File No: STD/1640 and STD/1641

August 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

STD/1640: Chemical in DP-HS-1008 Hi-Oxime

STD/1641: Chemical in DP-OMC-1045 Hi-Oxime

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/1640 STD/1641	BASF Australia Ltd	Chemical in DP-HS-1008 Hi-Oxime Chemical in DP-OMC-1045 Hi-Oxime	Yes	< 50 tonnes per annum	Reagent for solvent extraction of metals

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*
Flammable liquid (Category 4)	H227 - Combustible liquid
Skin corrosion / irritation (Category 2)	H315 - Causes skin irritation
Skin sensitisation (Category 1)	H317 - May cause an allergic skin reaction
Toxic to Reproduction (Category 1B)	H360 - May damage fertility or the unborn child

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*
Chronic Category 2	H411 – Toxic to aquatic life with long lasting effects

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
Based on the reported use pattern, 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:
  - Skin corrosion / irritation (Category 2): H315 - Causes skin irritation
  - Skin sensitisation (Category 1): H317 - May cause an allergic skin reaction
  - Toxic to Reproduction (Category 1B): H360 - May damage fertility or the unborn child

The above should be used for products/mixtures containing the notified chemicals, if applicable, based on the concentration of the notified 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 notified chemicals as introduced and in the formulated product:
  - Automated and closed systems for solvent metal extraction

- 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 as introduced and in the formulated product, and as residuals in the electrowinning process:
  - Avoid contact with skin and eyes
  - Avoid inhalation of mists or vapours
  - Clean up spills promptly

- 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 as introduced and in the formulated product:
  - Gloves
  - Goggles
  - Protective clothing
  - 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.

Storage

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

Transport and Packaging

- Due to the hazardous properties of the notified chemicals, introducers of the chemicals should consider their obligations under *Australian Code for the Transport of Dangerous Goods by Road and Rail* (ADG code) (NTC, 2017).

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
  - Metal extraction using the notified chemicals is proposed to be carried out in other than a closed system

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemicals have changed from reagent for solvent extraction of metals, 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 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 products containing 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)
BASF Australia Ltd (ABN: 62 008 437 867)
Level 12, 28 Freshwater Place
SOUTHBANK VIC 3006

NOTIFICATION CATEGORY
STD/1640 - Standard: Chemical other than polymer (more than 1 tonne per year)
STD/1641 - Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Chemical is being notified at the same time as a similar chemical.

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, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:

STD/1640 - Hydrolysis as a Function of pH, Adsorption/Desorption, Dissociation Constant, Autoignition Temperature, Flammability Limits, Explosive Properties, Oxidising Properties, Acute Dermal Toxicity, Acute Inhalation Toxicity, Repeated Dose Toxicity, Genotoxic Damage, Chromosome Damage, Ready Biodegradability, Bioaccumulation, Acute Algal Toxicity.

STD/1641 - Hydrolysis as a Function of pH, Adsorption/Desorption, Dissociation Constant, Flammability Limits, Autoignition Temperature, Explosive Properties, Oxidising Properties, Acute Dermal Toxicity, Acute Inhalation Toxicity, Repeated Dose Toxicity, Genotoxic Damage, Chromosome Damage, Bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
STD/1640 - Chemical in DP-HS-1008 Hi-Oxime
STD/1641 - Chemical in DP-OMC-1045 Hi-Oxime

MOLECULAR WEIGHT
STD/1640 - Value for the notified chemical < 500 g/mol
STD/1641 - Value for the notified chemical < 500 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa:
STD/1640 - Amber liquid to waxy solid with faint specific odour
STD/1641 - Amber liquid with mineral oil like odour

Property	Value		Data Source/Justification	
	STD/1640	*STD/1641*	*STD/1640*	*STD/1641*
Freezing Point	-14°C	-25°C	Measured	Measured
Boiling Point	Not able to be determined	Not able to be determined	Measured	Measured
Density	1011 kg/m^3 at 20°C	1014 kg/m^3 at 20°C	Measured	Measured

Property	Value		Data Source/Justification	
	STD/1640	*STD/1641*	*STD/1640*	*STD/1641*
Vapour Pressure	$< 1 \times 10^{-6}$ hPa at 25°C	< 1 hPa at 20°C	Measured	SDS
Water Solubility	0.073 ± 0.002 mg/L at 20 °C	< 0.07 mg/L at 20 °C	Measured	Measured
Hydrolysis as a Function of pH	Not determined	Not determined	The notified chemicals contain hydrolysable functionalities. These were found to slowly hydrolyse in acidic conditions.	
Partition Coefficient (n-octanol/water)	log Pow ≥ 7.1 at 20 °C	log Pow ≥ 7.1 at 20 °C	Measured*	Measured*
Adsorption/Desorption	Not determined.	Not determined	Expected to adsorb to soil and sludge based on the notified log Pow.	
Dissociation Constant	Not determined	Not determined	Contain dissociable functionalities, which are expected to remain mainly in their unionised state in the environmentally relevant pH range (4 – 9).	
Flash Point	75°C	65.5°C	Measured	Measured
Flammability Limits	Flammable	Flammable	SDS	SDS
Autoignition Temperature	Not determined	Not flammable	-	SDS
Explosive Properties	Not explosive	Not explosive	Contain no functional groups that would imply explosive properties.	
Oxidising Properties	Not oxidising	Not oxidising	Contain no functional groups that would imply oxidising properties.	

* The notified chemical is surface active and Pow measures are not truly relevant to surface active substances.

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

Reactivity
The notified chemicals are expected to be stable under normal conditions of use, but may slowly hydrolyse during use. Both chemicals should be kept away from sources of heat and electro-static discharge, while the chemical in DP-OMC-1045 Hi-Oxime (STD/1641) should not come in contact with strong acids, bases and oxidizing agents.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, 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***
Flammable liquid (Category 4)	H227 - Combustible liquid

4. 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 products containing the notified chemicals at < 90% concentration will be imported in 1000 L intermediate bulk containers (IBC) by sea.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 50	< 50	< 50	< 50	< 50

PORT OF ENTRY
Adelaide

IDENTITY OF RECIPIENT
BASF Australia Ltd
Level 12, 28 Freshwater Place
SOUTHBANK VIC 3006

TRANSPORTATION AND PACKAGING
The products containing the notified chemicals at < 90% concentration will be imported in 1000 L IBCs, as described above. The products containing the notified chemicals will be transported by road from the wharf and stored at the third party logistics warehouse. The material will be unloaded from the shipping container with the aid of a forklift and stored in the general chemical storage area until transported to customer sites. The sales product will be stored in a cool, dry area with adequate ventilation.

USE
The notified chemicals at up to 90% concentration will be used as organic reagents in a metal solvent extraction process as part of mineral processing. Solvent extraction occurs after the ore leaching process and prior to electrowinning of the metal. It is a continuous mix and separation process within the confines of a mixer-settler where solutions of the notified chemicals are pumped into the mixer and flow through the settler in an automatic process. The solvent extraction process for the purification of metals is usually located at the mines for primary concentration, however can also be used for secondary concentration of metals at smelters, depending on the feedstocks available for the notifier's customers.

OPERATION DESCRIPTION
The notified chemicals will not be manufactured in Australia, but will be imported as part of products at < 90%. Reformulation or repackaging will not occur. At the end-use site, the solution containing the notified chemicals at < 90% concentration will be transferred from the original packaging (IBCs) to on-site holding tanks using pumping equipment. Thereafter, all transfer processes will be fully automated and, in general, will use closed line systems. The solution containing the notified chemicals will be pumped into the process stream, where it will be used in a solvent extraction process in a series of tanks (i.e. a mixer-settler) in which the notified chemicals will be regularly recycled back into the process stream. The notified chemicals will be gradually lost into the process liquor, which will be sent to an on-site sludge dam and recycled through the sinter plant process stream.

Sampling during the metal extraction process is carried out with scoops attached to a wooden pole, which can pull samples out of the settler from a distance of 1-2 metres.

The notifier's customers in Australia are expected to use covered mixer-settlers to minimise evaporation of components of the product. The mixer-settlers may be located in buildings or outdoors.

Mixer-settlers are typically not cleaned *per se*, however, if there is gypsum precipitation or bottom CRUD formation these are removed by specialist contractors using fully enclosed chemical protection gear and breathing apparatus. Alternatively, a procedure known as a 'CRUD Float' can be used for floating of interfacial CRUD that requires no direct human contact.

The notified chemicals are not expected to be carried on to the electrowinning process, except in very small residual quantities. Any residual on the metal would be eventually decomposed as part of the purification of the metal at high temperatures.

Some degradation by hydrolysis of the notified chemicals may occur as part of use, resulting in the formation of lower molecular weight species in the mix, which may be hazardous. The notifier advised that the rate of hydrolysis of the notified chemicals is lower than that of other reagents for the same use.

5. HUMAN HEALTH IMPLICATIONS

5.1. Exposure Assessment

5.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	2 – 3	10 – 15
Plant operators	1	2 – 5
Maintenance workers	1	10 – 15
Supervisors	1	10 – 15

EXPOSURE DETAILS

Transport and Storage
Transport and storage workers are unlikely to be exposed to the notified chemicals. Dermal exposure may occur only in the case of accidental breach of the product packaging during unloading and transport to the customer sites or through spillage when the products are transferred from IBCs to on-site tanks.

End-use
Workers at the solvent extraction sites may be exposed to the notified chemicals at up to 90% concentration during transfer of the sales product from transport tanks to mixing tanks and during general maintenance operations, although exposure would be minimised by the largely automated processes and use of enclosed transfer lines and tanks.

It is expected that any worker exposure would be via the dermal and ocular routes. In the work scenarios described, inhalation exposure is not expected as the solvent extraction processes will be taking place in covered mixer-settlers, the notified chemicals are not deemed to be volatile, and there will be no flotation or air assisted processes which would generate mists in the solvent extraction processes. Dermal and ocular exposure to workers is expected to be minimised by the use of personal protective equipment (PPE), including coveralls, impervious gloves, eye protection and suitable respiratory protection (if ventilation is insufficient).

5.1.2. Public Exposure
The notified chemicals are intended for use in industrial settings by trained workers. The public may only be exposed to the notified chemicals in the unlikely event of an accident spillage during transport. Therefore, when used in the proposed manner, public exposure to the notified chemicals is not expected.

