and Transitional Provisions) Act 2019 (the Transitional Act) and Industrial Chemicals (Consequential Amendments and Transitional Provisions) Rules 2019 (the Transitional Rules).* The legislations are Acts of the Commonwealth of Australia. The Australian Industrial Chemicals Introduction Scheme (AICIS) 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 Agriculture, Water and the Environment.

This Public Report is available for viewing and downloading from the AICIS website. For enquiries please contact AICIS at:

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Executive Director
AICIS

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SUMMARY

The following details will be published on the AICIS website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1714 STD/1715	BASF Australia Ltd	1,3-Cyclohexanediamine, 4-methyl- (STD/1714) 1,3-Cyclohexanediamine, 2-methyl- (STD/1715)	Yes	< 5 tonnes per annum (combined)	Component of dishwashing detergents

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification
Based on the available information, the assessed chemicals are hazardous chemicals according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The hazard classification applicable to the assessed chemicals is presented in the following table.

Hazard Classification	*Hazard Statement*
Flammable liquid (Category 4)	H227 – Combustible liquid
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Skin corrosion/irritation (Category 1B)	H314 – Causes severe skin burns and eye damage

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

Hazard Classification	*Hazard Statement*
Acute toxicity (Category 3)	H402 - Harmful to aquatic life

Human Health Risk Assessment
Under the conditions of the occupational settings described, the assessed chemicals are not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the assessed chemicals are not considered to pose an unreasonable risk to public health.

Environmental Risk Assessment
On the basis of the PEC/PNEC ratio, the assessed chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemicals should be classified as follows:
  - Flammable liquid: H227 – Combustible liquid
  - Acute toxicity: H302 – Harmful if swallowed
  - Skin corrosion/irritation: H314 – Causes severe skin burns and eye damage

The above should be used for products/mixtures containing the assessed chemicals, if applicable, based on the concentration of the assessed chemicals present.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the assessed chemicals during reformulation processes:
  - Enclosed and automated processes
  - Local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the assessed chemicals 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 assessed chemicals during reformulation processes:
  - Protective clothing
  - Impervious gloves
  - Safety goggles
  - Respiratory protection, if inhalation exposure to aerosols may occur

  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 assessed chemicals 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.

Storage

- The handling and storage of the assessed chemicals 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 assessed chemicals should be handled by physical containment, collection and subsequent safe disposal.

Disposal

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

Regulatory Obligations

Specific Requirements to Provide Information
This risk assessment is based on the information available at the time of the application. The Executive Director may initiate an evaluation of the chemicals based on changes in certain circumstances. Under Section 101 of the IC Act the applicant of the assessed chemicals has post-assessment regulatory obligations to provide information

to AICIS when any of these circumstances change. These obligations apply even when the assessed chemicals are listed on the Australian Inventory of Industrial Chemicals (the Inventory).

Therefore, the Executive Director of AICIS must be notified in writing within 20 working days by the applicant or other introducers if:

- the final use concentration of the assessed chemicals exceeds 1% (combined) in household products;
- the function or use of the chemicals have changed from a component of liquid dishwashing detergents, or is likely to change significantly;
- the amount of chemicals being introduced has increased, or is likely to increase, significantly;
- the chemicals have begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemicals on human health, or the environment.

The Executive Director will then decide whether an evaluation of the introduction is required.

Safety Data Sheet
The SDS of the assessed chemicals provided by the applicant was reviewed by AICIS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND APPLICATION DETAILS

APPLICANT
BASF Australia Ltd (ABN: 62 008 437 867)
Level 12, 28 Freshwater Place
SOUTHBANK VIC 3006

APPLICATION CATEGORY
STD/1714: Standard: Chemical other than polymer (more than 1 tonne per year)
STD/1715: Standard: Chemical other than polymer (more than 1 tonne per year) – Chemical notified at the same time as a similar chemical

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
Data items and details taken to be protected information include import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for boiling point, hydrolysis as a function of pH, flammability, acute oral toxicity, eye irritation and biodegradability.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT
None

APPLICATION IN OTHER COUNTRIES
Europe (2013), Turkey (2015), Korea (2016) and Canada.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Sokalan® BAXX 210 (product containing assessed chemicals at 100% combined concentration)

CAS NUMBER
STD/1714: 13897-55-7
STD/1715: 13897-56-8

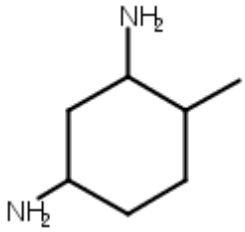
CHEMICAL NAME
STD/1714: 1,3-Cyclohexanediamine, 4-methyl-
STD/1715: 1,3-Cyclohexanediamine, 2-methyl-

OTHER NAME(S)
Other names for isomer mixture:
Methylcyclohexyldiamine (MCHDA)
Methyl-Diamine-Cyclohexane
Reaction product of 2,4-Dinitrotoluene and 2,6-Dinitrotoluene and hydrogen

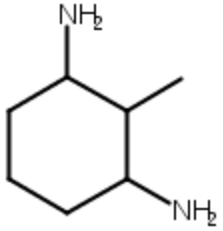
MOLECULAR FORMULA
STD/1714 and STD/1715: $C_7H_{16}N_2$

STRUCTURAL FORMULA

STD/1714:



STD/1715:



MOLECULAR WEIGHT
STD/1714 and STD/1715: 128.2 g/mol

ANALYTICAL DATA
Reference NMR, IR, HPLC, GC and UV/Vis spectra were provided (analysed as isomer mixture of the assessed chemicals)

3. COMPOSITION

DEGREE OF PURITY
The assessed chemicals are manufactured as an inseparable isomer mixture with a combined purity of > 98%.

Ratio of the isomers as reported by the applicant:
STD/1714: 70-90%
STD/1715: 10-30%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Cyclohexanamine, 2-methyl-		
CAS No.	7003-32-9	*Weight %*	≤ 0.5
Hazardous Properties	ECHA CLP:		
	H226 (Flammable liquid and vapour)		
	H302 (Harmful if swallowed)		
	H314 (Causes severe skin burns and eye damage)		
Chemical Name	Cyclohexanamine, 4-methyl-		
CAS No.	6321-23-9	*Weight %*	≤ 0.5
Hazardous Properties	H226 (Flammable liquid and vapour)		
	H314 (Causes severe skin burns and eye damage)		
Chemical Name	1,3-Benzenediamine, 4-methyl-		
CAS No.	95-80-7	*Weight %*	≤ 0.01
Hazardous Properties	HCIS:		
	H350 (May cause cancer)		
	H341 (Suspected of causing genetic defects)		
	H361 (Suspected of damaging fertility or the unborn child)		

H301 (Toxic if swallowed)
H312 (Harmful in contact with skin)
H373 (May cause damage to organs through prolonged or repeated exposure)
H317 (May cause an allergic skin reaction)
H411 (Toxic to aquatic life with long-lasting effects)

NON HAZARDOUS IMPURITIES (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

The following measured physical and chemical properties were obtained on the isomer mixture of the assessed chemicals.

