File No: STD/1652, STD/1653 & STD/1654

November 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

STD/1652: Chemical 1 in Evonik and Redox cleaning products

STD/1653: Chemical 2 in Evonik and Redox cleaning products

STD/1654: Chemical 3 in Evonik and Redox cleaning products

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1652 STD/1653 STD/1654	Redox Pty Ltd Evonik Australia	Chemical 1 in Evonik and Redox cleaning products Chemical 2 in Evonik and Redox cleaning products Chemical 3 in Evonik and Redox cleaning products	Yes	≤ 80 tonnes per annum	Ingredient in household and personal care products

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemicals are recommended for 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*
Serious eye damage /Eye irritation (Category 2A)	H319 – Causes serious eye irritation

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemicals are not considered to pose an unreasonable risk to the health of workers.

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemicals should be classified as follows:
  - Serious eye damage/eye irritation (Category 2A): H319 – Causes serious eye irritation

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemicals during reformulation:
  - Enclosed, automated processes, where possible
  - Adequate ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemicals during reformulation:

- Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemicals during reformulation:
  - Protective clothing
  - Impervious gloves
  - Safety glasses
  - Respiratory protection, if inhalation exposure 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 notified 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.

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemicals 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 chemicals are 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 concentration of the notified chemicals (combined) exceeds or is intended to exceed 1.5%
  - The notified chemicals are intended to be used in cosmetic products.

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemicals has changed from household and personal care products ingredient, or are 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 occupational health and safety, public health, or the environment.

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

Safety Data Sheet

The SDS of the notified chemicals provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Redox Pty Ltd (ABN: 92 000 762 345)
2 Swettenham Road
MINTO NSW 2566

Evonik Australia Pty Ltd (ABN: 31 145 739 608)
1 Ricketts Road
MOUNT WAVERLY VIC 3149

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year) – Group assessment

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, import volume, site of manufacture/reformulation and identity of manufacturer.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for all physico-chemical endpoints.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
STD/1652: EC (2016)
STD/1653: EC (2010)
STD/1654: EC (2010)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
STD/1652: Chemical 1 in Evonik and Redox cleaning products (contains ≤ 50 % concentration of Chemical 2 and Chemical 3 together)
STD/1653: Chemical 2 in Evonik and Redox cleaning products
STD/1654: Chemical 3 in Evonik and Redox cleaning products

MOLECULAR WEIGHT
STD/1652: < 500 g/mol
STD/1653: < 500 g/mol
STD/1654: < 500 g/mol

ANALYTICAL DATA
Reference NMR, IR, HPLC, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
STD/1652: ≤ 50 % (Chemical 2 and Chemical 3 together)
STD/1653: Unknown
STD/1654: Unknown

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellowish liquid (aqueous solution)*

Property	Value	Data Source/Justification
Melting Point/Freezing Point	-50 °C to - 20 °C	Analogue B data
Boiling Point	Aqueous Solution ~105 °C*	SDS* Analogue B data (boiling of analogue B was not observed below the temperature at which reaction and/or decomposition started 250 °C)
Density	1,070-1,090 kg/m^3 at 20 °C* 1,140 kg/m^3 at 20 °C	SDS* Analogue B data
Vapour Pressure	2.758 kPa at 25 °C (or 20 °C)	Analogue B data
Water Solubility	4.9 g/L at 20 °C	Measured*
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionalities but significant hydrolysis is not expected in the environmental pH range of 4-9.
Partition Coefficient (n-octanol/water)	log P_{ow} = -3.3.at 20 °C	Analogue A data
Surface Tension	26.32-42.82 mN/m at 20 °C	Measured*
Adsorption/Desorption	Not determined	Could bind to soil and sediments through hydrophobic and ion exchange mechanisms.
Dissociation Constant	Not determined	Contains ionisable functionalities which are likely to be ionised in the environmental pH range of 4-9.
Particle Size	Not determined	Introduced in aqueous solution
Flash Point	108 °C at 101.5 kPa	Analogue B data
Flammability	Not flammable	Analogue B estimate
Pyrophoric Properties	Not pyrophoric	Analogue B estimate
Autoignition Temperature	425 °C	Analogue B data
Explosive Properties	Not explosive	Analogue B data (additionally the notified chemicals contain no functional groups that imply explosive properties)
Oxidising Properties	Not oxidising	Analogue B data (additionally the notified chemicals contain no functional groups that imply oxidising properties)

* Chemical 1 in Evonik and Redox cleaning products (STD/1652)

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

Reactivity
The notified 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 notified chemicals are not 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.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemicals will not be manufactured in Australia. The notified chemicals will be imported into Australia at ≤ 50% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 80	≤ 80	≤ 80	≤ 80	≤ 80

PORT OF ENTRY
Throughout Australia

TRANSPORTATION AND PACKAGING
The imported products containing the notified chemicals at ≤ 50% concentration in 220 kg plastic drums or 1,000 kg Intermediate Bulk Containers (IBC) will be transported by road.