5.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemicals and on two acceptable analogues are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Test Method*	*Substance*	*Result and Assessment Conclusion*
Rat, acute oral toxicity	OECD TG 423	STD/1640	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute oral toxicity	OECD TG 423	STD/1641	LD50 > 5000 mg/kg bw; low toxicity
Rat, acute oral toxicity	OECD TG 423	STD/1641	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	in house method	Analogue 1	LD50 > 8410 mg/kg bw; low toxicity
Rat, acute dermal toxicity	in house method	Analogue 3	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation (*in vitro*)	OECD TG 431 & OECD TG 439	STD/1640	Irritating
Skin irritation (*in vitro*)	OECD TG 431 & OECD TG 439	STD/1641	Irritating
Rabbit, skin irritation	OECD TG 404	STD/1641	Irritating
Eye irritation (*in vitro*) Bovine Corneal Opacity and Permeability Test (BCOP Test) and EpiOcular Eye Irritation Test	OECD TG 437	STD/1640	non-irritating

Endpoint	*Test Method*	*Substance*	*Result and Assessment Conclusion*
Eye irritation (*in vitro*) Bovine Corneal Opacity and Permeability Test (BCOP Test) and EpiOcular Eye Irritation Test	OECD TG 437	STD/1641	non-irritating
Rabbit, eye irritation	OECD TG 405	STD/1641	slightly irritating
In Chemico Skin Sensitisation (DPRA Test.)	OECD TG 442C	STD/1640	inconclusive
In Vitro Skin Sensitisation (ARE-Nrf2 Luciferase Test.)	OECD TG 442D	STD/1640	evidence of sensitisation
In Vitro Skin Sensitisation (h-CLAT Test.)	OECD TG 442E	STD/1640	evidence of sensitisation
In Chemico Skin Sensitisation (DPRA Test.)	OECD TG 442C	STD/1641	inconclusive
In Vitro Skin Sensitisation (ARE-Nrf2 Luciferase Test.)	OECD TG 442D	STD/1641	evidence of sensitisation
In Vitro Skin Sensitisation (h-CLAT Test.)	OECD TG 442E	STD/1641	evidence of sensitisation
Rat, repeat dose Oral toxicity – 90 day	OECD TG 408	Analogue 1	NOAEL = 150 mg/kg bw/d
Mutagenicity – bacterial reverse mutation	OECD TG 471	STD/1640	non mutagenic
Mutagenicity – bacterial reverse mutation	OECD TG 471	STD/1641	non mutagenic
Genotoxicity – *in vitro* Gene Mutation HPRT	OECD TG 476	Analogue 1	non genotoxic
Genotoxicity – *in vitro* Gene Mutation HPRT	OECD TG 476	Analogue 3	non genotoxic
Genotoxicity – *in vitro* Chromosome Aberrations	OECD TG 473	Analogue 3	non-clastogenic
Rat, reproductive and developmental toxicity (Oral/Gavage)	OECD TG 421	Analogue 1	NOAEL = 30 mg/kg bw/d for developmental toxicity NOAEL = 100 mg/kg bw/d for general, systemic toxicity No NOAEL was established for reproductive toxicity

Toxicokinetics, metabolism and distribution

No toxicokinetics, metabolism or distribution studies were provided on the notified chemicals. For dermal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption. In addition absorption is likely to be low if the water solubility is below 1mg/L (ECHA, 2014). The absorption of the notified chemicals across biological membranes is likely to be limited by their moderate molecular weights (< 500 g/mol) and low water solubility (< 0.07 mg/L at 20°C). (However, dermal absorption may be enhanced by the irritating properties of the notified chemicals).

Acute toxicity

The notified chemicals were of low acute oral toxicity in rats. Acute dermal toxicity studies in New Zealand White rabbits using analogues indicated that the notified chemicals are of low acute dermal toxicity. No information is available on acute inhalation toxicity.

Irritation and sensitisation

Several skin irritation studies were carried out on the notified chemicals, both *in vitro* and an *in vivo* (in rabbits). All studies indicated that the notified chemicals meet the criteria for classification as skin irritants.

Eye irritation studies *in vitro* and an *in vivo* (in rabbits) indicated that the notified chemicals have some irritation potential, but do not meet the criteria for classification as eye irritants.

A battery of tests consisting of one *in chemico* and two *in vitro* cell based assays were conducted on each of the two notified chemicals to evaluate their skin sensitisation potential. The tests are part of an Integrated Approach

to Testing and Assessment (IATA) which address specific events on the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation.

The notified chemicals showed minimal chemical reactivity in the *in chemico* Direct Peptide Reactivity Assay (DPRA) which addresses first key event, the covalent binding of electrophilic chemical to nucleophilic centres in skin proteins. However, due to limited solubility of the test substances and the subsequent formation of emulsions, the results could be under-predictive and were considered inconclusive. Both of the notified chemicals gave positive results in the studies that measure the second key event (ARE-Nrf2 Luciferase Assay) and the third key event (*in vitro* h-CLAT assay). On the basis of the study results the notified chemicals are considered to be skin sensitisers.

Repeated dose toxicity
No repeated dose oral, dermal or inhalation studies were available for the notified chemicals. A 90-day repeated dose oral (gavage) toxicity study in rats has been carried out on an analogue according to OECD TG 408. Ten animals per sex per group were treated with the test substance at 0, 15, 50 and 150 mg/kg bw/day and a satellite/recovery test at 150 mg/kg bw/day. No mortalities or signs of substance-related toxic effects were observed. However, a statistically significant decrease in body weights in females at mid-dose group (50 mg/kg bw/day) and high-dose group (150 mg/kg bw/day) in week 13 and 10-13, respectively when compared to vehicle control were reported. A significant decrease in body weights of males was observed in the satellite high dose (150 mg/kg bw/day) from week 9-13 as compared to satellite vehicle control group. This effect was not observed in main dose groups and therefore, was not considered to be of toxicological significance by the study authors. The No Observed Adverse Effect Level (NOAEL) was established as 150 mg/kg bw/day by the study authors.

Mutagenicity/Genotoxicity
The notified chemicals were not mutagenic in bacterial reverse mutation studies. Neither of two analogues were genotoxic when tested in an *in vitro* gene mutation test using Chinese Hamster Ovary (CHO) cells. However there were some increases in the mutant frequency in one of the gene mutation tests that were discounted by the study authors, as they were not reproducible and may have been related to a low value for the solvent control. An analogue chemical was not clastogenic in an *in vitro* chromosome aberration test. Overall the notified chemicals do not show a strong concern for mutagenicity/genotoxicity.

Toxicity for reproduction/developmental
No studies for reproduction/developmental toxicity were provided for the notified chemicals. A reproduction/developmental toxicity screening test in rats was conducted on an analogue. The NOAEL for systemic toxicity was established as 100 mg/kg bw/day in this study, based on adverse effects observed at 300 mg/kg bw/day (refer to the summary study in Appendix B). The NOAEL for developmental toxicity was established as 30 mg/kg bw/day, based on foetal and pup mortality at 100 mg/kg bw/day. A NOAEL for reproductive toxicity was not able to be established, as adverse effects in the male reproductive system were seen at all dose levels.

Degradants
The solvent extraction mixture may also contain degradants (hydrolysis products) of the notified chemicals, which may be hazardous.

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***
Skin corrosion / irritation (Category 2)	H315 - Causes skin irritation
Skin sensitisation (Category 1)	H317 - May cause an allergic skin reaction
Toxic to Reproduction (Category 1B)	H360 - May damage fertility or the unborn child.

5.3. Human Health Risk Characterisation

5.3.1. Occupational Health and Safety

Based on the available information, the notified chemicals present a concern for a skin irritation and sensitisation and reproductive/developmental toxicity. Hazardous degradants may also be present. Therefore, worker exposure to the notified chemicals should be prevented.

However, exposure to workers during end-use applications should be limited by the use of engineering controls such as enclosed and automated processes. Skin or eye contact with the notified chemicals or mixture containing the notified chemicals should be avoided. Inhalation of vapour, aerosol or mist should also be avoided. The risk to the health of workers is expected to be further mitigated by the proposed use of PPE including protective gloves, full face mask and coveralls, and respiratory equipment if inhalation exposure could occur.

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.

5.3.2. Public Health

The notified chemicals are only intended for use in industrial settings. Therefore, when used in the proposed manner, the risk to public health from the notified chemicals is not considered to be unreasonable.

6. ENVIRONMENTAL IMPLICATIONS

6.1. Environmental Exposure & Fate Assessment

6.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The chemicals will be imported into Australia and used as an extractant in mineral processing for metal refining. Manufacture, reformulation and repackaging, will not take place in Australia. Storage tanks and mixer-settler units will be located in a bunded area, and adsorbent material will be used to contain and collect any onsite spill.

RELEASE OF CHEMICAL FROM USE

Solvent (organic phase) extraction of metals is a continuous mix and separation process conducted in a closed system. The metal dissolved in sulfuric acid is selectively concentrated into the organic phase comprising a diluent such as high flash point kerosene and the notified chemicals used as extractants. The concentrated and purified metal is back extracted from the organic phase with aqueous sulfuric acid (with subsequent electrowinning) to form metal sheets. The notified chemicals will slowly hydrolyse in contact with aqueous sulfuric acid. Minimal amounts of residual notified chemicals entrained in the aqueous sulfuric acid stream may adhere to metal sheets. The notifier indicates that the metal sheets may be further processed including subsequent smelting at 1200°C, which would thermally decompose the notified chemicals.

RELEASE OF CHEMICAL FROM DISPOSAL

The notified chemicals are expected to degrade in use with no disposal required under normal operating conditions.

6.1.2. Environmental Fate

The notified chemicals are poorly to moderately biodegradable (ultimate biodegradability) but not bioaccumulative. They are expected to slowly degrade by abiotic and biotic means to carbon dioxide, water and oxides of nitrogen. Under conditions of use they are expected to hydrolyse to simpler organic compounds.

6.1.3. Predicted Environmental Concentration (PEC)

Under normal conditions of use aquatic release is expected to be minimal.

6.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemicals and acceptable analogues are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Substance*	*Assessment Conclusion*
Fish Toxicity	LC50 > 43.8 µg/L (measured concentration)	STD/1640	Not harmful to fish to the limit of water solubility

Endpoint	*Result*	*Substance*	*Assessment Conclusion*
	LC50 = 17 mg/L(nominal concentration)	STD/1641	
Daphnia Toxicity	EC50 > 0.0295mg/L (measured concentration)	STD/1640	Harmful to aquatic invertebrates with long lasting effects
	EC50 > 5mg/L (measured concentration)	STD/1640	
	NOEC 0.189 mg/L (measured concentration)	Analogue 4	
Algal Toxicity	ErC50 8.1 mg/L	STD/1641	Toxic to algae
Inhibition of Bacterial Respiration	EC50 > 1000 mg/L	Analogue 3	Not inhibitory to microbial respiration

The results from ecotoxicological investigations on the analogue chemical indicate that the notified chemicals are chronically toxic to aquatic life with long lasting effects. Therefore, the notified chemicals are formally classified as "Chronic category 2. - Toxic to aquatic life with long lasting effects" under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* for acute and chronic toxicities (United Nations, 2009).

6.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the most sensitive endpoint for aquatic invertebrates. A safety factor of 50 was used given acute ecotoxicological endpoints are available for three trophic levels, and chronic ecotoxicological endpoints are available for two trophic levels. The PNEC applies to both notified chemicals (STD 1640 and STD 1641).

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
NOEC (Daphnia 21 days)	0.189	mg/L
Assessment Factor	50	
Mitigation Factor	1.00	
PNEC:	3.78	µg/L

6.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) has not been calculated as, based on the reported use of the notified chemicals as part of a metal solvent extraction process, release to the aquatic environment is expected to be limited. Therefore, the notified chemicals are not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

STD/1640

Melting Point/Freezing Point -14°C (glass transition)

Method	OECD TG 102 Melting Point/Melting Range Differential scanning calorimetry
Remarks	No melting temperature was found between -100°C and 50°C. A reproducible glass transition was found at -14°C in the 1st heating.
Test Facility	BASF (2014a)

Boiling Point Could not be determined.