APPEARANCE AT 20 °C AND 101.3 kPa: colourless to yellowish liquid

Property	*Value*	*Data Source/Justification*
Melting Point	Glass transition: -92 °C	Measured. No melting point observed
Boiling Point	210 °C	SDS
Density	939.5 kg/m^3 at 20 °C	Measured
Kinematic Viscosity	7.89 mm^2/s at 20 °C 4.18 mm^2/s at 40 °C	Measured
Vapour Pressure	0.017 kPa at 20 °C	Measured
Water Solubility	Miscible	Measured
Hydrolysis as a Function of pH	Stable to hydrolysis	Measured. Analogue chemical*
Partition Coefficient (n-octanol/water)	log Pow = 0.12 at 23 °C	Measured
Adsorption/Desorption	log K_{oc} = 1.2 at 23 °C at pH 7 log K_{oc} = 5.63 at 23 °C at pH 10	Measured
Dissociation Constant	pKa 1 = 10.4 at 24 °C pKa 2 = 8.4 at 24 °C	Measured
Flash Point	85.5 °C	Measured
Flammability	Not determined	Combustible liquid based on measured flash point
Autoignition Temperature	324 °C	Measured
Explosive Properties	Not explosive	Exothermic decomposition energy < 500 J/g as determined by differential scanning calorimetry
Oxidising Properties	Not oxidising	Based on chemical structure

*3-Aminomethyl-3,5,5-trimethylcyclohexylamine (CAS RN 2855-13-2)

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

Reactivity
The assessed chemicals are 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 assessed chemicals are recommended for physical hazard classification according to the *Globally Harmonised System of 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*
Flammable liquid (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemicals will not be manufactured in Australia. The assessed chemicals will be imported into Australia as a liquid at 100% combined concentration.

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

Import volume for isomer mixture:

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

PORT OF ENTRY
Melbourne and Sydney

IDENTITY OF RECIPIENTS
BASF Australia Ltd

TRANSPORTATION AND PACKAGING
The assessed chemicals (at 100% combined concentration) will be imported in 250 L steel drums and 1,000 L intermediate bulk containers (IBCs). The finished product containing the assessed chemicals (at ≤ 1% combined concentration) will be packaged in 1 L and 2 L plastic bottles. Transportation within Australia will be predominantly by road.

USE
The assessed chemicals will be used as a component of liquid dishwashing detergents at ≤ 1% combined concentration.

OPERATION DESCRIPTION
Reformulation for liquid dishwashing detergents
The assessed chemicals will typically be transferred by dip pipe or hose and pumped into a closed blending tank under local exhaust ventilation. After blending with other components, the finished liquid dishwashing detergents containing the assessed chemicals (at ≤ 1% combined concentration) will be transferred via automatic filling machines into appropriate containers for retail sale.

End-use
End-users (professional kitchen workers and the general public) will open the product container containing the assessed chemicals (at ≤ 1% combined concentration) and squirt the required amount of detergent into the sink for the washing of dishes and cutlery.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	2	30-50
Process operator	2	30-50
Quality control	2	30-50
Packaging	4-8	30-50
End-use		
– Retail staff	2	365
– Kitchen workers	8-12	240

EXPOSURE DETAILS
Transport and Storage

Transport and storage workers may come into contact with the assessed chemicals (at 100% combined concentration as introduced for reformulation, or ≤ 1% combined concentration in finished products), only in the unlikely event of an accidental breach of the product packaging.

Reformulation
Dermal and ocular exposure to the assessed chemicals (at ≤ 100% combined concentration) may occur during connection and disconnection of transfer lines, quality control, and cleaning and maintenance of equipment. Based on the low vapour pressure of the assessed chemicals (0.017 kPa at 20 °C), inhalation exposure to the assessed chemicals is not expected unless aerosols are formed. The applicant states that exposure is expected to be minimised through the use of enclosed and automated processes, and personal protective equipment (PPE) by workers such as protective clothing, eye protection, and impervious gloves.

End-use
Exposure of professional kitchen workers to the assessed chemicals (at ≤ 1% combined concentration) in end-use products may occur during measuring and dispensing of the liquid dishwashing detergent. The principal route of exposure will be dermal, while ocular exposure is also possible. Such professionals may use some PPE (gloves and protective clothing) to minimise repeated exposure. 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 assessed chemicals.

6.1.2. Public Exposure
Dermal and ocular exposure of the public to liquid dishwashing detergents containing the assessed chemicals (at ≤ 1% combined concentration) may occur through spills and splashes during handling.

Data on typical use pattern of dishwashing liquid (ACI, 2010) in which the assessed chemicals will be used is shown in the following table. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed for the assessed chemicals (ECHA, 2017). For calculation purposes, a lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used.

Household products (Direct dermal exposure)

Product type	*Frequency (use/day)*	*C (%)*	*Contact Area (cm^2)*	*Product Usage (g/cm^3)*	*Film Thickness (cm)*	*Time Scale Factor*	*Daily systemic exposure (mg/kg bw/day)*
Dishwashing liquid	3	1.0	1980	0.009	0.01	0.03	**0.0025**

Daily systemic exposure = Frequency × C × Contact Area × Product Usage × Film Thickness × Time Scale Factor × DA/ BW
(C = maximum intended combined concentration of the assessed chemicals; DA = dermal absorption; BW = body weight)

Using these assumptions results in an internal dose of 0.0025 mg/kg bw/day for the isomer mixture of the assessed chemicals.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the assessed chemical (STD/1714) and the isomer mixture of the assessed chemicals are summarised in the following table. For details of the studies, refer to Appendix B.

Toxicity studies on the assessed chemical (STD/1714)

Acute oral toxicity – rat	LD50 = 1,325 mg/kg bw; harmful
Skin irritation – *in vitro* EpiDerm™ model	corrosive
Mutagenicity – bacterial reverse mutation	non mutagenic

Toxicity studies on the isomer mixture of the assessed chemicals

Endpoint	***Result and Assessment Conclusion***
Acute dermal toxicity[1] – rat	LD50 > 3,420 mg/kg bw; low toxicity
Skin irritation – *in vitro* Corrositex® assay	corrosive
Skin sensitisation – mouse local lymph node assay (LLNA: BrdU-ELISA)[2]	evidence of sensitisation (EC1.6 = 1%)[3]

Endpoint	*Result and Assessment Conclusion*
Skin sensitisation – mouse local lymph node assay (LLNA: BrdU-ELISA)[4]	no evidence of sensitisation (up to 10% concentration)
Skin sensitisation – *in chemico* Direct Peptide Reactivity Assay (DPRA)	negative
Repeat dose oral toxicity – rat, 90 days	NOAEL = 100 mg/kg bw/day
Genotoxicity – *in vitro* mammalian cell gene mutation test (HPRT)	non mutagenic
Genotoxicity – *in vitro* chromosome aberration test in Chinese hamster V79 cells	non clastogenic
Combined repeated dose oral toxicity with reproduction/developmental screening – rat	NOAEL (systemic) = 25 mg/kg bw/day NOAEL (reproductive) = 200 mg/kg bw/day
Prenatal developmental toxicity – rat	NOAEL (maternal and prenatal developmental) = 100 mg/kg bw/day

[1]Hydrochloride salt of isomer mixture of the assessed chemicals
[2]Solvent: acetone:olive oil (4:1 v/v)
[3]Result considered non-reliable by study authors due to test substance reaction with solvent
[4]Sovent: propylene glycol

Toxicokinetics
Based on the low molecular weight of the assessed chemicals (128.2 g/mol) and partition coefficient (log Pow = 0.12 at 23 °C), absorption across biological membranes may occur.