USE
The notified chemicals will be used as surfactants ingredients in household and personal care products, such as laundry detergents, glass cleaners and bathroom and all-purpose cleaners and facial wash, shampoos, shower and bath preparations, and hygiene washes. The concentration of the notified chemicals in end-use cleaning products will be ≤ 1.5%.

OPERATION DESCRIPTION
The notified chemicals will not be manufactured within Australia.

Reformulation
Where imported as a blended bulk raw material (≤ 50% concentration) for reformulation, the notified chemicals will be transferred into the blending tank using metering pumps where it will be mixed with additional additives to form the finished household products. The notifier states that the mixing facilities are expected to be highly automated occurring within fully enclosed systems. After being reformulated, the finished products containing the notified chemicals will be transferred into end-use containers of various sizes suitable for retail packaging.

End-Use
The finished household and personal care products containing the notified chemicals (at ≤ 1.5% concentration) may be used by consumers and professionals, such as workers in home and industrial settings. Application of products could be by hand, spray or through the use of an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Warehouse	1.5	17
Raw incoming sampling	0.5	4
Lab Analyst	0.25	4
Operators	0.25	4
Compounder	0.5	6

EXPOSURE DETAILS

Transport and storage
Transport and storage workers are expected to only come into contact with the notified chemicals (at ≤ 50% concentration) in the unlikely event of an accident. In case of such accidental exposure, the main routes of exposure would be dermal and ocular.

Reformulation
Workers may be exposed to the notified chemicals during collection of samples, formulation of products, filling and packaging of the products into end-use containers and during cleaning and maintenance of the equipment.

Dermal and ocular exposure to the notified chemicals is expected. Such exposure will be minimised by the use of safe work practices and wearing personal protective equipment (PPE) including impervious gloves, coveralls and goggles. Inhalation exposure to the notified chemicals is not expected to occur due to the use of closed formulation process systems.

End-Use by Professional Cleaners
Exposure to the notified chemicals in end-use products (at ≤ 1.5% concentration) may occur in work areas where the service provided involves the use of cleaning products in the cleaning industry and personal care products in hair salon. The principal route of exposure will be dermal, while ocular and inhalation exposure are also possible. Such professionals may use some PPE to minimise repeated exposure. However, 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 chemicals.

6.1.2. Public Exposure

The notified chemicals will be sold to the general public only in the form of finished products (containing the notified chemicals at ≤ 1.5% concentration). Dermal and accidental ocular and inhalation exposure to the notified chemicals may occur during application of the household and personal care product. The exposure frequency and duration are expected to be low due to the expected infrequent use of cleaning products.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity*	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity*	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation*	slightly irritating
Eye irritation (*in vitro-Isolated Chicken Eye Method*)**	irritating
Guinea pig, skin sensitisation – Magnusson and Kligman Test*.	no evidence of sensitisation
Guinea pig, skin sensitisation – Magnusson and Kligman Test#	no evidence of sensitisation
Guinea pig, skin sensitisation – Delayed Contact Hypersensitivity Test#.	no evidence of sensitisation
Mouse, skin sensitisation –Hypersensitivity test	no evidence of sensitisation
Rat, repeat dose oral toxicity –**	NOAEL = 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation**	non mutagenic
Genotoxicity – *in vitro* Mammalian Chromosome Aberration*	non genotoxic
Genotoxicity – *in vivo* Cell Mutation at the Thymidine Kinase Locus ($TK^{+/-}$ in Mouse Lymphoma L5178Y Cells+	non mutagenic

*Analogue B
**Chemical 1 in Evonik and Redox cleaning products
+Analogue D
#Analogue C

Toxicokinetics, metabolism and distribution
No toxicokinetics data for the notified chemicals or analogues were provided. For dermal absorption, molecular weights below 100 g/mol. are favourable for absorption and molecular weights above 500 g/mol. do not favour absorption (ECHA, 2017). Dermal uptake is likely to be moderate to high if the water solubility is between 100-10,000 mg/L (ECHA, 2017). In addition evidence of skin sensitisation or irritation increase the probability of dermal absorption occurring (ECHA, 2017). Based on the low molecular weights (< 500 g/mol) and moderate water solubility (4.9 g/L at 20 °C) of the notified chemicals passage across biological membranes is expected to occur.

Acute toxicity
No acute oral, dermal or inhalation toxicity data for the notified chemicals were provided. Analogue B was found to be of low acute oral and dermal toxicity with LD50s > 2,000 mg/kg bw.

Irritation and sensitisation
The notified chemical (chemical 1 in Evonik and Redox cleaning products) was found to be an eye irritant in an evaluation of an in vitro eye irritation study using isolated chicken eye test.

In an acute skin irritation / corrosion study on analogue B (38.2%) on New Zealand White rabbits the analogue test substance was classified as mildly irritating.

No evidence of skin sensitisation was found in Magnusson and Kligman skin sensitisation tests performed on analogues B and C.