Method	OECD TG 103 Boiling Point Dynamic vapour pressure measurement
Remarks	At a forced pressure of 1 hPa and a temperature of approximately 160°C, continuously decreasing temperature was observed and a change of test item colour. This was indicative of a thermal change of the test item. Therefore, the normal boiling temperature could not be determined.
Test Facility	BASF (2015a)

Density 1011 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids Gas pycnometer method
Remarks	
Test Facility	BASF (2014b)

Vapour Pressure $< 1.0 \times 10^{-5}$ hPa at 25 °C

Method	OECD TG 104 Vapour Pressure Effusion method
Remarks	
Test Facility	BASF (2015b)

Water Solubility 0.073 ± 0.002 mg/L at 20 °C

Method	OECD TG 105 Water Solubility
Remarks	Modified Flask Method The HPLC column elution method could not be used because the substance is too viscous to be analysed by chromatography. A preliminary test was conducted by using a range of test item concentrations ranging from 10.29 to 10368 mg/L. No significant dissolution of test substance was observed in any test concentration. For the definitive test 125 mg/L was dissolved and stirred for up to 72 hours at 30°C. After a further 24 hours the samples were filtered (µm), dissolved in methanol and analysed by UV/VIS.
Test Facility	Competence Center Analytics BASF (2014-2015)

Partition Coefficient (n-octanol/water) log Pow ≥ 7.1 at 20 °C

Method	Variation of OECD TG 105 (flask method) (External method EM/00408/01, Competence Center Analytics, BASF)
Remarks	Single solubilities test in water and n-octanol. The water solubility of the test item is < 0.073 mg/L at 20 °C (see above). The test substance is too insoluble in water and too viscous to be conducted according to OECD TG 107 or 117. The log Pow value was hence estimated from the single solubilities in n-octanol and water. The test item and n-octanol were mixed in the ratios of 1:9, 1:1, and 9:1, and agitated on a roller mixer at room temperature overnight. The test item was miscible with n-octanol at all three ratios, ≡ 900g/1000mL.
Test Facility	Competence Center Analytics BASF

Flash Point 75°C

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	
Test Facility	Turner Laboratories (2017a)

Surface Tension 45 mN/m at 20°C

Method	OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Concentration: 90% saturation concentration Surface tension of purified water was used as a reference. The chemical was considered to be surface active.
Test Facility	BASF (2014c)

STD/1641

Melting Point/Freezing Point -25°C (glass transition)

Method	OECD TG 102 Melting Point/Melting Range Differential scanning calorimetry
Remarks	No melting temperature was found between -80°C and 50°C. A reproducible glass transition was found at -14°C in the 1st heating.
Test Facility	BASF (2014d)

Boiling Point Could not be determined.

Method	OECD TG 103 Boiling Point Dynamic vapour pressure measurement
Remarks	At a forced pressure of 9.95 hPa and a temperature of approximately 202°C, continuously increasing temperature was observed. This was indicative of a thermal change of the test item. Therefore, the normal boiling temperature could not be determined.
Test Facility	BASF (2014e)

Density 1014 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids Gas pycnometer method
Remarks	
Test Facility	BASF (2014f)

Vapour Pressure $< 1.5 \times 10^{-5}$ hPa at 25 °C

Method	OECD TG 104 Vapour Pressure Effusion method
Remarks	
Test Facility	BASF (2015c)

Water Solubility < 0.07 mg/L at 20 °C

Method	OECD TG 105 Water Solubility (External method EM/00408/01, Competence Center Analytics, BASF)
Remarks	Flask Method The HPLC column elution method could not be used because the substance is too viscous to be analysed by chromatography. A preliminary test was conducted by using a range of test item concentrations ranging from 100.4 to 9823 mg/L. No significant dissolution of test substance was observed in any test concentration. For the definitive test 500 mg/L was dissolved and stirred for up to 72 hours at 30°C. After a further 24 hours the samples were filtered (µm), dissolved in methanol and analysed by UV/VIS.
Test Facility	Competence Center Analytics BASF (2014-2015)

Partition Coefficient (n-octanol/water) log Pow ≥ 7.1 at 20 °C

Method	Variation of OECD TG 105 (flask method) (External method EM/00408/01, Competence Center Analytics, BASF)
Remarks	Single solubilities test in water and n-octanol. The water solubility of the test item is < 0.07 mg/L at 20 °C (see above). The test substance is too insoluble in water and too viscous to be conducted according to OECD TG 107 or 117. The log Pow value was hence estimated from the single solubilities in n-octanol and water. The test item and n-octanol were mixed in the ratios of 1:9, 1:1, and 9:1, and agitated on a roller mixer at room temperature overnight. The test item was miscible with n-octanol at all three ratios, ≡ 900g/1000mL.
Test Facility	Competence Center Analytics BASF

Flash Point 65.5°C

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	
Test Facility	Turner Laboratories (2017b)

Surface Tension 41 mN/m at 20°C

Method	OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Concentration: 90% saturation concentration Surface tension of purified water was used as a reference. The chemical was considered surface active.
Test Facility	BASF (2015d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral (STD/1640)

TEST SUBSTANCE	Notified chemical (STD/1640)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method, December 17, 2001 EC Council Regulation No 440/2008 B.1 Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/ Wistar strain Crl:WI (Han) SPF
Vehicle	Corn oil
Remarks - Method	Toxicity of the test item was tested by stepwise treatment of two groups of 3 female animals. Initially, three females were given a single dose of the chemical at 2000 mg/kg bw by oral gavage. No mortality occurred in the first group, 3 females in the second group were administered the chemical at 2000 mg/kg bw. The study was carried out according to GLP.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3F	2000	0/3
2	3F	2000	0/3

LD50	> 2000 mg/kg bw
Signs of Toxicity	No mortality occurred in both groups. The mean body weight of the animals in the first group increased within the normal range throughout the study period. In the second group all animals revealed a loss of body weight within the first three days after administration, which reached a normal level of increase in two of the animals on day 7. The third animal showed a stagnation of body weight on day 7. The body weight of all three animals increased within the normal range during the second week.
Effects in Organs	There were no macroscopic pathological findings in any of the six animals sacrificed at the end of the observation period. No histological examinations were performed.
Remarks - Results	Clinical observations revealed impaired general state, piloerection and diarrhoea in all animals of both groups. In addition, all animals in group one exhibited cowering position, while dyspnoea and reduced defecation were seen from days 1 to 3 in the animals of group two. Dehydration and smeared fur were seen in 2/3 of group two animals, from day 2 to day 3.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Bioassay (2015a)

B.2. Acute toxicity – oral (STD/1641)

TEST SUBSTANCE	Notified chemical (STD/1641)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method, March 3, 1996 EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/ Wistar strain SPF
Vehicle	Olive oil
Remarks - Method	The study was carried out stepwise, with one group of three male and then three female animals given a dose of 5000 mg/kg bw by oral gavage. The animals were fasted for approximately 18 hours prior to dosing and for a

further 3 hours after the dosing. The dosing of male and female animals occurred on different days.

Each animal was observed 1, 3 and 6 hours after administration and thereafter daily for a period of 14 consecutive days. Body weight was recorded on days 0, 7 and 14. All animals were killed by inhalation of CO_2 on day 14 and subjected to a gross necropsy examination.

The study was carried out according to GLP. The dosage of 5000 mg/kg bw was higher than the upper limit of 2000 mg/kg bw specified in TG 423.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3M	5000	1/3 M
	3F	5000	1/3 F

LD50	> 5000 mg/kg bw
Signs of Toxicity	One male and one female rat died on day 2 and 4, respectively. The remaining four animals showed slight to moderate signs of toxicosis. The surviving rats had a normal body weight gain during the study period.
Effects in Organs	Two dead animals showed bleeding in the stomach, empty gut and light lungs.
Remarks - Results	Decreased motor activity, diarrhoea, pinched abdomen and piloerection were observed at different intensities in all animals from 1 hour after the administration of the test item.

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

TEST FACILITY Frey-Tox (2001a)

B.3. Acute toxicity – oral (STD/1641)

TEST SUBSTANCE Notified chemical (STD/1641)

METHOD OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method, 2001
EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method

Species/Strain	Rat/ Wistar strain SPF
Vehicle	Corn oil Ph.Eur.
Remarks - Method	No significant protocol deviations. Single oral administration by gavage.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3F	2000	0/3
2	3F	2000	0/3

LD50	> 2000 mg/kg bw
Signs of Toxicity	Impaired general state and piloerection were noted in all animals on the day of dosing and until day 2. Dyspnoea was observed in all animals on day 1. Diarrhoea was observed 1 hour after dosing.
Effects in Organs	The mean body weight of the animals increased within the normal range throughout the study period. There were no macroscopic pathological findings in all sacrificed animals at the end of the observation period
Remarks - Results	No mortality occurred in either test group.

CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Bioassay (2015b)

B.4. Acute toxicity – dermal (Analogue 1)

TEST SUBSTANCE	Analogue 1
METHOD	Stated to be according to CFR 1500.40. Similar to OECD TG 402 Acute Dermal Toxicity or its Limit test
Species/Strain	Rabbits, New Zealand white
Vehicle	Administered undiluted
Type of dressing	Occlusive
Remarks - Method	Thirty adult animals (15 males and 15 females) were assigned to five test groups with 3 males and 3 females per group. Dosages were determined in a preliminary test. The back of each rabbit was clipped free of hair over an area constituting 30% of the total body surface. Just prior to the application of the test material, abrasion of the skin was performed with the tip of a 23 gauge needle on the exposure sites of 2 males and 1 female per each of the 5 groups. The skins of the remaining male and 2 females per group were left intact. Skin applications were made on the intact and abraded skin of all testing animals. The test material was applied at levels of 8.41, 10, 12.60, 15.87 and 20 g/kg bw under a square gauze patch. A layer of plastic wrap was applied to the torso of the animals to prevent evaporation of the test material. This layer was secured with masking tape and covered with a protective cloth bandage binder. The test material remained in contact with the skin for 24 hours. Observations for mortality, local reactions and behavioural abnormalities were continued for 14 days following the skin applications. Body weights were recorded on day 1, 7 and 14. At the end of the observation period, all animals that died or were sacrificed moribund were subjected to necropsy. It was not recorded whether the deaths occurred in animals with or without abrasion.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3M/3F	8,410	0/6
2	3M/3F	10,000	3/6 (2M and 1F)
3	3M/3F	12,600	1/6 (1F)
4	3M/3F	15,870	2/6 (1M and 1F)
5	3M/3F	20,000	3/6 (2M and 1F)

LD50	> 8,410 mg/kg bw
Signs of Toxicity - Local	Back skin was red, swollen or burned, mouth and nose red in colour, animals pulling matted fur from bodies and dead skin off their back.
Signs of Toxicity - Systemic	Decreased activity, ataxia, no faeces or diarrhoea, appetite decreased progressively with increased doses of the test item applied.
Effects in Organs	There were no organ abnormalities observed in the dose group of 8,410 mg/kg bw. At 10,000 mg/kg bw and above, the following changes were observed in the organs of some of the tested animals: Lungs: dark and mottled Liver: dark with blanched areas Kidneys: pale and vascularised Spleen: dark

	Gall Bladder: appear very enlarged Stomach and Large Intestine: filled with air and hard material
Remarks - Results	At 8410 mg/kg bw no animals died within the 14 days observation period when the test sample was applied dermally to the intact and abraded skin of rabbits. However, there was inconsistent death pattern at higher dose levels, leading to conclusion that delayed mortality occurred in this study. Considering that all animals showed progressive decrease in body weight throughout the study, the authors argued that the delayed mortality could be due to a combination of toxic effect of the test material and the decreased appetite.