Acute Toxicity
No acute oral toxicity studies were provided of the assessed chemicals. However, the assessed chemical (STD/1714) has been reported to be harmful by the oral route with an LD50 of 1,410 ml/kg (equivalent to 1,325 mg/kg bw) (Smyth *et al*., 1969).

The hydrochloride salt of the isomer mixture of the assessed chemicals was found to be of low acute dermal toxicity in rats.

Irritation and Sensitisation
The assessed chemical (STD/1714) was found to be corrosive to the skin in an *in vitro* study using the EpiDerm™ reconstructed human epidermis model. In another *in vitro* study (Corrositex® biobarrier membrane assay), the isomer mixture of the assessed chemicals was found to be corrosive. Based on the results of these studies, the assessed chemicals warrants classification as a Category 1B skin corrosive under the GHS.

No eye irritation studies were provided of the assessed chemicals. Substances that are corrosive to the skin are considered to induce irreversible damage to the eyes.

The isomer mixture of the assessed chemicals was found to be a skin sensitiser in a local lymph node assay (LLNA: BrdU-ELISA), where a mixture of acetone and olive oil (4:1, v/v) was used as a vehicle. The EC1.6 value (estimated concentration required to produce a stimulation index of 1.6 – a positive response for skin sensitisation) was determined to be 1%. However, the study authors reported that this vehicle interfered with the assessed chemicals and thus the prediction of the skin sensitisation potential was regarded to be non-reliable. In a follow-up local lymph node assay (LLNA: BrdU-ELISA) using propylene glycol as vehicle, the isomer mixture of the assessed chemicals was found not to be a skin sensitiser at up to 10% concentration. The isomer mixture of the assessed chemicals also gave a negative response in the *in chemico* Direct Peptide Reactivity Assay (DPRA), the first key event (molecular initiating) of the adverse outcome pathway (AOP) for skin sensitisation.

Repeated Dose Toxicity
A repeated dose oral (gavage) toxicity study on the isomer mixture of the assessed chemicals was conducted in rats, in which the test substance was administered at 5, 25 and 100 mg/kg bw/day. The No Observed Adverse Effect Level (NOAEL) was established as 100 mg/kg bw/day, based on the absence of treatment related adverse effects up to the highest dose tested.

Mutagenicity/Genotoxicity
The assessed chemical (STD/1714) was not mutagenic in a bacterial reverse mutation assay. No bacterial reverse mutation study was submitted for the assessed chemical (STD/1715).

The isomeric mixture of the assessed chemicals was neither mutagenic in a gene mutation test in Chinese hamster ovary cells nor clastogenic in a chromosomal aberration test in Chinese hamster V79 cells.

Toxicity for Reproduction
In a combined repeated dose oral toxicity study with the reproduction/developmental toxicity screening test in rats, the isomer mixture of the assessed chemicals was administered at 25, 100 and 200 mg/kg bw/day. The NOAEL was established as 25 mg/kg bw/day for systemic toxicity, based on the premature death of a female at 100 mg/kg bw/day, and correlation between increased weights and histopathological findings of accessory sex organs. The NOAEL for reproductive toxicity was established as 200 mg/kg bw/day.

In a prenatal developmental toxicity study in rats, the isomer mixture of the assessed chemicals was administered by gavage in dams at concentrations of 5, 25 and 100 mg/kg bw/day on gestation days 6-19. A NOAEL for maternal and prenatal developmental toxicity was established as 100 mg/kg bw/day, the highest dose tested in this study.

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

Hazard Classification	*Hazard Statement*
Acute Toxicity (Category 4)	H302 ─ Harmful if swallowed
Skin Corrosion (Category 1B)	H314 ─ Causes severe skin burns and eye damage

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The assessed chemicals are corrosive and harmful by the oral route. Adverse systemic effects were also noted following repeated oral exposure.

Reformulation
Exposure of workers to the assessed chemicals (at 100% combined concentration) may occur during transfer and blending operations. Therefore, caution should be exercised when handling the assessed chemicals during reformulation processes. Provided that adequate control measures are in place to minimise worker exposure, including the use of enclosed and automated processes and PPE (protective clothing, eye protection, impervious gloves and respiratory protection, if inhalation exposure may occur), the risk to workers from use of the assessed chemicals is not considered to be unreasonable.

End-use
Professional kitchen workers will handle the assessed chemicals (at < 1% combined concentration), similar to public use. Therefore, the risk to workers who regularly use products containing the assessed chemicals 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 assessed chemicals in liquid dishwashing detergents (at ≤ 1% combined concentration). The main route of exposure is expected to be dermal with some potential for accidental ocular exposure.

Local effects
The assessed chemicals are corrosive to the eyes and skin. Given the low proposed use concentrations (at ≤ 1% combined concentration) and further dilution of the assessed chemicals in the wash water, corrosive effects are not expected.

Systemic effects
The potential systemic exposure to the public from the use of the assessed chemicals in liquid dishwashing detergents was estimated to be 0.0025 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 25 mg/kg bw/day established from a combined repeated dose oral toxicity study with the reproductive and developmental toxicity screening test on the isomer mixture of the assessed chemicals, the margin of exposure (MOE) was estimated to

be 10,000. A MOE value greater than or equal to 100 is considered acceptable to account for intra and inter-species differences.

Therefore, based on the information available, the risk to the public associated with use of the assessed chemicals at ≤ 1% combined concentration in liquid dishwashing detergents 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 assessed chemicals will be imported into Australia for reformulation into finished dishwashing detergents. The reformulation and repackaging are expected to be highly automated and occur within a fully enclosed environment, with minimal environmental release. Release of the assessed chemicals to the environment in the event of accidental spills or leaks during reformulation, storage and transport is expected to be absorbed on suitable materials and disposed of to landfill in accordance with local government regulations. Empty import containers will be collected by an approved waste contractor for reuse or disposal of in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

During use as a component of finished dishwashing detergents, almost the entire volume of the assessed chemicals is expected to be released to sewers. Spills are expected to be cleaned up with an appropriate sorbent material, which is expected to be disposed of to landfill, or spills may be washed to sewers. Residues of the assessed chemicals in the empty containers are likely to be rinsed and be added into the dish washing water via the sink, or disposed of to landfill with the empty containers.

RELEASE OF CHEMICAL FROM DISPOSAL

Small amounts of the assessed chemicals may remain as residues in empty containers, which are expected to be disposed of to landfill along with the empty containers.

7.1.2. Environmental Fate

The majority of the assessed chemicals is expected to enter the sewer system before potential release to surface waters on a nationwide basis. The assessed chemicals are not expected to be readily biodegradable based on an analogue chemical (3-aminomethyl-3,5,5-trimethylcyclohexylamine, CAS RN 2855-13-2) (OECD, 2004), and do not significantly adsorb to sewage sludge. For further details on the adsorption on activated sludge studies, refer to Appendix C.