Repeated dose toxicity
In a four week oral toxicity repeated dose study in rats followed by two week recovery period test, the No Observed Adverse Effect Level (NOAEL) of chemical 1 was established to be the highest dose tested 500 mg/kg bw/day.

Mutagenicity/Genotoxicity
The bacterial reverse mutation test (Ames test) assessed the mutagenic potential of chemical 1 in Evonik and Redox cleaning products in several bacterial strains. Based on the results obtained in this study, it can be concluded that the test item does not induce point mutations or frame-shifts in the genome of the bacterial strains used with or without metabolic activation regardless of the procedure. Data provided for analogue B and E showed them to be non-genotoxic in an *in vitro* Mammalian Chromosome Aberration test or in an *in vitro* Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y cells mutation test respectively.

Health hazard classification
Based on the available information, the notified chemicals are recommended for 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*
Serious eye damage /Eye irritation (Category 2A)	H319 – Causes serious eye irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemicals are expected to be irritating to the eye and slight skin irritants. There is potential for dermal and ocular exposure of workers to the notified chemicals (at ≤ 50% concentration) during reformulation processes. Exposure should be minimised through the stated use of enclosed, automated processes, local exhaust ventilation and PPE.

Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemicals are not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health

The public may have dermal or ocular exposure to the notified chemicals (at ≤ 1.5% concentration) through the use of household and general personal care products. The risk of irritant effects from the notified chemicals at these concentrations is considered to be low.

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemicals will be imported as a blended bulk raw material for reformulation into the end-use household and industrial cleaning products. The reformulation processes are expected to involve automated blending operation in an enclosed environment, followed by automated filling of the finished products into end-use containers. Wastes containing the notified chemicals generated during reformulation are expected to be disposed of in accordance with local government regulations. Release of the notified chemicals in the event of accidental spills or leaks during import, reformulation, storage and transport are also expected to be collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemicals are expected to be released to sewers across Australia as a result of their uses in cleaning products.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemicals in empty import and end-use containers are likely to either share the fate of the containers and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Following their uses in cleaning products, the majority of the notified chemicals are expected to enter sewers across Australia. The ready biodegradation test conducted on Chemical 1 shows that it is readily biodegradable (90% degradation over 28 days in OECD 301B test). Therefore, the notified chemicals are expected to be removed effectively by biodegradation at sewage treatment plants (STPs) before potential release to surface waters nationwide. For details of the biodegradability study, refer to Appendix C. A very minor proportion of the notified 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. As the notified chemicals contain ionic functionalities and are surface active, they could bind to soil through hydrophobic and ion exchange mechanisms. The notified chemicals are not expected to be bioaccumulative in the environment due to their ready biodegradability. In the aquatic and soil compartments, the notified 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 predicted environmental concentration (PEC) has been calculated to assume the worst case scenario with 100% release of the notified chemicals into sewer systems nationwide over 365 days per annum. It is also assumed under the worst-case scenario that there is no removal of the notified chemicals during sewage treatment processes. The resultant 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	80,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	80,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	219.18	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	44.9	µg/L
PEC - Ocean:	4.49	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 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 44.9 µg/L may potentially result in a soil concentration of approximately 0.3 mg/kg. Due to the notified chemicals ready biodegradability, annual accumulation is not expected.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on Chemical 1 and Analog C are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity of Chemical 1	96 h LC50 > 289 mg/L	Not harmful to fish
Daphnia Toxicity of Analog C	48 h EC50 = 7.8 mg/L	Toxic to aquatic invertebrates
Algal Toxicity of Analog C	72 h EC50 = 17.2 mg/L	Harmful to Algae

The above ecotoxicological endpoints of Analog C indicate that the notified chemicals could be toxic to aquatic invertebrates. Therefore, the notified chemicals are provisional classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) as "Acute Category 2; Toxic to aquatic life" (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The most sensitive endpoint from the above ecotoxicity tests is 48 h EC50 for Daphnia, and this was selected for the calculation of the predicted no-effect concentration (PNEC). An assessment factor of 100 was used in this case given acute endpoints for three trophic levels are available as a general indication of potential toxicity.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
48 h EC50 for Daphnia	7.8	mg/L
Assessment Factor	100	
Mitigation Factor	1	
PNEC:	78	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the PEC and PNEC:

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	44.9	78	**0.58**
Q - Ocean	4.49	78	**0.058**

The conservative risk quotients (Q = PEC/PNEC) have been calculated to be less than 1 for both the riverine and marine compartments, indicating that the notified chemicals are unlikely to reach ecotoxicologically significant concentrations in the aquatic environment based on its maximum annual importation quantity and the assessed use pattern. Therefore, based on the calculated risk quotient, the notified chemicals are not expected to pose an unreasonable risk to the aquatic environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point -50 °C to - 20 °C

Method	OECD TG 102 Melting Point/Melting Range European Economic Community (EEC), EEC-Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.1 Melting/Freezing Temperature
Remarks	The test was performed to analogue B at 40.1% concentration using Q100 differential scanning calorimeter (DSC).
Test Facility	Notox B.V. (2006a)