CONCLUSION Analogue 1 is of low acute toxicity via the dermal route.

TEST FACILITY FDRL (1980)

B.5. Acute toxicity – dermal (Analogue 3)

TEST SUBSTANCE Analogue 3

METHOD Method is based on Federal Register, Vol. 43,No 168, 22/8/78, Section 163.81-2 and is similar to OECD TG 402 Acute Dermal Toxicity

Species/Strain	Rabbits, New Zealand white
Vehicle	Administered as supplied
Type of dressing	Occlusive
Remarks - Method	Similar to OECD TG 402 Method

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5M, 5F	> 2000	1F/10
2	2M, 2F	0	0/4

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No abnormalities observed
Signs of Toxicity - Systemic	No abnormalities observed
Effects in Organs	No abnormalities were observed except for the one dead female animal: (overall cyanotic: Lungs appeared normal with some small pooling of blood and believed to be agonal). Liver was friable, pale and appeared mottled. Peritoneum was dehydrated and very dark and abdominal skin was hard and cracked with significant erythema seen.
Remarks - Results	1 dead F

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

TEST FACILITY Pharmichem Testing Services, lnc. (1980)

B.6. Irritation – *in vitro* skin corrosion and skin irritation (STD/1641)

TEST SUBSTANCE Notified chemical (STD/1641)

METHOD OECD TG 431 *In vitro* Skin Corrosion – Reconstructed human epidermis (RHE) test method (2013)
OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2013)

Vehicle	Administered as supplied (Undiluted)
Remarks - Method	Two *in vitro* assays were part of this *in vitro* skin irritation and corrosion test strategy: The Skin Corrosion Test (SCT) and Skin Irritation Test (SIT). EpiDerm™ tissue samples for the corrosion test were incubated with the

test substance for 3 minutes and 1 hour, respectively, followed by a 3 hour incubation. EpiDerm™ tissue samples for the irritation test were incubated with the test substance for 1 hour followed by incubation and post-incubation stages.

A colorimetric test was used to determine the destruction of tissue measuring the metabolic activity of the tissue after exposure / incubation. The reduction of mitochondrial dehydrogenase activity, measured by reduced formazan production after incubation with a tetrazolium salt (MTT).

As the test substance was found to react with MTT, an additional MTT reduction control KC (freeze-killed controls) was introduced. However, the result of the KC did not indicate an increased MTT reduction (difference to KC of the negative control is not greater than 0.1). Thus the KC was not used for viability calculation for corrosion. The KC was not reported for the evaluation of skin irritation, based on the fact that the uncorrected values already indicated that the test substance is an irritant.

RESULTS

Corrosion test

Test material	*Mean OD_{570} of triplicate tissues (3 min exposure/1 hour exposure)*	*Relative mean Viability (%) (3 min exposure/1 hour exposure)*
Negative control	1.839/1.874	100/100
Test substance	1.718/1.642	93/88
Positive control	0.244/0.098	13/5

Irritation test

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	2.767	100	0.49
Test substance	0.310	11	1.49
Positive control	0.073	3	0.18

OD = optical density; SD = standard deviation

Remarks - Results

Corrosion Test:
The mean viability of the test-substance treated tissues determined after an exposure period of 3 minutes was 93%, and it was 88% after an exposure period of 1 hour. The criteria for the study indicate that the test substance is non-corrosive because mean tissue viability is ≥ 50% after 3 minutes and ≥15% after 1 hour.

Irritation test:
The mean viability of the test-substance treated tissues determined after an exposure period of 1 hour with about 42 hours post-incubation was 11%. The criteria for the study indicate that the test substance is an irritant because mean tissue viability is ≤ 50%.

CONCLUSION

The notified chemical was irritating to the skin under the conditions of the test.

TEST FACILITY

BASF (2014)

B.7. Irritation – *in vitro* skin corrosion and skin irritation (STD/1640)

TEST SUBSTANCE

Notified chemical (STD/1640)

METHOD

OECD TG 431 *In vitro* Skin Corrosion - Reconstructed human epidermis (RHE) test method (2013)

OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2013)

Vehicle: Administered as supplied (Undiluted)

Remarks - Method: Two *in vitro* assays were part of this *in vitro* skin corrosion and irritation test strategy: The Skin Corrosion Test (SCT) and Skin Irritation Test (SIT).

EpiDerm™ tissue samples for the corrosion test were incubated with the test substance for 3 minutes and 1 hour, respectively, followed by a 3-hour post incubation stage. EpiDerm™ tissue samples for the irritation test were incubated with the test substance for 1 hour followed by incubation and post-incubation stages.

A colorimetric test was used to determine the destruction of tissue measuring the metabolic activity of the tissue after exposure / incubation. The reduction of mitochondrial dehydrogenase activity was measured by reduced formazan production after incubation with a tetrazolium salt (MTT).

As the chemical was found to react directly with MTT, an additional MTT reduction control KC (freeze-killed controls) was introduced. The final mean viabilities of the test substance for the corrosion and irritation test are given after KC correction in all test runs.

RESULTS

Corrosion test

Test material	*Mean OD_{570} of triplicate tissues (3 min exposure/1 hour exposure)*	*Relative mean Viability (%) (3 min exposure/1 hour exposure)*	*SD of relative mean viability (3 min exposure/1 hour exposure)*
Negative control	2.069/1.863	100/100	4.2/2.4
Test substance	2.119/2.076	99.7/94.7*	13.8/9.0
Positive control	0.349/0.179	16.9/9.6	0.1/0.0

*Corrected for MTT direct reduction

Irritation test

Test material	*Mean OD_{570} of triplicate tissues 1^{st} test run/2^{nd} test run*	*Relative mean Viability (%) 1st test run/2nd test run*	*SD of relative mean viability 1st test run/2nd test run*
Negative control	3.108/2.750	100/100	6.1/5.1
Test substance	0.677/0.370	12.5/6*	1.1/7.6
Positive control	0.086/0.081	2.8/3.0	0.1/0.1

OD = optical density; SD = standard deviation
*Corrected for MTT direct reduction

Remarks - Results

Corrosion Test:
The final mean viability of the test-substance treated tissues determined after an exposure period of 3 minutes was 99.7%, and it was 94.7% after an exposure period of 1 hour. The criteria for the study indicate that the test substance is not corrosive because mean tissue viability is ≥50% after 3 minutes and ≥15% after 1 hour.

Irritation test:
1st test run:
The final mean viability of the test-substance treated tissues determined after an exposure period of 1 hour with about 42 hours post-incubation was 12.5%. The acceptance criteria for the negative control tissues (mean OD570 of the negative control = 3.1) was not met in this run and a 2nd test run was performed

2nd test run:
The final mean viability of the test-substance treated tissues determined after an exposure period of 1 hour with about 42 hours post-incubation was 6.0%. All acceptance criteria were met. The criteria for the study indicate that the test substance is an irritant because mean tissue viability was ≤ 50%.

CONCLUSION The notified chemical was irritating to the skin under the conditions of the test.

TEST FACILITY BASF (2015a)

B.8. Irritation – skin (STD/1641)

TEST SUBSTANCE Notified chemical (STD/1641)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion (1992)
Species/Strain Rabbit/Albino Chbb:HM(SPF)
Number of Animals 3 Females
Vehicle Administered as supplied (Undiluted)
Observation Period 21 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	3.33	3.33	3.33	4	>14 d	0 (21 d)
Oedema	3.67	3.67	3.67	4	>14 d	0 (21 d)

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

Remarks - Results All animals showed well defined erythema and moderate oedema after 24 hours of exposure to the test substance. A severe erythema and oedema was observed after 48 and 72 hours of test substance exposure termination. Seven days after termination of exposure all animals showed a strong yellow crust. Granulation tissues as well as a slight to severe erythema and crust of scales were observed on animals at 14 days after exposure termination. At day 21 all animals were free of any signs of skin irritation.

CONCLUSION The notified chemical is irritating to the skin.

TEST FACILITY Frey Tox (2001)

B.9. Irritation – *in vitro* BCOP test and EpiOcular eye irritation test (STD/1640)

TEST SUBSTANCE Notified chemical (STD/1640)

METHOD OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants (2013)
Commission Regulation (EU) No 1152/2010 OECD (2014a) Draft Proposal for a New Test Guideline (EpiOcularTM)
Vehicle Administered as supplied (Undiluted)
Remarks - Method No significant protocol deviations.

Two *in vitro* assays were part of this *in vitro* eye irritation test strategy: The Bovine Corneal Opacity and Permeability Test (BCOP Test) and EpiOcular Eye Irritation Test.

Three corneas were treated with the test substance for 10 minutes followed by a 2-hours post-incubation period.

Two EpiOcular™ tissue samples were incubated with the test substance for 30 minutes followed by a 2-hours post-incubation period. The ability of the test substance to directly reduce MTT was tested. Killed tissues were included in the study to measure this effect.

The negative control (NC) used for the BCOP test was de-ionized water and the Positive Control PC was 100% ethanol. The NC for the EpiOcular test was sterile de-ionized water and the PC was methyl acetate.

RESULTS

BCOP

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Vehicle control	2.3	0.004	2.4
*Test substance**	0.0	0.000	0.0
*Positive control**	31.7	1.452	53.4

SD = Standard deviation; IVIS = *in vitro* irritancy score *Corrected for background values

EpiOcular

Test material	*Mean OD570*	*Mean % Viability*	*Intertissue Variability*
Vehicle control	1.710	100	1.5
Test substance	1.840	91.4*	4.8
Positive control		28.2	0.3

*Adjusted on basis of killed tissues, to account for direct MTT reduction.

Remarks - Results

BCOP Test: The mean IVIS (*In Vitro* Irritancy Score) of the test-substance treated corneas was 0.0. The IVIS of the test substance was < 3 and therefore is not warranting classification for eye corrosion or irritation.

EpiOcular Test: The mean viability of the test-substance treated tissues was 91.4%. As the test substance shows a mean tissue viability of > 60%, it does not warrant a hazard classification as for eye irritation.

CONCLUSION

The notified chemical was not an eye irritant under the conditions of the test.

TEST FACILITY

BASF (2015b)

B.10. Irritation – *in vitro* BCOP test and EpiOcular eye irritation test (STD/1641)

TEST SUBSTANCE

Notified chemical (STD/1641)

METHOD

OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants
Commission Regulation (EU) No 1152/2010 OECD (2014a) Draft Proposal for a New Test Guideline (EpiOcularTM)

Vehicle

Administered as supplied (Undiluted)

Remarks - Method

No significant protocol deviations.

Two *in vitro* assays were part of this *in vitro* eye irritation test strategy: The Bovine Corneal Opacity and Permeability Test (BCOP Test) and EpiOcular Eye Irritation Test.

Three corneas were treated with the test substance for 10 minutes followed by a 2-hours post-incubation period.

Two EpiOcular™ tissue samples were incubated with the test substance for 30 minutes followed by a 2-hours post-incubation period. The ability of the test substance to directly reduce MTT was tested. Killed tissues were included in the study to measure this effect.