A proportion of the assessed chemicals may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. The assessed chemicals in landfill and soils are expected to have medium mobility based on the soil adsorption coefficient. The assessed chemicals are not expected to be bioaccumulative based on the measured partition coefficient (log Pow = 0.12). In the aquatic and soil compartments, the assessed chemicals are expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The use pattern will result in most of the assessed chemicals being washed into the sewer. The predicted environmental concentration (PEC) has been calculated assuming the realistic worst-case scenario with 100% release of the assessed chemicals into sewer systems nationwide over 365 days per annum. The extent to which the assessed chemicals are removed from the effluent in STP processes based on the properties of the assessed chemicals has not been considered for this scenario, and therefore no removal of the assessed chemicals during sewage treatment processes, is assumed. The PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	5,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	5,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	13.70	kg/day

Water use	200.0	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River:	2.81	µg/L
PEC – Ocean:	0.28	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 assessed chemicals in this volume are 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 2.81 µg/L may potentially result in a soil concentration of approximately 18.7 µg/kg. Assuming accumulation of the assessed chemicals in soil for 5 and 10 years under repeated irrigation, the concentration of assessed chemical in the applied soil in 5 and 10 years may be approximately 0.094 mg/kg and 0.187 mg/kg, respectively.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the isomer mixture of the assessed chemicals are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 > 120 mg/L	Not harmful to fish
Daphnia Toxicity	EC50 = 34.1 mg/L	Harmful to *Daphnia magna*
Algal Toxicity	EC50 > 220 mg/L	Not harmful to algal growth
Inhibition of Bacterial Respiration	EC50 > 875 mg/L	Not harmful to bacterial respiration
Chronic Toxicity	NOEC = 3.2 mg/L	Not harmful to *Daphnia magna*

Based on the above ecotoxicological endpoints, the assessed chemicals are expected to be acutely harmful to invertebrates. The assessed chemicals are not readily biodegradable, but the chronic toxicity has demonstrated no harmful effects to daphnia and the chronic classification is not required, as there is no other indication of chronic aquatic toxicity. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the assessed chemicals are formally classified as "Acute Category 3; Harmful to aquatic life".

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) was calculated using the most sensitive endpoint for ecotoxicity (*Daphnia magna*, NOEC = 3.2 mg/L) with an assessment factor of 50 as all three acute measured endpoints and a chronic endpoint are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEC (*Daphnia*)	3.2	mg/L
Assessment Factor	50	
Mitigation Factor	1.00	
PNEC	64	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) was calculated based on the predicted PEC and PNEC.

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	2.81	64	**0.04**
Q – Ocean	0.28	64	**< 0.01**

The risk quotient for discharge of treated effluents containing the assessed chemicals to the aquatic environment indicates that the assessed chemicals are unlikely to reach ecotoxicologically significant concentrations in surface waters. Therefore on the basis of the PEC/PNEC ratio, the assessed chemicals are not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Glass transition: -92 °C

Method	OECD TG 102 Melting Point/Melting Range
Remarks	Differential scanning calorimetry method. No melting point observed.
Test Facility	BASF (2011a)

Density 939.5 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	Densimetry oscillation method
Test Facility	BASF (2008a)

Kinematic Viscosity 7.49 mm^2/s at 20 °C
4.18 mm^2/s at 40 °C

Method	OECD TG 114 Viscosity of Liquids
Remarks	Capillary viscosimetry method
Test Facility	BASF (2008b)

Vapour Pressure 0.017 kPa at 20 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Dynamic method under nitrogen atmosphere
Test Facility	BASF (2009a)

Water Solubility Miscible

Method	OECD TG 105 Water Solubility
Remarks	Flask Method. Miscible at all concentrations.
Test Facility	BASF (2011a)

Hydrolysis as a Function of pH $t_{1/2}$ > 1 year (Analogue)

Method	OECD TG 111 Hydrolysis as a Function of pH
Remarks	After 5 days under the accelerated conditions of 50 ºC, the rate of hydrolysis of the test substance was < 10% at pH 4, 7 and 9. The test substance is expected to be hydrolytically stable.
Test Facility	OECD (2004)

Partition Coefficient (n-octanol/water) log Pow = 0.12 at 23 °C at pH 12

Method	OECD TG 107 Partition Coefficient (n-octanol/water)
Remarks	Flask Method
Test Facility	BASF (2011a)

Adsorption/Desorption – screening test log K_{oc} = 1.2 at 23 °C at pH 7
log K_{oc} = 5.63 at 23 °C at pH 10

Method	OECD TG 121 Adsorption – Desorption Using a Batch Equilibrium Method
Remarks	Kow method: Modular HPLC system with refractive index
Test Facility	BASF (2011a)

Adsorption/Desorption < 10% DOC after 72 h

Method	ISO 18749 Adsorption on Activated sludge – Batch test using specific analytical methods

Remarks TOC Analyser: degree of adsorption by measurement of dissolved organic carbon (DOC).
Test Facility BASF (2013a)

Dissociation Constant pKa 1 = 10.4 and pKa 2 = 8.4 at 24 °C

Method OECD TG 112 Dissociation Constants in Water
Remarks The dissociation constants were determined using potentiometric titration (HNP method)
Test Facility BASF (2011a)

Flash Point 85.5 °C

Method EC Council Regulation No 440/2008 A.9 Flash Point
Remarks Pensky-Martens closed cup method
Test Facility BASF (2011b)

Autoignition Temperature 324 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks Determined by using the apparatus described in EN 14522
Test Facility BASF (2011b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Hydrochloride salt of the assessed chemicals
METHOD	OECD TG 402 Acute Dermal Toxicity (1987)
Species/Strain	Rat/Wistar/Crl:WI (Han) SPF
Vehicle	Double distilled water
Type of dressing	Semi-occlusive
Remarks – Method	No protocol deviation

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5M/5F	1,710	0/10
2	5M/5F	3,420	0/10

LD50	3,420 mg/kg bw
Signs of Toxicity – Local	No local effects were observed
Signs of Toxicity – Systemic	No systemic clinical signs were observed during clinical examination
Effects in Organs	No signs of toxicity were observed at necropsy
Remarks – Results	The mean body weight of the animals increased within the normal range throughout the study period in both dosage groups. Mean body weight of the female animals were constant during the first post-exposure observation week but increased during the second week within the normal range in both dosage groups.
CONCLUSION	The test substance is of low acute toxicity via the dermal route.
TEST FACILITY	Bioassay (2009)

B.2. Skin Irritation – *In Vitro* Human Skin Model Test

TEST SUBSTANCE	Assessed chemical (STD/1714)
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Human Skin Model Test (2004) EpiDerm™ Reconstructed Human Epidermis Model
Vehicle	None
Remarks – Method	No protocol deviations. The assessed chemical directly reduced MTT and therefore, additional MTT-reduction freeze-killed controls (KC) were incorporated into the testing. However, the result of KC did not indicate an increased MTT reduction (difference to KC of the negative control is not greater than 0.1) and thus, KC was not used for viability calculation for corrosion. Positive and negative controls were run in parallel with the test substance: − Negative control (NC): deionised water − Positive control (PC): 8N potassium hydroxide