Boiling Point Not determined

Method	OECD TG 103 Boiling Point European Economic Community (EEC), EEC-Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.2 Boiling temperature
Remarks	Evaporation of water from the test substance (analogue B at 40.1% concentration) was observed between approximately 25°C and 175°C and reaction and/or decomposition of this test substance was observed above approximately 250 °C: boiling of analogue B was not observed below the temperature at which reaction and/or decomposition started.
Test Facility	Notox B.V. (2006a)

Density 1,140 kg/m3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids European Economic Community (EEC), EEC-Directive 92/69 EEC, A.3 Relative Density
Remarks	A glass pycnometer with a nominal volume of 10 ml was used for analogue B at 40.1% concentration. The temperature of measurement was 20.0 °C.
Test Facility	Notox B.V. (2006b)

Vapour Pressure 2.758 kPa at 20 °C

Method	OECD TG 104 Vapour Pressure European Economic Community (EEC), EEC-Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.4 Vapour Pressure
Remarks	Static technique method for analogue B at 40.1% concentration
Test Facility	Notox B.V. (2006c)

Water Solubility 4.9 g/L at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks	Test concentration of 0.5 g/L to 50 g/L. The water solubility expressed by the critical micelle concentration.
Test Facility	Dr U Noack-Laboratorien (2015a)

Partition Coefficient (n-octanol/water) log P_{ow} = -3.3 at 20 °C and pH = 7.1

Method	OECD TG 107 Partition Coefficient (n-octanol/water). EEC directive 92/69 A.8 Partition Coefficient.
Remarks	Flask Method
Test Facility	NOTOX B.V. (2006d)

Surface Tension 26.32 to 42.82 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: 1.11 g/L
Test Facility	Dr U Noack-Laboratorien (2015a)

Flash Point > 108 °C at 101.5 kPa

Method	European Economic Community (EEC), EEC-Directive 92/69 EEC, A.9 Flash Point
Remarks	A Pensky-Martens Closed Cup automatic flash-point tester was used for analogue B at 40.1% concentration. No flammable vapour/air mixture was produced up to the point where the test substance started to boil at 108 °C.
Test Facility	Notox B.V. (2006e)

Flammability Not flammable

Method	European Economic Community (EEC), EEC directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.12 "Flammability (contact with water)
Remarks	Based on the composition of the test substance (analogue B at 40.1% concentration), the molecular structure of the main component and the presence of water (50%) in the test substance. It was concluded that analogue B is incapable of developing a dangerous amount of (flammable) gas in contact with air, damp air or water.
Test Facility	Notox B.V. (2006f)

Pyrophoric Properties Not pyrophoric

Method	European Economic Community (EEC), EEC directive 92/69 EEC, A.13 Pyrophoric properties of solids and liquids.
Remarks	Based on the structure, experience in handling and the presence of water in the test substance the study authors determined that analogue B is not pyrophoric.
Test Facility	Notox B.V. (2006g)

Autoignition Temperature 425 °C

Method	European Economic Community (EEC), EEC directive 92/69 EEC, A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks	The temperature in the test vessel containing analogue B at 40.1% concentration was lowered gradually from a sufficiently high temperature until a temperature at which there was no more ignition occurred.
Test Facility	Notox B.V. (2006h)

Explosive Properties Not explosive

Method	European Economic Community (EEC), EEC-Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.14 Explosive Properties.
Remarks	Based on the composition of the test substance analogue B (40.1%), the molecular structure of the major component and the presence of water (56.1%), it was concluded that the substance is not explosive
Test Facility	Notox B.V. (2006i)

Oxidizing Properties Not oxidising

Method	European Community (EC), Commission directive 2004/73/EC, Part A, Methods for the determination of physico-chemical properties, A.21: "Oxidizing properties (liquids)"
Remarks	Based on the composition of the test substance analogue B at 40.1% concentration and the molecular structures of both the major component and the presence of water (56.1%), it was concluded that the test substance has no oxidising properties.
Test Facility	Notox B.V. (2006j)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Analogue B (38.2%)
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/White Wistar
Vehicle	The test substance administered as supplied /tap water
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	6F	300	0/6
2	6F	2000	0/6

LD50	> 2000 mg/kg bw
Signs of Toxicity	No acute oral toxicity was observed after administration of 300 and 2000 mg/kg bw of the test substance
Effects in Organs	The necropsy results showed no treatment related morphological pathological findings. Average body weight gain in both animal groups was 19.6%
Remarks - Results	No death was observed in both animal groups
CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	Stockhausen (2005a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Analogue B (40.1%)
METHOD	OECD TG 402 Acute Dermal Toxicity EC, Council Directive 67/548/EEC, Annex V, B.3 (1992) "Acute Toxicity (Dermal)"
Species/Strain	Rat/Wistar Crl:WI
Vehicle	None
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations. The test substance was administered to each animal of both sexes by a single dermal application at 2000 mg/kg bw for 24 hours.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No abnormalities were found at macroscopic post mortem examination of the animals. However, chromodacryorrhea (bloody tears) was noted among four animals on days 1 and 2. Scales were seen in the treated skin-area of four animals between days 3 and 12.
Signs of Toxicity - Systemic	No sign of systemic toxicity effects was observed.
Effects in Organs	The body weights gain was within the range expected for rats used in this type of study during the observation period.
Remarks - Results	No death was observed in both animal groups

CONCLUSION The test substance is of low acute toxicity via the dermal route.