The negative control (NC) used for the BCOP was de-ionized water and the Positive Controls (PC); were 100% ethanol and 100% dimethylformamide respectively. For the EpiOcular test, NC was sterile de-ionized water and PC was methyl acetate.

RESULTS

BCOP

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Vehicle control	3.7	0.000	3.7 (1.8)
*Test substance**	0.0	0.000	0.0 (0)
*Positive control1**	32.0	1.789	58.9 (4.6)
*Positive control2**	107.8	0.962	122.2 (6.5)

SD = Standard deviation; IVIS = *in vitro* irritancy score *Corrected for background values

Epiocular

Test material	*Mean OD_{570}*	*Mean % viability*	*Inter-tissue variability %*
Vehicle control	2.137	100	14.7
Test substance	1.763	82*	2.9
Positive control	0.565	26	3.6

*Adjusted on basis of killed tissues, to account for direct MTT reduction.

Remarks - Results

BCOP Test: The mean IVIS (*In Vitro* Irritancy Score) of the test-substance treated corneas was 0.0. The IVIS of the test substance was < 3 and therefore classification is not warranted for eye corrosion or irritation.

EpiOcular Test: The mean viability of the test-substance treated tissues was 82%. As the test substance showed a mean tissue viability of > 60%, it does not require a hazard classification for an eye irritation.

CONCLUSION

The notified chemical was an eye irritant under the conditions of the test.

TEST FACILITY

BASF (2014b)

B.11. Irritation – eye (STD/1641)

TEST SUBSTANCE

Notified chemical (STD/1641)

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion (1987)
EC Council Regulation 1997 B.5 Acute Toxicity (Eye Irritation)

Species/Strain: Albino Rabbit/ Chbb:HM(SPF)
Number of Animals: 3 females
Observation Period: 21 days
Remarks - Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.33	1.67	2.33	3	> 14 d	0 (21 d)
Conjunctiva: chemosis	1.33	1.00	1.67	2	> 72 h	0 (21 d)
Conjunctiva: discharge	0.33	0	0	2	> 24 h	0 (21 d)
Corneal opacity	0.00	0.00	0.00	0	0	0 (21 d)

Iridial inflammation	0.00	0.00	0.00	1	> 1h	0 (21 d)

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

Remarks - Results: Well-defined signs of irritation were observed on the treated eyes. All effects were fully reversible within 21 days.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: Frey Tox (2001b)

B.12. Skin Sensitisation – *in chemico* DPRA test (STD/1640)

TEST SUBSTANCE: Notified chemical (STD/1640)

METHOD: OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA; 2015)

Remarks - Method: No significant deviations from the OECD test guideline.

The test substance was dissolved at a 100 mM concentration in acetonitrile. Acetonitrile was used as the vehicle control. Ethylene glycol dimethacrylate (50 mM in acetonitrile) was used as positive control. Solvent reference controls were setup and used in parallel to sample preparation in order to verify the validity of the test run. The test substance was incubated in dark with the peptide solutions for 24 ± 2 h at room temperature for the reaction to take place. The ratios of test substance: peptides were 1:10 cysteine peptide and 1:50 lysine peptide. After incubation, peptide depletion was monitored by HPLC coupled with a UV detector at wavelength of 220 nm using a reverse-phase HPLC column.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle	0.00*	0.00*
Test Substance	-0.41 ±2.53	-2.31 ± 0.08
Positive Control	52.61 ± 5.99	12.67 ± 0.50

* – normalised to 100%; SD = Standard Deviation

Remarks - Results: No co-elution of the test substance and peptides occurred.

Negative depletions were considered to be “zero” for calculation of the mean peptide depletion, which was thus calculated to be 0.0% (negative prediction for skin sensitisation).

Based on the test results alone, the test substance shows a minimal chemical reactivity in the DPRA under the test conditions used. However, it was noted that due to the limited solubility of the test substance the samples with both peptides were emulsions and that the result could therefore be under-predictive. Following OECD TG 442C a “negative” result should be considered “inconclusive” in this case.

The positive controls and references fulfilled all quality criteria confirming the validity of the test.

CONCLUSION: The test result was inconclusive in this adverse outcome pathway (AOP) key event (KE) 1 assay.

TEST FACILITY: BASF (2015c)

B.13. Skin Sensitisation – *in vitro* ARE-Nrf2 luciferase test (STD/1640)

TEST SUBSTANCE: Notified chemical (STD/1640)

METHOD: OECD TG 442d *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (2015)

Remarks - Method: No significant deviations from the OECD test guideline. KeratinoSens™ test method was used. The results from Experiments 2 & 3 were used, as excessive cytotoxicity occurred in Experiment 1.

Dimethyl sulphoxide (DMSO) was used as the vehicle control. DL-Lactic acid in DMSO was used as negative control. Ethylene glycol dimethacrylate in DMSO was used as the positive control. Two independent experiments were conducted with samples tested in triplicates in each test. The mean ± standard deviations for cell viability and luciferase induction calculated from three independent experiments are depicted below.

The CV75 value (estimated concentration that affords 75% cell viability) was determined by linear regression from the concentration response curve to be approximately 2.7 µg/mL of the preliminary cytotoxicity assessment test to be used for the 2 experiments (Relative Luciferase induction and relative viability in LuSens cells after 48 hours exposure to the test substance).

RESULTS

Sample	*Concentration (µg/mL) Experiment 2 and 3*	*% Cell viability (mean ± SD, n=3) Experiments 2/3*	*% Luciferase Induction (mean ± SD, n=3) Experiments 2/3*
Vehicle Control		100.0/100.0 ± 4.9/5.3	1/1 ± 0.11/0.19
Negative Control	450	100.4/108.9 ± 4.4/6.9	0.94/0.96 ± 0.11/0.11
Test substance	0.4	128.5/117.4 ± 1.6/6.0	1.32/1.30 ± 0.05/0.14
	0.5	126.6/108.3 ± 7.1/8.4	1.40/1.34 ± 0.09/0.07
	0.6	134.3/124.0 ± 7.3/1.3	1.41/1.18 ± 0.15/0.02
	0.8	125.8/119.9 ± 5.3/6.5	1.54/1.41 ± 0.29/0.09
	0.9	127.3/104.5 ± 4.2/4.4	1.63/1.52 ± 0.09/0.12
	1.1	100.8/89.6 ± 8.0/9.0	1.64/1.60 ± 0.29/0.11
	1.3	49.0/32.2 ± 6.9/10.1	1.99/1.72 ± 0.14/0.28
	1.6	0.1/0.1 ± 0.6/0.4	-0.04/-0.02 ± 0.07/0.04
Positive Control	18	98.9/101.8 ± 3.2/2.6	6.52/6.88 ± *0.46/1.00*

* – normalised to 100%

Remarks - Results: Luciferase induction > 1.5 at cell viability ≥ 70% is required for identification as a skin sensitiser. This criterion was met for both the positive control and for multiple concentrations of the test substance.

In experiment 1 the EC1.50 was calculated by linear regression from the results of the 0.6 µg/mL and the 0.8 µg/mL concentrations to be 0.7 µg/mL. In experiment 2 the EC1.50 was calculated by linear regression from the results of the 0.8 µg/mL and the 0.9 µg/mL concentrations to be 0.9 µg/mL.

CONCLUSION: The test substance was considered to be a skin sensitiser in this AOP KE2 assay.

TEST FACILITY: BASF (2015c)

B.14. Skin Sensitisation – *in vitro* h-CLAT test (STD/1640)

TEST SUBSTANCE: Notified chemical (STD/1640)

METHOD

Draft Guideline similar to OECD TG 442e *In Vitro* Skin Sensitisation: human Cell Line Activation Test (h-CLAT; 2015)

Vehicle: 0.2% DMSO in culture medium

Remarks - Method: No significant deviations from the OECD test guideline were evident.

The potential of test substance to induce the cell membrane markers CD86 and CD54 expression was evaluated in the Human Cell Line Activation Test (h-CLAT). For this purpose the test substance was incubated with human pro-monocytic cell line THP-1 for approximately 24 hours at 37 °C and membrane markers expression were measured by flow cytometry. Stimuli mediated increase in expression of the cell surface markers CD86 and CD54 was measured using fluorescence tagged antibodies.

A pre-test was performed in order to determine the concentrations suitable for the three main experiments. The main tests were conducted to evaluate the ability of the test substance to induce expression of CD54 and CD86. The following concentrations were used: 2.2, 2.7, 3.2, 3.9, 4.6, 5.6, 6.7, and 8.0 µg/mL

Positive Control: 1- chloro-2,4-dinitrobenzene (DNCB), 4.0 µg/mL in DMSO

Negative Control: Lactic acid (LA), 1000 µg/mL in culture medium

Test acceptance criteria:
Cell Viability: > 50% for Positive control, > 75% for test substance and > 90% for negative and vehicle controls

RESULTS

Sample	*Concentration (µg/mL)*	*RFI* CD86 Mean (%) Experiments 1/2/3*	*RFI* CD54 Mean (%) Experiments 1/2/3*	*Relative Viability (%) Experiments 1/2/3*
Vehicle Control		100.0/100.0/100.0	100.0/100.0/100.0	100.0/100.0/100.0
Negative Control	1000	70.3/84.1/78.6	109.0/104.2/99.5	101.2/99.8/100.2
Test substance	2.2	103.6/119.3/118.9	97.9/99.4/104.9	100.4/100.2/100.4
	2.7	81.5/116.2/101.8	70.5/90.6/87.5	100.9/100.5/100.5
	3.2	119.2/100.6/109.2	111.9/91.7/117.9	101.2/100.3/100.3
	3.9	110.5/99.9/107.0	88.2/112.7/87.6	100.9/100.2/100.4
	4.6	119.3/141.8/115.3	91.5/133.3/106.0	101.0/99.6/100.0
	5.6	138.0/162.1/118.4	138.3/164.6/113.5	94.0/93.5/99.3
	6.7	249.2/202.5/147.9	176.7/208.9/208.7	72.1/80.5/93.0
	8.0	255.1/306.8/262.1	160.5/103.9/318.0	12.5/29.6/56.2
Positive Control	4	256.5/322.3/357.1	252.2/787.7/422.2	90.9/86.9/86.0

*Relative fluorescence intensity

Remarks - Results: The EC150 (the concentration resulting in a RFI of 150) for CD86 was calculated to be 5.7 µg/mL (experiment 1), 4.6 µg/mL (experiment 2) or 6.7 µg/mL (experiment 3), respectively. The EC200 (the concentration resulting in a RFI of 200) for CD54 was calculated to be 6.4 µg/mL (experiment 2) or 6.6 µg/mL (experiment 3), respectively. In experiment 1, the RFI of 200 for CD54 expression was not exceeded.

After 24 hours of exposure to the test substance, CD86 and CD54 expression was induced to >150 and >200 respectively in THP-1 cells, with at least 50% viability, in at least two independent experiments. From this it was concluded by the study author that test substance does induce dendritic cell activation.

(Precipitates were not observed in any concentration of the three

experiments after 24 hours of the dissolution of the test substance in DMSO).

CONCLUSION The test substance was considered a skin sensitiser in the AOP KE3 assay.

TEST FACILITY BASF (2015c)

B.15. Skin Sensitisation – *in chemico* DPRA test (STD/1641)

TEST SUBSTANCE Notified chemical (STD/1641)

METHOD OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA; 2015)

Remarks - Method No significant deviations from the OECD test guideline.