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*		*Relative Mean Viability* (%)*	
	3 min	*1 hr*	*3 min*	*1 hr*
Negative control	1.878	1.823	100	100
Test substance	0.489	0.173	26	10
Positive control	0.435	0.128	24	7

*Tissue viability as percentage of mean optical density of negative control; OD = optical density

Remarks – Results	In comparison to the negative control, the mean viability of the test substance treated tissues was 26% and 10% after an exposure period of 3 minutes and 1 hour, respectively. According to the study guideline, based on the mean tissue viability of < 50% after 3 minutes exposure, the assessed chemical should be classified for skin corrosion/irritation (Category 1) under the GHS.
CONCLUSION	The test substance was considered corrosive to the skin under the conditions of the test.
TEST FACILITY	BASF (2009b)

B.3. Skin Irritation – *In Vitro* Corrositex® Assay

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 435 *In vitro* Membrane Barrier Test Method for skin corrosion (2006) Corrositex® Biobarrier Membrane test system
Vehicle	None
Remarks – Method	No protocol deviations. A categorisation screen test was performed to assess the appropriate scoring scale (category 1 - high acid/alkaline reserve; category 2 – low acid/alkaline reserve) for the test substance. The test substance was assigned to timescale 1. Positive and negative controls were run in parallel with the test substance: − Negative control: 10% citric acid − Positive control: Sodium hydroxide, solid

RESULTS

Test Material	*Break-through time (min:s)*				
	Vial 1	*Vial 2*	*Vial 3*	*Vial 4*	*Mean*
Negative control	NB*	-	-	-	-
Test substance	5:01	4:35	7:30	8:10	6:19
Positive control	10:14	-	-	-	-

*NB = no break-through within 60 min observation period

Remarks – Results	The mean break-through time determined for the test substance in the *in vitro* membrane barrier test was 6 minutes and 19 seconds. According to the study guideline, based on a break-through time of > 3 minutes and ≤ 1 hour, the test substance should be classified for skin corrosion/irritation (Category 1B) under the GHS. The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The test substance was considered corrosive to the skin under the conditions of the test.
TEST FACILITY	BASF (2011c)

B.4. Skin Sensitisation – LLNA

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 442B Skin Sensitisation: Local Lymph Node Assay BrdU-ELISA (2010)
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	acetone:olive oil (4:1, v/v)
Preliminary study	Yes
Positive control	α-Hexylcinnamaldehyde (≥ 95%) in acetone:olive oil (4:1), conducted in parallel with the test substance
Remarks – Method	No protocol deviation. Preliminary tests were conducted using 2.5%, 5%, 10%, 25%, 50% and 100% of test substance to justify the dose concentrations for the main study. At 100% and 50% test substance concentration signs of systemic toxicity were observed. Ear thickness and ear weight were exceeded at 10%, 20% and 5% test substance concentration. In addition to this, scaling, incrustations and very slight erythema were also observed. No increase in ear thickness or ear weight was observed at 2.5%, therefore, 0.5%, 1% and 2% concentrations were used for the main study. A test substance is regarded as a sensitiser in the LLNA: BrdU-ELISA if exposure to one or more test substance concentration results in a 1.6-fold or greater increase in incorporation of BrdU compared with concurrent controls, as indicated by the stimulation index (SI). The estimated test item concentration required to produce a SI of 1.6 is referred to as the EC1.6 value.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Mean BrdU labelling index*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5F	0.080	1.0
0.5	5F	0.120	1.5
1	5F	0.127	1.6
2	5F	0.241	3.0
Positive Control			
25	5F	0.316	4.0

EC1.6	1%
Remarks – Results	No unscheduled mortalities or signs of systemic toxicity were observed during the study period. Scaling was observed in animals at 2% concentration of the test substance. Body weight change were within the range commonly recorded for the animals of this age and strain. There was a significant increase in lymph node weights and cell counts at 1% and 2% concentrations, indicative of skin sensitisation to the test substance. In an amendment to the report (Bioassay, 2014b), the study authors state the results of the study are regarded to be non-reliable based on evidence showing a clear adduct formation between the solvent (acetone) and the amino group of the test substance.

CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance, however the results of the study are regarded to be non-reliable by the study authors due to interference with solvent.
TEST FACILITY	Bioassay (2013)

B.5. Skin Sensitisation – LLNA

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 442B Skin Sensitisation: Local Lymph Node Assay BrdU-ELISA (2010)
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	Propylene glycol
Preliminary study	Yes
Positive control	α-Hexylcinnamaldehyde (≥ 95%) in propylene glycol, conducted in parallel with the test substance
Remarks – Method	No protocol deviation

A preliminary test was conducted using 2.5%, 5%, 10% and 25% of test substance to justify the dose concentrations for the main study.

At 25% test substance concentration both test animals showed local signs of irritation as indicated by increased ear weights of > 25%.

A test substance is regarded as a sensitiser in the LLNA: BrdU-ELISA if exposure to one or more test substance concentration results in a 1.6-fold or greater increase in incorporation of BrdU compared with concurrent controls, as indicated by the stimulation index (SI). The estimated test item concentration required to produce a SI. of 1.6 is referred to as the EC1.6 value.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Mean BrdU labelling index*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5F	0.105	1.0
2	5F	0.091	0.9
5	5F	0.108	1.0
10	5F	0.113	1.1
Positive Control			
25	5F	0.295	2.8

Remarks – Results	No unscheduled mortalities or signs of systemic toxicity were observed during the study period.

The stimulation index was below the threshold of 1.6 in all test groups, indicating a non-sensitising response.

Slight reduction in bodyweight gain was observed in animals at all three doses. Sporadic body weight loss was justified by the author by the increased activity due to environmental enrichment.

CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.
TEST FACILITY	Bioassay (2014b)

B.6. Skin Sensitisation – *In Chemico* DPRA Test

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	Similar to OECD TG 442c In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)
Vehicle	De-ionised water
Remarks – Method	Ethylene glycol dimethacrylate (at 50 mM in de-ionised water) was used as the positive control. No significant protocol deviations.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle Control	0.00 ± 3.20	0.00 ± 1.55
Test Substance	5.77 ± 2.28	-5.49 ± 0.81
Positive Control	82.20 ± 4.77	14.40 ± 1.65

SD = Standard Deviation

Remarks – Results	The mean peptide depletion as average of cysteine- and lysine-peptide depletions was calculated as 2.88% (negative depletion was considered to be zero for calculation of the mean peptide depletion), indicating minimal reactivity (negative prediction for skin sensitisation). The positive and vehicle controls performed as expected, confirming the validity of the test.
CONCLUSION	The test substance was considered to have minimal reactivity for peptide depletion under the conditions of the test, showing negative results in the first key event (molecular initiating) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.
TEST FACILITY	BASF (2013b)

B.7. Repeat Dose 90-day oral toxicity – Rat

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (1998)
Species/Strain	Rats/Wistar Crl:WI(Han)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week
Vehicle	Ultrapure or deionised water
Remarks – Method	No significant protocol deviation. The dose levels were selected based on results of the OECD TG 422 screening study below.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M/10F	0	0
Low Dose	10M/10F	5	0
Mid Dose	10M/10F	25	0
High Dose	10M/10F	100	0

Mortality and Time to Death

No mortalities were observed during the study.