TEST FACILITY NOTOX (2006k)

B.3. Irritation – skin

TEST SUBSTANCE Analogue B (38.2%)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)
EC Directive 2004/10/EC B.4 Acute Toxicity (Skin Irritation)

Species/Strain	Rabbit/New Zealand White
Number of Animals	1Male & 2Females
Vehicle	None
Observation Period	7 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	0	0	0	1	< 24h	0
Oedema	0	0	0	0	7days	0

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

Remarks - Results Slight erythema but no oedema was observed in one animal 1 h after removal of the test substance. No erythema and oedema were observed between 24 and 72 hours.

No systemic toxicity was observed during the study.

Body weight gain was positive and within the normal range.

CONCLUSION The test substance is slightly irritating to the skin.

TEST FACILITY Stockhausen (2005b)

B.4. Irritation – eye (*in vitro)* (Isolated Chicken Eye Test)

TEST SUBSTANCE Chemical 1 in Evonik and Redox cleaning products

METHOD Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants OECD TG 438 (ICE) Test

Vehicle	Saline
Remarks - Method	Three main parameters were measured to disclose possible adverse effects: corneal thickness (expressed as corneal swelling relative to control values), corneal opacity and fluorescein retention of damaged epithelial cells. In addition, histopathology of the corneas was performed.

RESULTS

Test material	***Mean Corneal Thickness*** *Swelling % (Time in min)*	***Mean Corneal opacity*** *(min)*	***Mean Fluorescein Retention*** *(min)*
Negative control	0	0(30), 0(75), 0(120), 0(180), 0(240)	0(30)
Test substance	6(30), 9(75), 9(120), 10(180), 14(240)	1.8(30), 2.0(75), 2.0(120), 2.3(180), 2.3(240)	2.2(30)

Positive control	41(30), 43(75), 46(120), 47(180), 47(240)	4.0(30), 4.0(75), 4.0(120), 4.0(180), 4.0(240)	3(30)

OD = optical density

Remarks - Results

After administration of the test substance to isolated chicken eyes, a small lump of the test substance adhered to the cornea of one eye. After approximately 5 minutes, the eye was rinsed with saline. Mean percentage of corneal swelling was calculated for all observation time points. The test substance caused:

- a slight corneal swelling (14%);
- moderate to severe opacity (2.3); and
- moderate or moderate to severe fluorescein retention (2.2)

Based on the highest mean score of 14% ICE class II is applicable for the test substance.

Based on the highest mean score for corneal opacity (2.3) and fluorescein retention (2.2), ICE class III is applicable for the test substance.

The prolonged exposure to the test substance showed severe opacity and moderate to severe fluorescein retention.

Based on 2 ICE class scores of III and ICE class score of II, the eye irritation hazard classification for the test substance cannot be predicted according to the Test Guideline (TG). However, it is not a Cat. 1 eye irritant or a non-irritant.

Negative control eye did not show any corneal effects demonstrating that the general conditions during the test were adequate.

The positive control (NaOH) caused severe corneal effects demonstrating the ICE test valid to detect severe eye irritants.

Microscopic examination of the treated cornea with the test substance showed very slight erosion of the epithelium. The treated cornea with negative control showed no abnormalities and the treated cornea with the positive control caused severe erosion of the epithelium, severe necrosis of the stroma and necrosis of the endothelium.

CONCLUSION

The test substance was considered to be irritating to the eye under the conditions of the test.

TEST FACILITY

TNO Triskelion (2014)

B.5. Skin sensitisation - Magnusson and Kligman Test

TEST SUBSTANCE

Analogue B (38.2%)

METHOD

OECD TG 406 Skin Sensitisation - Magnusson and Kligman test
EC Directive 2004/10/EC Skin Sensitisation

Species/Strain

Guinea pig/ Pirbright White, BOR DHPW

PRELIMINARY STUDY

Maximum Non-irritating Concentration: 70% (w/w)
intradermal: 0.5% of 2% (no skin reaction); 3.5% and 5% (elicited a slight reaction).
topical: 50% provoked a slight reaction and 30% showed no reaction

MAIN STUDY

Number of Animals

Test Group: 10 Control Group: 5

Vehicle

0.9% NaCl solution

Positive control

Conducted in parallel with the test substance

INDUCTION PHASE	Induction Concentration: intradermal: 0.1 mL of 3.5% (w/w) topical: 30%
Signs of Irritation	Intradermal application of the 3.5 % caused slight to moderate erythema and oedema formation. The topical application of 50% of the test substance caused slight erythema and oedema formation.
CHALLENGE PHASE	
challenge	topical: 30% (The challenge application was carried out on control and test group animals 21 days after the dermal application).
Remarks - Method	No significant protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: challenge*	
		24 h	*48 h*
Test Group			
10 F	30%	0/10	0/10
Control Group			
5F	Nil	0/5	0/5