The test substance was dissolved at a 100 mM concentration in acetonitrile, using a theoretical molecular weight. Acetonitrile was used as for both vehicle control and negative control. Ethylene glycol dimethacrylate in acetonitrile was used as positive control. Solvent reference controls were setup and used in parallel to sample preparation in order to verify the validity of the test run. The test substance was incubated in dark with the peptide solutions for 24 ± 2 h at room temperature for the reaction to take place. The ratios of test substance: peptides were 1:10 cysteine peptide and 1:50 lysine peptide. After incubation, peptide depletion was monitored by HPLC coupled with a UV detector at wavelength of 258 nm for measuring UV absorbance and 220 nm/258 nm for the area ratio measurement using a reverse-phase HPLC column.

The test substance was soluble in acetonitrile. The samples with the test substance and the peptide stock solutions were emulsions. After 24 hours precipitates were noticed in the samples of the C-peptide then all samples were centrifuged prior to HPLC analysis.

RESULTS

Sample	*Cysteine Peptide Mean Depletion (% ± SD) at 220 nm*	*Lysine Peptide Mean Depletion (% ± SD) at 220 nm*
Negative Control	0.00 ± 2.03	0.00 ± 2.65
Test Substance	-8.95 ± 1.56	-0.79 ± 1.55
Positive Control	59.06 ± 5.44	16.77 ± 1.10

SD = Standard Deviation

Remarks - Results Negative depletions were considered to be “zero” for calculation of the mean peptide depletion, which was thus calculated to be 0.0% (negative prediction for skin sensitisation). No co-elution of test substance and peptides occurred, as demonstrated by the consistent values of the peak area ratios 220 nm/258 nm.

Based on the test results alone, the test substance shows a minimal chemical reactivity in the DPRA under the test conditions used. However, it was noted that due to the limited solubility of the test substance the samples with both peptides were emulsions and that the result could therefore be under-predictive. Following OECD TG 442C a “negative” result should be considered “inconclusive” in this case.

The positive controls and references fulfilled all quality criteria confirming the validity of the test.

CONCLUSION The test result was inconclusive in this AOP KE 1 assay.

TEST FACILITY BASF (2015c)

B.16. Skin Sensitisation – *in vitro* ARE-Nrf2 luciferase test (STD/1641)

TEST SUBSTANCE Notified chemical (STD/1641)

METHOD Draft method similar to OECD TG 442d *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (2015)

Remarks - Method No significant deviations from the OECD test guideline. KeratinoSens™ test method was used.

Dimethyl sulphoxide (DMSO) was used as the vehicle control. DL-Lactic acid in DMSO was used as negative control. Ethylene glycol dimethacrylate in DMSO was used as positive controls. Three independent experiments were conducted with samples tested in triplicates in each test. The mean ± standard deviations for cell viability and luciferase induction calculated from three independent experiments are depicted below.

The CV75 value (estimated concentration that affords 75% cell viability) was determined by linear regression from the concentration response curve to be approximately 2.5 µg/mL of the preliminary cytotoxicity assessment test to be used for the 2 experiments (experiment 2 and 3 in the table below - Relative Luciferase induction and relative viability in LuSens cells after 48 hours exposure to the test substance.

RESULTS

Sample	*Concentration (µg/mL)*	*Mean Relative Viability (%) Experiments 2/3*	*Mean Luciferase Fold Induction Experiments 2/3*
Vehicle Control		100.0/100.0 ± 4.9/5.3	1.00/1.00
Negative Control	450	108.5/109.7	0.69/1.07
Test substance	0.6	99.1/110.1	1.46/2.28
	0.7	90.8/105.5	1.45/1.90
	0.9	107.4/114.5	1.55/1.99
	1.0	98.6/106.1	1.86/1.95
	1.2	74.8/91.7	1.78/2.41
	1.5	36.8/33.5	2.13/2.31
	1.8	1.5/0.2	0.40/0.05
	2.1	0.0/-0.1	-0.15/-0.14
Positive Control	18	92.2/97.0	6.16/6.16

* – normalised to 100%

Remarks - Results An increase in Luciferase activity > 1.5 in LuSens cells was induced in at least two consecutive concentrations with at least 70% viability and statistical significance in at least two independent experiments. Therefore the test substance has a keratinocyte activating potential.

The test substance was soluble in DMSO at all concentrations used in the main test and soluble in 1% DMSO in medium 3 (final concentrations). After 48 hours precipitates were not noticed in any concentration used.

CONCLUSION The test substance was considered to be a skin sensitiser in this AOP KE2 assay.

TEST FACILITY BASF (2015c)

B.17. Skin Sensitisation – *in vitro* h-CLAT test (STD/1641)

TEST SUBSTANCE Notified chemical (STD/1641)

METHOD Draft Guideline similar to OECD TG 442e *In Vitro* Skin Sensitisation: human Cell Line Activation Test (h-CLAT; 2015)

Vehicle Culture medium

Remarks - Method No significant deviations from the OECD test guideline were evident.

The potential of test substance to induce the cell membrane markers CD86 and CD54 expression was evaluated in the Human Cell Line Activation Test (h-CLAT). For this purpose the test substance was incubated with human pro-monocytic cell line THP-1 for approximately 24 hours at 37 °C and membrane markers expression were measured by flow cytometry. Stimuli mediated increase in expression of the cell surface markers CD86 and CD54 was measured using fluorescence tagged antibodies.

A pre-test was performed in order to determine the concentrations suitable for the three main experiments. The main tests were conducted to evaluate the ability of the test substance to induce expression of CD54 and CD86. The following concentrations of the test substance were used: 1.5, 1.8, 2.2, 2.6, 3.1, 3.7, 4.5, and 5.4 µg/mL

Positive Control: 1- chloro-2,4-dinitrobenzene (DNCB), 4.0 µg/mL
Negative Control: Lactic acid (LA), 1000 µg/mL
Test acceptance criteria:
Cell Viability: > 50% for Positive control, > 75% for test substance and > 90% for negative and vehicle controls

RESULTS

Sample	*Concentration (µg/mL)*	*RFI* CD86 Mean (%) Experiments 1/2/3*	*RFI* CD54 Mean (%) Experiments 1/2/3*	*Relative Viability (%) Experiments1/2/3*
Vehicle Control		100.0/100.0/100.0	100.0/100.0/100.0	100.0/100.0/100.0
Negative Control	1000	70.3/84.1/78.6	109.0/104.2/99.5	101.2/99.8/100.2
Test substance	1.5	159.5/192.4/129.1	315.2/154.1/236.6	98.4/98.7/97.8
	1.8	150.9/170.8/126.3	164.1/164.3/167.0	98.5/98.4//97.7
	2.2	163.9/177.8/133.3	274.8/169.6/227.5	98.0/98.1/96.7
	2.6	146.6/164.0/134.3	166.3/154.5/232.5	97.2/98.1/96.2
	3.1	139.9/189.3/141.1	146.2/145.0/113.9	97.1/96.6/93.6
	3.7	163.2/209.8/128.9	244.8/126.0/110.3	95.6/94.1/91.4
	4.5	161.2/204.1/141.2	110.4/149.1/118.2	94.0/92.8/87.9
	5.4	161.6/230.8/160.6	116.0/143.1/138.1	88.0/88.1/75.7
Positive Control	4	232.1/244.8/236.2	317.9/370.4/373.8	86.2/83.5/84.4

*Relative fluorescence intensity

Remarks - Results CD86 and CD54 expression was induced in THP-1 cells after 24 hours of exposure to test substance affording at least 50% viability in at least two independent experiments. Therefore the test substance does induce dendritic cell activation. The criteria for RFI and cell viability were met in all three experiments for CD86, and in experiments 1 and 3 for CD54.

The test substance was soluble in culture medium at concentrations used in the main test. No precipitates were noticed after 24 hours of the test substance in any concentration.

CONCLUSION The test substance was considered a skin sensitiser in the AOP KE3 assay.

TEST FACILITY BASF (2015c)

B.18. Repeat dose toxicity – oral (Analogue 1)

TEST SUBSTANCE Analogue 1

METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents EC Directive 88/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species
Species/Strain	Rat/Wistar (Crl: WI)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week
Vehicle	Corn oil
Physical Form	Liquid (viscous oil)
Remarks - Method	No significant protocol deviations. The dose selection was based on the results of the 14-day range finding study conducted on the analogue chemical.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control (vehicle)	10M; 10F	0	0
low dose	10M; 10F	15	0
mid dose	10M; 10F	50	0
high dose	10M; 10F	150	0
Satellite vehicle control	10M; 10F	0	0
Satellite high dose	10M; 10F	150	0
Control (untreated)	10M; 10F	0	0
Control (untreated)	10M; 10F	0	0

Mortality and Time to Death

Mortality was not observed in any group during the treatment.

Clinical Observations

No treatment-related clinical signs were observed during the treatment period. No significant changes in food consumption were observed in treatment groups when compared to controls.

There was statistically significant body weight decreases in males of the satellite high dose group (150 mg/kg bw/day) from week 9-13 as compared to satellite vehicle control group. This was not observed in main dose groups and therefore, was not considered to be of toxicological significance by the study authors.

Females at mid and high-dose groups showed a statistically significant decrease in body weights in weeks 13 and 10-13, respectively when compared with the vehicle control group. However, body weights of animals in the 50 mg/kg bw/day group were comparable to the body weights of animals in the untreated groups on week 13.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

No statistically significant changes were observed in treated males at any dose group when compared with control males. Males in the satellite high dose group were found to have a statistically significant decrease in haemoglobin, haematocrit, neutrophils and an increase in mean corpuscular haemoglobin concentration (MCHC) and lymphocytes, when compared to satellite vehicle control group. These effects were not observed in the main dose groups, hence considered to be non-treatment related by the study authors.
High-dose females had a statistically significant increase in neutrophils and increased lymphocytes as compared to vehicle controls. Females in the satellite dose groups did not show these effects.

Clinical Biochemistry

No significant changes were observed in main male dose groups. However, satellite high-dose males showed a statistically significant decrease in total protein and sodium levels as compared to satellite vehicle controls.

Statistically significantly increased urea and blood urea nitrogen (BUN) levels were observed in all treated females and decrease in alkaline phosphatase was noted in high dose females when compared with the vehicle control group. Satellite high dose females were noted with a statistically significantly decreased calcium levels as compared to satellite vehicle controls, but were comparable with untreated controls.

Functional Observational Battery
High dose males and mid and low dose females demonstrated a statistically significant increase in the activity level as compared to vehicle controls. Satellite high dose males demonstrated statistically significant decrease in auditory response at amplitude 1 (90 decibels) and amplitude II (105 decibels) when compared to satellite vehicle control animals. This decreased auditory response was comparable to the control animals and hence were not considered to be treatment-related or of toxicological importance.

Urinalysis
Urinalysis data showed no changes in the composition of urine in any of the treated groups except mid-dose females on day 1. This effect was not observed in any other treatment group and was not considered to be biologically adverse by the study authors.

Effects in Organs
In males, there were no statistically significant changes in mean organ weights in any treatment group.
In females, statistically significant increase in the relative weight of brain and adrenals and absolute weight of adrenals and spleen were observed when compared to the vehicle control group. These effects were not observed in satellite groups and did not correlate with microscopic findings, hence were not considered to be treatment-related.