Clinical Observations

Salivation occurred temporarily and immediately after the high-dose treatment (9/10 M; 1/10 F) on several days, beginning on day 55. However, the effect was considered due to the substance taste or local affection of the upper digestive tract.

Increase in mean body weight on day 63 and mean body weight change on days 35 and 63 were observed in low dose female rats. As there was no clear dose-response relationship and these differences did not occur over the complete course of treatment, the observation was considered not treatment related by the study authors.

A deviation from 'zero values' in an isolated low dose female rat during the neurobehavioral assessment was also considered not treatment related by the study author.

Statistically significant decreases in mean motor activity were observed in low and mid dose male animals (70.5% and 74.3% compared with control groups, respectively). This effect was not evident in female animals and in the absence of dose-response relationship, the change was not considered to be toxicologically significant by the study authors.

Ophthalmological examinations showed a scar on the upper edge of the right eyeball in one female in high dose group. Other ophthalmological findings were also observed in both treated and control groups and thus they were considered incidental.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

The following effects were statistically significant (compared with the control group):

- decreased mean relative reticulocyte counts (11.8%) in the mid dose male group, but they were not dose-dependent; therefore, the change was regarded as incidental
- decreased potassium levels in the high dose male group (6.4%), but they were reported within the historical control range

Effects in Organs

Although the following effects were statistically significantly different from the controls, they were considered incidental or not treatment related due to lack of a dose-response relationship or corresponding histopathology, or they were within historical control ranges.

- increased absolute and relative epididymal weights in the high dose group (12% and 19%, respectively)
- decreased absolute and relative thyroid weights in all treated male animals (13–17%)
- increased absolute and relative pituitary weights in all treated female animals (13–18%)
- increased relative liver and kidney weights in the high dose female group (8%)

Remarks – Results

Based on the low level of changes and lack of dose-response relationship, the study authors considered the effects observed were not toxicologically significant.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 100 mg/kg bw/day, the highest dose tested in this 90-day rat study.

TEST FACILITY	BASF (2017a)

B.8. Genotoxicity – Bacteria

TEST SUBSTANCE	Assessed chemical (STD/1714)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test (1997) EC Directive No. 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria Plate incorporation (Test 1) and pre incubation procedures (Test 2 and 3)
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 and *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	Test 1a

With and without metabolic activation: 20 – 5,000 µg/plate (all tester strains)

Test 1b
With metabolic activation: 0.8 – 200 µg/plate (TA98)

Test 1c
With metabolic activation: 10 – 2,500 µg/plate (TA98)

Test 2
Without metabolic activation: 20 – 5,000 µg/plate (all tester strains)

With metabolic activation: 20 – 5,000 µg/plate (TA1535, TA1537, TA100 and WP2uvrA); 0.8 – 200 µg/plate (TA98)

Test 3
With and without metabolic activation: 8 – 2,000 µg/plate (TA1535, TA1537 and TA100); 0.4 – 1,000 µg/plate (TA98)

Vehicle: Dimethylsulfoxide (DMSO)

Remarks – Method: Positive control:
with S9-mix: 2-aminoanthracene (all tester strains)
without S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine (TA1535 and TA100), 4-nitro-o-phenylenediamine (TA98), 9-aminoacridine (TA1537) and 4-nitroquinoline-N-oxide (WP2uvrA)

Preliminary toxicity test was not conducted.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1a	Not conducted	≥ 2,500	> 5,000	Negative
Test 2	Not conducted	≥ 500	> 5,000	Negative
Test 3	Not conducted	≥ 100	> 2,000	Negative
Present				
Test 1a	Not conducted	≥ 100	> 5,000	Negative
Test 1b	Not conducted	≥ 0.8	> 200	Negative
Test 1c	Not conducted	≥ 1,250	> 2,500	Negative
Test 2	Not conducted	≥ 20	> 5,000	Negative
Test 3	Not conducted	≥ 500	> 2,000	Negative

Remarks – Results: Bacteriotoxic effect was observed in strain TA98 (with metabolic activation) at doses ≥ 100 and ≥ 0.8 µg/plate (standard plate test) in two separate experiments; however, the study authors attributed this effect to a technical error that may have occurred while using S9 mix.

No relevant increase in the number of revertant colonies of any of the tested strains were observed following treatment with the test substance at any dose level, with or without metabolic activation, in either mutation test.

The positive controls induced a distinct increase of revertant colonies during the study indicating the validity of the test system.

CONCLUSION: The test substance was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY: BASF (2009c)

B.9. Genotoxicity – *In vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test (1997) EC Directive No 440/2008; B.17 Mutagenicity - *In vitro* Mammalian Cell Gene Mutation Test
Species/Strain	Chinese hamster
Cell Type/Cell Line	Chinese hamster ovary (CHO) cells
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat livers
Vehicle	Ham's F12 medium
Remarks – Method	No significant protocol deviations. Positive control: with S9-mix: methylcholanthrene without S9-mix: ethylmethanesulfonate

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0*, 40.6, 81.3, 162.5*, 325*, 650*, 1300*	4 h	7-9 days
Test 2	0*, 40.6, 81.3, 162.5*, 325*, 650*, 1300*	24 h	7-9 days
Present			
Test 1	0*, 40.6, 81.3, 162.5*, 325*, 650*, 1300*	4 h	7-9 days
Test 2	0* 250*, 500*, 1000*, 1300*	4 h	7-9 days

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	Not conducted	> 1,300	> 1,300	Negative
Test 2	Not conducted	> 1,300	> 1,300	Negative
Present				
Test 1	Not conducted	> 1,300	> 1,300	Negative
Test 2	Not conducted	> 1,300	> 1,300	Negative

Remarks – Results	The test substance did not cause any increases in the mutant frequencies with or without S9 mix. No cytotoxicity was observed up to the highest concentration tested. The positive controls gave a satisfactory response and the vehicle controls were within the historical control range, confirming the validity of the test system.
CONCLUSION	The test substance was not mutagenic to CHO cells under the conditions of the test.
TEST FACILITY	BASF (2011d)

B.10. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1997)

	EC Directive 440/2008/EC B.10 Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test
Species/Strain	Chinese hamster
Cell Type/Cell Line	V79 cells
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat livers
Vehicle	Minimal essential medium with Earle' salts (MEM)
Remarks – Method	No significant protocol deviations. A preliminary test was conducted at a concentration range of 10.2 to 1,300 µg/mL, with 18 h harvest time after 4 h- and 18 h- exposure periods without S9 mix, and after 4 h-exposure time with S9 mix. Positive control: with S9-mix: cyclophosphamide without S9-mix: ethylmethanesulfonate

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*,40.6, 81.3, 162.5*, 325*, 650*, 1,300	4 h	18 h
Test 2	0*, 40.6*, 81.3*, 162.5*, 325, 650, 1,300	18 h	18 h
Test 2a	0*, 162.5, 325*, 650, 1300	18 h	28 h
Present			
Test 1	0*, 81.3, 162.5, 325*, 650*, 1,300*	4 h	18 h
Test 2	0*, 81.3, 162.5*, 325*, 650*, 1,300	4 h	28 h