Remarks - Results	The challenge application of the test substance at a concentration of 30% did not cause any irritation in test animals. A formation of crusts at the injection sites of the test substance was observed from the intradermal application (3.5%).
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	Stockhausen (2005c)

B.6. Skin sensitisation - Magnusson and Kligman Test

TEST SUBSTANCE	Analogue C
METHOD	OECD TG 406 Skin Sensitisation - Magnusson and Kligman Test
Species/Strain	Guinea pig/ Pirbright White Bor: DHPW (SPF)
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 1% topical: 30%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 20
Vehicle	Water
Positive control	Conducted in parallel with the test substance
INDUCTION PHASE	Induction Concentration: intradermal: Not specified topical: Saturated solution in Freund's complete adjuvant
Signs of Irritation	No record of effects following induction were included in the study report.
CHALLENGE PHASE	
challenge	topical: 30%
Remarks - Method	No significant protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: challenge*	
		24 h	*48 h*
Test Group			
10M, 10F	30%	0/20	4/20

Control Group			
10M, 10F	30%	0/20	5/20

Remarks - Results: Preliminary Study (range finding test):
intradermal: 0.1, 0.5% (no skin reaction), 1% (immoderate erythema), and 5% (elicited a black discolouration of the injection sites).
topical: 5% did not produce a skin reaction or excessive inflammation (Occlusive patch).
Main study:
At the 48 hour observation 4 female animals in the test group, and 5 female animals in the control group showed slight irritation.

CONCLUSION: There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.

TEST FACILITY: IBR (1990)

B.7. Skin sensitisation – Magnusson and Kligman Test

TEST SUBSTANCE: Analogue C

METHOD: Similar to OECD TG 406 Skin Sensitisation - Magnusson and Kligman Test

Species/Strain: Guinea-Pig/ Pirbright white
PRELIMINARY STUDY: Maximum Non-irritating Concentration: Not reported
MAIN STUDY
Number of Animals: Test Group: 15 Control Group: 5
Vehicle: Water
Positive control: Not conducted in parallel with the test substance.
INDUCTION PHASE: Induction Concentration:
intradermal: 0.5%
topical: 60%
Signs of Irritation: Temporary and slight dermal irritation was observed following both the intradermal and topical applications.
CHALLENGE PHASE
1st challenge: topical: 10%
Remarks - Method: The control animals were inducted similarly to the test animals omitting the intradermal injections and topical application.

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: challenge*		
		24 h	*48 h*	*72 h*
Test Group				
15M	10%	2/15	0/15	0/15
Control Group				
5M	10%	0/5	0/5	0/5

Remarks - Results: Dermal reactions were observed in two treated animals after 24h hour observation after challenge. Dryness and sloughing of the epidermis was observed in two control animals and one test animal after 72 hours of the challenge application.

CONCLUSION: There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.

TEST FACILITY: HRC (1980)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Chemical 1 in Evonik and Redox cleaning products
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents EC Directive No. 440/2008 B7 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rats/ Sprague Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days Recovery Period
Vehicle	Purified water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	5M, 5F	0	0/10
low dose	5M, 5F	100	0/10
mid dose	5M, 5F	300	0/10
high dose	5M, 5F	500	0/10
control recovery	5M, 5F	0	0/10
high dose recovery	5M, 5F	500	0/10

Mortality and Time to Death
No mortality was observed during the period of the test.

Clinical Observations
No test substance-related, findings were observed with regard to body weight parameters (food consumption and body weight gain) at all dose levels during the study. No toxicological relevant changes were observed in clinical signs or the neurotoxicity assessment and motor activity measurements.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Lymphocytosis was observed in one female dosed at 300 mg/kg bw/day and three females dosed at 500 mg/kg bw/day. Monocytosis was observed in one female dosed at 300 mg/kg bw/day and two females dosed at 500 mg/kg bw/day. Individual changes were between 61% and 93% for lymphocytes and 134% and 150% for monocytes when compared with control values. After the recovery period
Lymphocytosis and neutrophilia were observed in one female after the recovery period. These changes were of 49% and 3.7 fold, respectively when compared to mean control data. Other treated animals showed complete reversibility of lymphocyte counts to normal and monocytes data were comparable with controls.

There were no toxicologically relevant changes to the clinical chemistry parameters that were measured.

Effects in Organs
No differences were reported in terminal body and organ weights between treated and control animals and no treatment-related changes were noted at macroscopic and microscopic observations.

Remarks – Results
No mortality occurred. No clinical signs or treatment related effects in neurotoxicity assessment and no changes in body weight and food consumption were noted.

The lymphocytosis and monocytosis seen in female animals dosed at 300 and/or 500 mg/kg bw/day showed reversibility at the end of the recovery period or comparability to control data.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 500 mg/kg bw/day.