All groups including the vehicle control and untreated control animals had non-significant recurrent lesions in the thymus (multifocal red spots), mandibular and cervical lymph nodes (enlarged and red).

Histopathological findings included statistically significant recurrence of pancreatic haemorrhage in high-dose female group (150 mg/kg bw/day). These findings were reported as spontaneous, incidental and usually observed in Wistar rats of this age.

Remarks – Results
Survival and food consumption were not affected by the treatment.

Based on the low level of changes seen and lack of dose-response relationship, the study author considered the effects observed were not to be treatment related and toxicologically insignificant.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 150 mg/kg bw/day in Wistar rats.

TEST FACILITY IIBAT (2009)

B.19. Genotoxicity – bacteria (STD/1640)

TEST SUBSTANCE	Notified chemical (STD/1640)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test (1997) Commission Regulation (EC) No 440/2008; B.13 / B.14 Mutagenicity – Reverse Mutation Test using Bacteria Plate incorporation procedure/Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA 1535, TA 100, TA 1537, TA 98 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 33 - 5700 µg/plate b) Without metabolic activation: 33 - 5700 µg/plate
Vehicle	Acetone
Remarks - Method	No significant protocol deviations. Standard plate (Test 1) and pre-incubation method (Test 2) were used.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*

Absent				
Test 1	> 5,000		≥ 1,000	Negative
Test 2		> 2,850	≥ 1,000	Negative
Present				
Test 1	> 5,000		≥ 1,000	Negative
Test 2		> 2,850	≥ 1,000	Negative

Remarks - Results

A weak bacteriotoxic effect was occasionally observed depending on the strain and test conditions from about 2850 µg/plate onward (pre-incubation test).

Precipitation of the test substance was found from 1000 µg/plate onward with and without S9 mix.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

CONCLUSION

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

TEST FACILITY

BASF (2015e)

B.20. Genotoxicity – bacteria (STD/1641)

TEST SUBSTANCE

Notified chemical (STD/1641)

METHOD

OECD TG 471 Bacterial Reverse Mutation Test (1997)
Commission Regulation (EC) No 440/2008; B.13 / B.14 Mutagenicity – Reverse Mutation Test using Bacteria
Plate incorporation procedure/Pre incubation procedure

Species/Strain: *Salmonella typhimurium*: TA 1535, TA 100, TA 1537, TA 98
Escherichia coli: WP2uvrA

Metabolic Activation System: S9 mix from phenobarbital/β-naphthoflavone induced rat liver

Concentration Range in Main Test:
a) With metabolic activation: 33 - 5700 µg/plate
b) Without metabolic activation: 33 - 5700 µg/plate

Vehicle: DMSO

Remarks - Method: No significant protocol deviations. Standard plate (Test 1) and pre-incubation (Test 2) methods were used.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in: Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000 for all strains except ≥ 333 for TA 100		≥ 1,000	Negative
Test 2		≥ 100	≥ 2,500	Negative
Present				
Test 1	> 5,000		≥ 1,000	Negative
Test 2		≥ 100	≥ 2,500	Negative

Remarks - Results

A slight decrease in the number of his+ was observed in the standard plate test only in tester strain TA 100 without metabolic activation from about 333 µg/plate onward. In the preincubation assay bacteriotoxicity (decrease in the number of his+ or trp+ revertants) was occasionally observed depending on the strain and test conditions from about 100 µg/plate onward.

Precipitation of the test substance was found from 1000 µg/plate onward in the standard plate test and from 2500 µg/plate onward in the

preincubation test both with and without S9 mix.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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

TEST FACILITY BASF (2015f)

B.21. Genotoxicity – *in vitro* gene mutation test (Analogue 1)

TEST SUBSTANCE Analogue 1

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 ovary (CHO) cells
Cell Type/Cell Line	(CHO) V79 cells
Metabolic Activation System	S9 mix from phenobarbital- and β-naphthoflavone induced rat liver
Vehicle	Acetone
Remarks - Method	No significant protocol deviations. The cultures were incubated for the respective exposure period at 37 °C, 1.5% (v/v) CO2

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0.02, 0.05*, 0.09*, 0.19*, 0.38*, 0.75*, 1.5*, 3.0, 6.0	4 hours	7-9 days
Test 2	0.05*, 0.09*, 0.19*, 0.38*, 0.75, 1.25, 1.5	4 hours	7-9 days
Present			
Test 1	0.75, 1.5*, 3.0*, 6.0*, 12.0*, 18.0*	4 hours	7-9 days
Test 2	0.75, 1.5, 3.0*, 6.0*, 12.0*, 18.0*, 24.0	4 hours	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	≥ 191.3	≥ 1.5	≥ 191.3	Negative
Test 2		≥ 0.75	-	Negative
Present			≥ 191.3	
Test 1	≥ 191.3	> 18.5	≥ 191.3	Negative
Test 2		≥ 24.0	≥ 191.3	Negative

Remarks - Results The vehicle controls indicated mutant frequencies within the range expected for the CHO cell line.

Both positive control substances, ethyl methanesulfonate (EMS) and 7,12-dimethylbenz[a]-anthracene (DMBA), showed the expected increase in the frequencies of forward mutations.

No substantial and reproducible dose dependent increase of the mutation frequency was observed up to the maximum concentration with or without metabolic activation.

Relevant cytotoxic effects indicated by a relative cloning efficiency or cell density below 50% occurred at 0.75 µg/mL in test1 without metabolic activation and at 18.0 µg/mL in test 1 with metabolic activation. In test 2 cytotoxic effects were noted at 0.38 µg/mL without metabolic activation.

A very steep cytotoxic gradient was noted with metabolic activation with virtually no cytotoxicity at 18.0 µg/mL. At the next higher concentration of 24.0 µg/mL the cell density was too low for mutation frequency analysis.

CONCLUSION The test item was not clastogenic to CHO cells treated *in vitro* under the conditions of the test.

TEST FACILITY Harlan (2013)

B.22. Genotoxicity – *in vitro* gene mutation test (Analogue 3)

TEST SUBSTANCE Analogue 3

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 ovary (CHO) cells
Cell Type/Cell Line	V79 (CHO) cells
Metabolic Activation System	S9 mix from phenobarbital- and β-naphthoflavone induced rat liver
Vehicle	Tetrahydrofurane (THF)
Remarks - Method	No significant protocol deviations. The cultures were incubated for the respective exposure period at 37°C, 1.5% (v/v) CO2

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0.1*, 0.2*, 0.4*, 0.8*, 1.6, 2.3, 3.1	4 hours	7-8 days
Test 2	0.08, 0.16, 0.31, 0.63, 1.25*, 2.50*, 5.00*, 7.50*, 10.0*, 15.00, 20.00	24 hours	7-8 days
Present			
Test 1	1.6*, 3.1*, 6.3*, 12.5*, 18.8, 25.0, 37.0	4 hours	7-8 days
Test 2			

*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	≥ 23.4 (4 h)		≥ 375	
Test 1		≥ 1.6	-	Negative
Test 2		≥ 18.8	-	Negative
	≥ 23.4 (24 h)		≥ 93.4	
Present	≥ 23.4		≥ 187.5	
Test 1		≥ 15.0	-	Negative

Remarks - Results The vehicle controls indicated mutant frequencies within the range expected for the CHO cell line.

Both positive control substances, ethyl methanesulfonate (EMS) and 7,12-dimethylbenz[a]-anthracene (DMBA), showed the expected increase in the frequencies of forward mutations.

No substantial and reproducible dose dependent increase of the mutation frequency was observed in both main experiments. However an increase in mutation frequency (x15) was seen at 0.8 µg/mL in one of two cultures in test 1 in the absence of metabolic activation. A linear regression analysis identified a possible dose dependent increase ($p < 0.05$) in mutant colonies in cultures I and II of Test 1 in the absence of metabolic activation. There were also isolated incidences where the increase in mutant colonies was slightly higher than a 3-fold increase which was used as a criterion for

	mutagenicity in the study. The study authors discounted the effects as they were not reproducible and/or may have been related to a low value for the solvent control.
CONCLUSION	The analogue was not considered by the study authors to be clastogenic to CHO cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2010a)

B.23. Genotoxicity – *in vitro* chromosome aberration assay (Analogue 3)

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1997) Commission Regulation (EC) No 440/2008; B.10 *In vitro* Mammalian Chromosome Aberration Test
Species/Strain	*Chinese hamster*
Cell Type/Cell Line	V79
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Tetrahydrofuran (THF)
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.2, 0.5, 0.9	4 h	18 h
Test 2	1.9, 3.8, 7.5	18 h	18 h
Present			
Test 1	3.8, 7.5, 15.0	4	18 h
Test 2	-	-	-

*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	> 1.9	≥ 0.9	-	Negative
Test 2	>.3.6	≥ 7.5	-	Negative
Present				
Test 1	> 60.0	> 15	-	Negative

Remarks - Results	Clear toxicity was observed in test 1 and 2 in the absence and presence of metabolic activation with S9 mix at the highest evaluable concentration. In the presence of S9 mix no cytotoxicity was observed up to the highest evaluable concentrations. There was no evidence of an increase in polyploid metaphases after treatment with the test substance as compared to the control cultures. The level of aberrant cells for the test substance was generally within the range of the solvent controls, and did not show a dose-related increase. However, in Test 1 in the absence of metabolic activation, the aberrant cells at the highest dose slightly exceeded the historical solvent controls, with no statistical significance. In Test 2, the aberrant cells at the mid dose also slightly exceeded the historical controls, with statistical significance ($p < 0.05$). It was considered that this result occurred because of a relatively low solvent control value in Test 2. The study authors considered that the test substance did not induce relevant structural chromosome aberrations in the Chinese hamster V79 cell line.

	Both positive control substances, ethyl methanesulfonate (EMS) and cyclophosphamide(CPA), and vehicle controls gave satisfactory responses, confirming the validity of the test system.
CONCLUSION	The test substance was not clastogenic to V79 cells Chinese hamster cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2010b)

B.24. Reproduction and Developmental Toxicity (Analogue 1)

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 421 Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat/Crl:WI(Han)
Route of Administration	Oral – gavage
Exposure Information	Exposure days: Males - 51 days (2-weeks prior to mating and during mating period (2 weeks); and 3-weeks post mating) Females - 57 days (2-weeks prior to mating and during mating period and gestation period until the F1 generation reached day 4 of post-partum).
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations. The test animals were dosed once daily.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M; 10F	0	0/20
Low dose	10M; 10F	30	1/20 (1F)
Mid dose	10M; 10F	100	0/20
High dose	10M; 10F	300	0/20

Mortality and Time to Death

One female rat in the low dose group was sacrificed moribund because of piloerection, pale skin, blood in bedding and inability to deliver. No other mortalities were noted in any other treatment groups.

No high-dose males were able to produce implants due to infertility associated with reduced sperm quality and pathological alterations in the sexual organs. One male in the low-dose group did not generate implants.