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 650	≥ 650	> 1,300	Negative
Test 2	≥ 650	≥ 325	> 1,300	Negative
Test 2a		≥ 325	> 1,300	Negative
Present				
Test 1	≥1300	≥ 650	> 1,300	Negative
Test 2		≥ 650	> 1,300	Negative

Remarks – Results	In Test 1 in the absence of S9 mix a dose-related increase in the aberration rates (excluding gaps) was observed. However, the study authors considered this finding biologically irrelevant, given that the values were equal or below the respective negative control value and within the historical control range of the test facility. In both main tests, no statistically significant increases in the frequency of chromosome aberrations were observed in the presence or absence of metabolic activation. The positive controls gave a satisfactory response and the vehicle controls were within the historical control range, confirming the validity of the test system.
CONCLUSION	The test substance was not clastogenic to Chinese hamster V79 cells under the conditions of the test.
TEST FACILITY	BASF (2011e)

B.11. Reproductive and developmental toxicity – Rat

TEST SUBSTANCE	Isomer mixture of the assessed chemicals

METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996)
Species/Strain	Wistar/Crl:WI (Han)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: Males (M): 38 days (14-day pre-mating, mating and approximately 7-day post-mating) Females (F): 56 days (14-day premating, mating to gestation days (GD) 0–20, postnatal days (PND) 0–4 or lactation days (LD) 1–4 until one day before sacrifice Dose regimen: 7 days per week
Vehicle	Deionised water
Remarks – Method	No significant protocol deviations were noted. The dose levels of 25, 100 and 250 mg/kg bw/day were selected based on the recommendation of the study sponsor (the applicant of the test substance). Because of the severe clinical findings, the high dose 250 mg/kg bw/day was reduced to 200 mg/kg bw/day from study day 7 onwards.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M/10F	0	0/20
Low Dose	10M/10F	25	0/20
Mid Dose	10M/10F	100	1/20
High Dose	10M/10F	200	2/20

Mortality and Time to Death

Two high dose and one mid dose female rats were found dead/moribund on GD 4 and GD 21, respectively. While the high dose revealed signs of systemic toxicity (including premature deaths and impaired body weights), the cause of the single premature death in mid dose group was unknown.

Clinical Observations

Salivation occurred temporarily and immediately after the high-dose treatment in several animals, and thus the effect was considered due to the substance taste or local affection of the upper digestive tract.

At 200 mg/kg bw/day, the following adverse effects were considered treatment-related:

- semiclosed eyelids (both sexes of all animals from study day 1 onwards), piloerection (2/10 M and 1/10 F on several study days, and 2/10 F on GD 1-4), respiratory sounds (2/10 M on several study days)
- semiclosed eyelids (4/5 M and 2/5 F) were also seen during functional observation battery examination
- laboured respiration, respiratory sounds, hypothermia and poor general state (2/10 F) during premating and gestation
- decreased mean food consumption in female rats during gestation (14%) and lactation (12%)
- decreased mean body weight and body weight change over the entire treatment period, with a maximum in male animals during premating (7%) and in female animals during gestation (31%) and during lactation (10%)
- decreased mean terminal body weight (6%) in male animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No treatment related changes among haematological and urinalyses parameters were observed.

The following clinical chemistry effects were statistically significantly different from the controls and considered related to treatment:

- decreased total protein (6%) and albumin (4.9%) in male animals at the high dose
- decreased glucose levels in female animals at the mid (12.2%) and high (16.4%) doses.

Reproductive/developmental findings

Fertility indices ranged between 90–80% at the mid and high dose, respectively, although the variations in males were considered within the historical control data, while those in females were attributed to either sperm-negative or not becoming pregnant. Gestation indices were 88%, 89% and 75% for the low, mid and high dose treatment, respectively. The study authors claimed that they were reduced because the calculation did not include the 3 dead/moribund, but sperm-positive female animals.

Live birth index was comparable with control, although a single stillborn pup was seen in the high dose group.

No treatment related effects on the pup viability, sex ratio and pup weight were observed. Necropsy showed dextrocardia (1 pup in the low dose group) and discoloured liver (2 pups in the mid and high dose groups). These findings were considered spontaneous and without biological relevance by the study authors.

Effects in Organs

The following effects were statistically significantly different from the controls:

- decreased absolute weights of thyroid glands (17% at ≥ 200 mg/kg bw/day) in male animals
- increased relative weights of epididymides (10 % at ≥ 200 mg/kg bw/day) and epididymal tails (at 14–15% at ≥ 100 mg/kg bw/day)
- increased absolute and relative weights of seminal vesicles (22–53% and 24–64% at ≥ 100 mg/kg bw/day, respectively)
- increased absolute and relative weights of prostate glands (24% and 25–21% at ≥ 100 mg/kg bw/day, respectively).

Histopathological findings of coagulating glands and seminal vesicles (at ≥ 100 mg/kg bw/day), and prostate glands (at ≥ 25 mg/kg bw/day) were found correlated with their macroscopically enlarged glands and and/or increased absolute and relative weights. As these findings did not influence reproductive functionality, these were regarded as treatment related but non-adverse reproductive effects by study authors.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 25 mg/kg bw/day for systemic toxicity in this study, based on the premature death of a female in the mid dose group and correlation between increased weights and histopathological findings of accessory sex organs.

The NOAEL for reproductive toxicity was established as 200 mg/kg bw/day, the highest dose tested in this study.

TEST FACILITY BASF (2011f)

B.12. Prenatal developmental toxicity – Rat

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 414 Prenatal Developmental Toxicity (2001)
Species/Strain	Rat/Wistar Crl:WI[Han]
Route of Administration	Oral – gavage
Exposure Information	Exposure days: gestation days (GD) 6–19
Vehicle	Ultrapure water
Remarks – Method	No significant protocol deviations. The dose levels were selected based on results of the OECD TG 422 screening study above. Due to technical reasons, the study was carried out in two cohorts.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	24F	0	0/24
Low dose	25F	5	0/25
Mid dose	24F	25	0/24
High dose	25F	100	0/25

Mortality and Time to Death

All dams survived to scheduled necropsy.

Effects on Dams

No clinical signs or changes of general behaviour were considered to be related to treatment at up to 100 mg/kg bw/day.

A statistically significantly increased food consumption (7.5% higher than control) in low dose group on GD 6-8 was considered incidental by the study authors. The mean (corrected) body weights of dams at all doses were comparable with the controls.

At 100 mg/kg bw/day, increased absolute and relative monocyte counts were observed (12.5% and 14.8%, respectively). Given there was no change in other differential blood cell or total white blood cell counts, this effect was regarded as treatment-related but not adverse by the study authors. Lower creatinine (7.2%) was also reported at this high dose; however, the mean value was within the historical control data.

No other treatment related effects on liver, kidney, or uterus weights, pregnancy rates, numbers of corpora lutea, implantations, pre- and post-implantation loss, resorption, litter size, foetal sex ratios, or gross pathological changes were observed.

Effects on Foetus

One female foetus from the mid dose group showed gastroschisis. One female foetus exhibited limb hyperextension and another male foetus from the high dose group had hydronephrosis and hydroureter. The observations (including other reported foetal malformations and variations) were within the historical control data and without a dose-response relationship, and thus they were considered not related to treatment.