TEST FACILITY RTC (2016)

B.9. Genotoxicity – Bacterial Reverse Mutation Test

TEST SUBSTANCE	Chemical 1 in Evonik and Redox cleaning products
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Council Regulation (EC) No 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria Plate incorporation procedure and Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, and *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Phenobarbital/β-Naphthoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 3-5,000 µg/plate b) Without metabolic activation: 3-5,000 µg/plate
Vehicle	Deionised water
Remarks - Method	No significant deviations from the OECD guidelines. The pre-experiment is reported as main experiment 1, since the criteria are met (Evaluable plates (> 0 colonies) at five concentrations or more in all strains used).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5000 (except TA100 ≥ 5000)			
Test 1		≥ 5000	> 5000	Negative
Test 2		≥ 5000)	> 5000	Negative
Present	> 5000			
Test 1		> 5000	> 5000	Negative
Test 2		≥ 5000)	> 5000	Negative

Remarks - Results	The plates incubated with the test item showed normal background growth up to 5000 µg/plate with or without S9 mix in all strains tested. Toxic effects, as a reduction in the number of revertants (below the indication factor of 0.5), were absent in nearly all strains, except in strain TA 100 where toxic effects were observed at 5000 µg/plate without S9 mix in experiment 1 and with or without S9 mix in experiment 2 No precipitation of the test item occurred up to the highest investigated dose. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. The positive controls showed a significant increase of induced revertant colonies, thus confirming the integrity of S9-mix and the test system.
CONCLUSION	The notified chemical (Chemical 1) was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Harlan (2014)

B.10. Genotoxicity – *in vitro* - Mammalian Chromosome Aberration

TEST SUBSTANCE	Analogue B (38.2%)
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	Chinese hamster
Cell Type/Cell Line	V79 cells
Metabolic Activation System	S9 fraction from Phenobarbital/β-Naphthoflavone induced rat liver.
Vehicle	Cell culture medium
Remarks - Method	No significant deviations from the OECD guidelines were noted. The treatment interval was 4 hours with and without metabolic activation. Two parallel cultures were set up. 100 metaphases per culture were scored for structural chromosomal aberrations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 250, 500, 1000*, 2500*, 5000*	4 h	20 h
Test 2	0*, 125, 250, 500, 1000*, 2500*, 5000*	4 h	20 h
Present			
Test 1	0*, 250, 500, 1000*, 2500*, 5000*	4 h	20 h
Test 2	0*, 1000*, 2000*, 3000*, 4000*, 5000*	4 h	20 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	> 5000	> 5000	> 5000	Negative
Test 2		> 5000	> 5000	Negative
Present				
Test 1	> 5000	> 5000	> 5000	Negative
Test 2		> 5000	> 5000	Negative

Remarks - Results	In experiment 1 and 2 no precipitate of the test item was noted in all dose group and no toxic effects of the test item were noted up to the highest dose group. No biologically significant decrease of mitotic cells (relative mitotic index below 70%) was noted in all dose groups evaluated with and without metabolic activation compared to the vehicle control. No increase in the frequencies of polyploid cells was found after treatment with the test item as compared to the control groups. The positive controls showed a significant increase in number of chromosome aberrations confirming the integrity of S9-mix and the test system.
CONCLUSION	The test substance was not clastogenic to Chinese hamster V79 cells treated *in vitro* under the conditions of the test.
TEST FACILITY	BSL BIOSERVICE (2005)

B.11. Genotoxicity – *in vitro* Cell Mutation Test at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells

TEST SUBSTANCE	Analogue D
METHOD	OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test EC Directive No. 440/2008 B.17: Mutagenicity – *In vitro* Mammalian Cell Gene Mutation Test
Species/Strain	Mouse Lymphoma L5178Y Cells
Metabolic Activation System	S9 fraction from Phenobarbital/β-Naphthoflavone induced rat liver.
Vehicle	Deionised water
Remarks - Method	No significant deviations from protocols. A test item is classified as mutagenic if the induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control. Experiment 1 was performed with and without liver microsomal activation and a treatment period of 4 h. Experiment 2 was performed with a treatment period of 24 hours in the absence of metabolic activation and 4 hours in the presence of metabolic activation. The highest applied concentration in the pre-experiment (5000 µg/mL) was limited by the solubility properties of the test item in aqueous medium. The concentration range of the main experiments was limited by the cytotoxic potential of the test item.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	2.4*; 4.9*; 9.8*; 19.5*; 39.0*, 58.5, 78.0	4 h	48 h	52 h
Test 2	2.5, 5.0, 10*; 20*; 40*; 50*; and 60*	24 h	48 h	72 h
Present				
Test 1	4.9*; 9.8*; 19.5*; 39.0*; 78.0*, 117.0, 156.0	4 h	48 h	52 h
Test 2	10, 20, 40*; 80*; 100*; 110*; and 120*	4 h	48 h	52 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	≥ 78.1	≥ 19.5	> 5000	Negative
Test 2	≥ 39.1	≥ 40	> 5000	Negative
Present				
Test 1	≥ 312.5	≥ 117.0	> 5000	Negative
Test 2		≥ 40	> 5000	Negative

RESULTS	No substantial and reproducible dose dependent increase in mutant colony numbers was observed in both experiments 1 and 2. The positive controls showed a significant increase in number of chromosome aberrations confirming the integrity of S9-mix and the test system.
Doses Producing Toxicity	Relevant cytotoxic effects indicated by a relative total growth of less than 50% in both parallel cultures were observed in the absence of metabolic activation at 39 µg /mL in experiment 1 following 4 hour treatment and at 40 µg /mL and above in experiment 2 following 24 hours treatment.