Remarks – Results

Administration of the test substance by oral gavage at up to 100 mg/kg bw/day during GD 6–19 produced no treatment-related prenatal developmental toxicity in Wistar rats.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for maternal and prenatal developmental toxicity was established as 100 mg/kg bw/day, the highest dose tested in this study.

TEST FACILITY BASF (2017b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 203 Fish, Acute Toxicity Test, Acute, static
Species	Zebrafish (*Danio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Total Organic Carbon (TOC), HPLC
Remarks – Method	No protocol deviations. Following a preliminary range finding test, the main study was conducted as a limit test at 120 mg/L. The test solution was adjusted for acceptable media pH with 1M HCl.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control		7	0	0	0	0	0
120		7	0	0	0	0	0

EC50	> 120 mg/L at 96 hours
NOEC	≥ 120 mg/L at 96 hours
Remarks – Results	Oxygen saturation concentration was > 80% as all validity criteria were met. Analytical concentrations were verified and measured concentrations in test solutions were within ± 20% of the nominal concentration. No additional adverse effects were observed in any treatment. All test solutions were clear and colourless during the test.
CONCLUSION	The test substance is not harmful to fish.
TEST FACILITY	BASF (2011g)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test –Acute, static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not recorded
Analytical Monitoring	None
Remarks – Method	Screening test. Stock solution was clear and colourless. The pH of 9.8 was adjusted to pH 7.4. Test concentrations were prepared from dilution of stock solution.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number and percent Immobilised*	
Nominal	*Actual*		*48 h (No.)*]	*48 h (%)*
Control		20	1	5
0.1		20	0	0
1		20	1	5

10	20	1	5
100	20	18	90

EC50	10-100 mg/L at 48 hours
NOEC	10 mg/L at 48 hours
Remarks – Results	The study indicates that all validity criteria were met, but no details were recorded. Statistical analysis was conducted using the trimmed Spearman-Karber method.

CONCLUSION	The test substance is harmful to *Daphnia magna*.
TEST FACILITY	BASF (2010)

C.2.3. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test –Acute, static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	176 - 256 mg $CaCO_3$/L
Analytical Monitoring	TOC
Remarks – Method	No protocol deviations. Following a preliminary study, the main study was conducted with a range of concentration below. Potassium dichromate was also used as a reference substance as part of a quality assurance program. Test solutions were made from dilution of stock solution.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number and percent Immobilised*	
Nominal	*Actual*		*48 h (No.)]*	*48 h (%)*
Control	-	20	0	0
10	8.2	20	0	0
22	21.1	20	6	30
46	43.8	20	12	60
100	99.8	20	20	100
220	217.5	20	20	100

EC50	34.1 mg/L at 48 hours
NOEC	10 mg/L at 48 hours
Remarks – Results	Validity criteria were met. Oxygen concentration was > 3 mg/L in control and test vessels. The 24 h EC50 of the reference substance was 1.10 mg/L which is within the acceptable range of 0.6-2.1 mg/L. Statistical analysis was conducted using TOXRAT professional and EC50 was calculated using the probit method.

CONCLUSION	The test substance is harmful to *Daphnia magna*.
TEST FACILITY	BASF (2011h)

C.2.4. Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction test

Species	*Daphnia magna*
Exposure Period	21 d - Semi-static
Auxiliary Solvent	
Water Hardness	Total hardness 176 – 256 mg/L
Analytical Monitoring	
Remarks – Method	Semi-static with renewal of 3 times per week.

Survival of parental daphnids and number of offspring released per female daphnid (*Daphnia magna*)

Test Day	A	B	C	D	E	F	G	H	I	J	Number of Adult Daphnids Immobilized	Percent Survival
Total Number of Offspring Released per Daphnid												
21												
Nominal Conc. (mg/L)												
0	160	160	160	164	146	153	155	166	162	130	0	100
0.1	169	178	169	173	180	159	158	161	165	160	5	100
0.32	170	185	173	167	166	182	171	160	157	167	2	100
1.0	148	161	148	150	170	173	151	168	134	136	6	100
3.2	124	137	126	132	166	156	151	149	149	9	11	100
10	159	153	1.38	146	150	T	T	129	132	145	53	80

T = Parent daphnid died during the test

Nominal loading retested, daphnid survival and cumulative mean number of offspring released, mean total body length and dry weight of daphnids (*Daphnia magna*)

Test Day 21

Nominal Loading Rate (mg/L)	*Mean Percent Survival*	*Mean Number of Offspring Released Per Female (SD)*	*Mean Total Body Length (mm) (SD)*	*Mean Dry Weight (mg) (SD)*
Control	6.9	156	4.44	-
NOELR* (mg/L)	3.2(reproduction)	and 10(mortality)		

* No-Observed-Effect Loading Rate

Remarks – Results	Validity criteria were met and no deviations from test guidelines were observed. The EC50 (24 h) of the reference substance potassium dichromate was 0.71 mg/L ,which is within range of 0.6 – 2.1. EC50 and NOEC values of the test substance were statistically calculated using Dunnett's test or Fishers exact test. Reproduction at 3.2 mg/L was not statistically different than the control. No abnormal behaviour was observed in any of the test treatments.
CONCLUSION	The test substance is chronically harmful to *Daphnia magna*
TEST FACILITY	BASF (2018)

C.2.5. Algal Growth Inhibition Test

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation No 440/2008 C.3 Algal Inhibition Test
Species	Green algae (*Desmodesmus subspicatus*)
Exposure Period	72 hours
Concentration Range	Nominal: Control, 10, 22, 46, 100 and 220 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	TOC

Remarks – Method	Potassium dichromate was used as a reference substance (72 h ErC50 = 0.91 mg/L).

RESULTS

Biomass		*Growth*	
EyC50 (mg/L at 72 h)	*NOEyC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOErC (mg/L)*
165	22	> 220	22

Remarks – Results	All validity criteria were met. The increase in biomass, mean coefficient of variation for section growth rates and coefficient of variation of average specific growth rates were 56-fold, 8% and 2.9%, respectively. Statistical analysis was conducted using TOXRAT professional. EC50 and NOEC were calculated using the probit method and Dunnett's multiple t-test.
CONCLUSION	The test substance is not harmful to algal growth.
TEST FACILITY	BASF (2011g)

C.2.6. Inhibition of Microbial Activity

TEST SUBSTANCE	Isomer mixture of the assessed chemicals
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 from a municipal waste water treatment plant
Exposure Period	3 hours
Concentration Range	Nominal: 62.5, 125, 250, 500 and 1,000 mg/L
Remarks – Method	No protocol deviations. 3,5-Dichlorophenol was used as a reference substance. Test concentrations were not analytically determined.
RESULTS	
EC50	> 870 mg/L
NOEC	77 mg/L
Remarks – Results	All validity criteria were met. The EC50 of reference substance was 10.3 mg/L which is within an expected range. The coefficient of variation in the control samples was 6.4% (≤ 30% O_2 consumption). Statistical analysis was conducted using the software TOXRAT Professional.
CONCLUSION	The test substance is not harmful to microbial respiration.
TEST FACILITY	BASF (2012)

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