	In the presence of metabolic activation: No reproducible cytotoxic effects were noted in experiment 1 and ≥ 100 µg/mL in experiment 2. The isolated minor reduction of the relative total growth to 43.5 % in the first culture of experiment 1 with metabolic activation was not considered a toxic effect since no comparable reduction was observed in the parallel culture under identical conditions.
Genotoxic Effects	The mutation frequency did not reproducibly reach or exceed the threshold of 126 above the mutation frequency of the corresponding solvent control in any of the experimental parts.
Remarks - Results	The positive controls showed a significant increase in number of mutant colonies confirming the integrity of S9-mix and the test system.
CONCLUSION	The test substance was not clastogenic under the conditions of this *in vitro* Mammalian Cell Gene Mutation Test.
TEST FACILITY	Harlan CCR (2009)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Chemical 1
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Activated sludge from a local STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 by titrimetric method
Remarks - Method	No significant deviations from the test guidelines were reported. The test item was added directly to the test vessels. A toxicity control was run.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	35	6	47
14	68	14	68
21	86	21	74
28	90	28	90

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium acetate surpassed the threshold level. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days was 90%.
CONCLUSION	The test substance is readily biodegradable.
TEST FACILITY	Dr U Noack-Laboratorien (2005)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Chemical 1
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static
Species	*Danio rerio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	231 mg $CaCO_3$/L
Analytical Monitoring	Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS)
Remarks – Method	No significant deviations from the test guidelines were reported. The test media was renewed daily. The test concentration was measured at 0 h, 24 h, 72 h and 96 h.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*
Nominal	*Actual*		*96 h*
Control	< LOQ*	7	0
289	313	7	0

*LOQ: Limit of Quantification of 100µg/L

LC50	> 289 mg/L at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The measured values of the test substance were in the range of 99 to 108% of the nominal values. During the test, the dissolved oxygen was ≥ 84%.
CONCLUSION	The test substance is not harmful to fish.
TEST FACILITY	Dr U Noack-Laboratorien (2015b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Analogue C
METHOD	EC Council Regulation 92/96/EEC C.2 Acute Toxicity for Daphnia - Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	Total organic carbon (TOC) was analysed by TOC-500 analyser
Remarks - Method	No significant deviations from the test guidelines were reported. The test solutions were prepared from the dilution of a stock solution. A positive control (K_2CrO_7) was run less than one month prior to the current study.

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*Immobilised (%)*	
		24 h	*48 h*
Control	19	0	0
3.5	20	15	20
6	20	10	20
10	20	90	100
17	21	67	76
30	20	55	75

LC50	7.8 mg/L (95% CL: 4.7 – 13 mg/L) at 48 hours (calculated by probit analysis).
Remarks - Results	All validity criteria for the test were satisfied. During the test, the dissolved oxygen concentration was ≥ 7.4 mg/L at 20 °C (≥ 81 % saturation; USGS 2011). An LC100 of 2 mg/L was calculated for the positive control and this was regarded to sufficiently demonstrate the sensitivity of the test species. The TOC results indicate that the test substance was stable during the test and nominal concentration were used in the endpoint calculation. The increase in percentage immobilisation was not a monotonic response with respect to concentration.
CONCLUSION	The test substance is toxic to aquatic invertebrates.
TEST FACILITY	Huls (1996)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Analogue C
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation 92/69/EC C.3 Algal Inhibition Test
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 3.13, 6.25, 12.5, 25, 50, 100 mg/L

	Actual at t = 0 h: 2.51, 5.18, 10.8, 23.8, 44.9, 101 mg/L
Auxiliary Solvent	None
Water Hardness	0.24 mmol Ca+Mg/L
Analytical Monitoring	Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS)
Remarks - Method	No significant deviations from the test guidelines were reported. The test concentration was measured at 0 h and 72 h.

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
17.2 (95% CL: 16.4 – 17.9)	6.25	31.9 (95% CL: 30.2 – 33.7)	12.5

Remarks - Results	All validity criteria for the test were satisfied. The mean cell density in the control increased by 98 times. At the start of the test, the measured values of the test substance were in the range of 80 to 101% of the nominal values. At the end of the test, they were in the range of 68 to 84%.

CONCLUSION The test substance is harmful to alga.

TEST FACILITY Dr U Noack-Laboratorien (2006)

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