Effects on Parental Animals

Females

The mean daily food consumption of females in the mid dose group was statistically significantly lower (about 40%) than the control group during lactation period (PND 1-4). During the gestation period, the mean body weights of mid-dose females were statistically significantly lower (up to 14% below the control group on GD 14 and 20). There were statistically significantly lower body weights in this dose group during GD 7-20 (up to 47% decrease) and GD 10-20 (about 38% decrease) when compared with the controls. Mean body weights of treated females at 30 mg/kg bw/day and at 300 mg/kg bw/day were similar to those of the control females. Females at 300 mg/kg bw/day were found to have statistically significant decreased mean corpuscular volume (MCV), increased mean corpuscular haemoglobin concentrations (MCHC), increased alanine aminotransferase (ALT) activity, decreased red blood cell (RBC) counts, haemoglobin and haematocrit values, regenerative anaemia, increased relative reticulocyte counts and increased triglyceride and total bilirubin values compared with control females.

Fertility effects observed in high-dose females included marred ovaries, mild to moderately increased atretic follicles, inactivity of interstitial cells in ovaries of six females, increased number of cysts in nine females and a decreased number of corpora lutea in nine females all correlated to decreased organ size and reduced mean organ weights (~ 48% compared to controls). One female in the mid-dose group showed atretic follicles and decreased number of corpora lutea and was sacrificed in a moribund state as it could not give birth. Significant

increase in mean absolute weights of the adrenal gland (51%), liver (14%) and pituitary gland (26%) were observed at 300 mg/kg bw/day. Statistically significantly decreased number of implantation sites (n=59 when compared to 123 on control group) and increased post-implantation loss (with mean of 51.61% when compared to 3.88% control group) were reported at 100 mg/kg bw/day.

Males

During pre-mating period, mean food consumption of the high-dose males were statistically significantly lower (24%) when compared with the control group. Food consumption in males in the low and mid-dose groups was unaffected. Mean body weights of high-dose males were statistically significantly lower (7% below the control males) and during the mating and post-mating periods by 7-13%. Haematology findings in high-dose males were indications of regenerative anaemia such as decreased RBC counts, haemoglobin and haematocrit values, as well as increased relative reticulocyte counts, prolonged prothrombin time. Increased ALT and creatinine levels and decreased cholesterol levels were also reported in high dose males.

All treatment groups included increased incidences of abnormal sperm and decreased total sperm head counts in the cauda epididymides. Reduced absolute weights of testes (28%), epididymides (38%), prostate (65%) and seminal vesicles (81%) were reported at 300 mg/kg bw/day. Other effects observed in high dose males were moderated tubular degeneration (two males); slight reduction of tubular size in left testes (seven males); slight to extreme oligospermia (four males); and minimal to slight debris in seminiferous tubules in left epididymides (three males). Mid-dose males had decreased absolute weights of prostates (32%), seminal vesicle (35%) and epididymides (13%). Low-dose males had 16-18% decrease in the mean weight of seminal vesicles. Pituitary gland weight was increased in all treated males (statistically significant at 300 mg/kg bw/day).

Effects on Foetus

No pregnancies were observed and no pups were delivered at 300 mg/kg bw/day. Decreased mean number of delivered pups and increased number of dead/cannibalised pups, (viability index 14% below the control) were reported at 100 mg/kg bw/day. No effects were reported in F1 pups at 30 mg/kg bw/day.

Remarks - Results

A few treated animals in mid- and high dose groups showed transient salivation immediately after treatment during the whole study period. This was considered by the study authors to be due to unpleasant taste of the test substance or by local irritation of upper digestive tract and not a sign of systemic toxicity. Reduced terminal body weights in high dose group (both genders) ranged within the historical control data, the study authors considered the increased in relative kidney weights in the high-dose group as a consequence to the lower body weights when compared to the controls.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for systemic toxicity was established as 100 mg/kg bw/day in this study, based on adverse effects observed at 300 mg/kg bw/day.

The NOAEL for developmental toxicity was established by the study authors as 30 mg/kg bw/day, based on developmental toxicity classification and foetal and pup mortality at 100 mg/kg bw/day.

No NOAEL for reproductive toxicity was able to be established, as adverse effects in the male reproductive system were seen at all dose levels.

TEST FACILITY BASF (2014c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ultimate biodegradability (Analogue 2)

TEST SUBSTANCE	Analogue 2
METHOD	Modified OECD TG 301D. Two phase closed bottle test. Biological oxygen demand for insoluble substances (BODIS).
Inoculum	Sewage sludge from municipal STP
Exposure Period	28 d
Concentration Range	Nominal: 100 mg/L Actual: Not determined mg/L
Remarks – Method	The test item was directly added, at a concentration of 100 mg/L to the inoculum. Sodium acetate, also at 100 mg/L, was used as a reference control. Incubation was then for 28 d. The extent of degradation was determined as the ratio of the biochemical oxygen demand to either the theoretical oxygen demand (ThoD) or chemical oxygen demand (COD).

RESULTS

Test substance (ThOD)		*Sodium Acetate (COD)*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	75
14	0	14	83
21	0	21	83
28	1	28	83

Remarks – Results	The validity criteria for the test were met.
CONCLUSION	The test item is poorly biodegradable.
TEST FACILITY	Henkel KGaA, (1996)

C.1.2. Ultimate biodegradability (STD/1641)

TEST SUBSTANCE	Notified Chemical (STD/1641)
METHOD	Modified OECD TG 301D. Two phase closed bottle test. Biological oxygen demand for insoluble substances (BODIS).
Inoculum	Sewage sludge from municipal STP
Exposure Period	28 d
Concentration Range	Nominal: 100 mg/L Actual: Not determined mg/L
Remarks – Method	The test item was directly added, at a concentration of 100 mg/L to the inoculum. Sodium acetate, also at 100 mg/L, was used as a reference control. Incubation was then for 28 d. The extent of degradation was determined as the ratio of the biochemical oxygen demand to either the theoretical oxygen demand (ThoD).

RESULTS

Test substance		*Sodium Acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	4	7	68
14	6	14	76
21	10	21	85
28	15	28	89

Remarks – Results	The validity criteria for the test were met.
CONCLUSION	The test item is moderately biodegradable (ultimate biodegradability).
TEST FACILITY	Henkel KGaA, (2000a)

C.1.3. Bioaccumulation (Analogue 3)

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test EC Directive 98/73/EC C.13 Bioconcentration: Flow-Through Fish Test
Species	*Oncorhynchus mykiss* (rainbow trout)
Exposure Period	Exposure: 28 days Depuration: 9 days
Auxiliary Solvent	Acetonitrile
Concentration Range	Nominal: 7-35 mg/L Actual: Not determined mg/L
Analytical Monitoring	
Remarks - Method	A stock solution of the test item was made up at 100 mg/L in methanol. This was diluted in a solution of acetonitrile/HPLC water (50:50) containing 2% formic acid to obtain the nominal concentrations: 7 and 35 µg/L. These two test concentrations were administered to the fish (each group consisting of 85 fish) via water. A third group of fish, which were not exposed to the test item, acted as a negative control. The amount of the test item with respect to body weight and lipid content were measured. A limit of quantification of 0.35 µg/g body weight was established.
RESULTS	
Bioconcentration Factor	138 (7 µg/L) and 159 (35 µg/L)
CT50	
Remarks - Results	Recovery rates between 91 and 103% were obtained from fish and water during method validation. No significant lethal or non-lethal effects, or anomalous feeding behaviour, were found amongst the control and test item groups. The uptake phase was marked by a distinct increase in the uptake of test substance within 6 days, followed by a clear plateau. After transfer to the depuration phase, the level of test substance rapidly diminished, reaching a level below that of quantification after 4 (7 µg/L) and 8 days (35 µg/L).
CONCLUSION	The analogue and by inference, the notified chemical is not bioaccumulative.
TEST FACILITY	Dr U. Noack-Laboratorien, (2009)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish (STD 1640)

TEST SUBSTANCE	Notified Chemical (STD 1640)
METHOD	OECD TG 203 Fish, Acute Toxicity Test Static EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish
Species	*Danio rero* (zebrafish)
Exposure Period	96 h
Auxiliary Solvent	
Water Hardness	10-250 mg $CaCO_3$/L
Analytical Monitoring	
Remarks – Method	A preliminary test was conducted with six fish each for test item, with a nominal loading rate of 100 mg/L (saturated solution), and a negative control. The fish were inspected daily. No mortalities were observed. The

definitive test used seven fish for each of the test item and a negative control. Observations were made at 2, 24, 78 and 96 h. The concentration of the test item was measured in the test concentration and control at the commencement of the study and at 96 h.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*2h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	7	0	0	0	0	0
100	0.043	7	0	0	0	0	0

Nominal concentration mg/L	*Measured concentrations (µg/L) after x h*	
	0 h	96 h
0	< LOQ	< LOQ
100	206	9.31

LOQ – limit of quantification

The geometric mean of the measured concentrations at 0 h and 96 h for the nominal 100 mg/L concentration was 43.8 µg/L.

LC50 > 43.8 µg/L at 96 hours

NOEC (or LOEC) ≥ 43.8 µg/L at 96 hours

Remarks – Results The validity criteria for the test were met. Oxygen saturation was 100% for the duration of the experiment.

CONCLUSION The notified chemical is not toxic to fish to the limit of water solubility.

TEST FACILITY Dr U. Noack-Laboratorien, (2015a)

C.2.2. Acute toxicity to fish (STD 1641)

TEST SUBSTANCE Notified chemical (STD 1641)

METHOD OECD TG 203 Fish, Acute Toxicity Test Semi-Static
EU guideline 92/69/EWG

Species *Danio rero* (zebrafish)

Exposure Period 96 h

Auxiliary Solvent None

Water Hardness Not recorded

Analytical Monitoring

Remarks – Method A preliminary range finding test was conducted with ten fish each for test item, with a nominal loading rates of 1 and 100 mg/L.

Six nominal test concentrations of the test substance were used in the definitive limit test and for each concentration 10 fish were used.

The solutions were renewed every 24 h. Concentrations of the nominal 10, 30 and 100 mg/L solutions were measured after 24 h.

RESULTS

Concentration (mg/L)	*Number of Fish*	*Mortality (%)*				
Nominal		*0h*	*24 h*	*48 h*	*72 h*	*96 h*
0	10	0	0	0	0	0
1	10	0	0	0	0	0
3	10	0	0	0	0	0
10	10	0	0	0	0	0
30	10	0	0	20	90	100
100	10	0	0	100	100	100

Nominal loading concentration (mg/L)	*Measured concentrations (mg/L) after x h* 0 h	24 h
10	0.2 / 0.2	0.0 / 1.1
30	0.1 / 0.2	0.4 / 1.5
100	2.0 / 1.3	1.8 / ND

LC50 17 mg/L nominal value estimated using half-logarithmic interpolation

Remarks – Results The validity criteria for the test were met. Oxygen saturation was ≥ 81% for the duration of the experiment.

CONCLUSION The notified chemical (STD 1641) is harmful to fish.

TEST FACILITY Henkel KGaA, (2001)

C.2.3. Acute toxicity to aquatic invertebrates (STD 1640)

TEST SUBSTANCE Notified chemical (STD 1640)

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

Species *Daphnia magna*
Exposure Period 48 hours *[acute study]*
Auxiliary Solvent None
Water Hardness 160 – 180 mg $CaCO_3$/L
Analytical Monitoring
Remarks – Method A saturated solution of the test item with a nominal loading rate of 100 mg/L was prepared in water by slow stirring.

A preliminary range finding test was performed using a negative control and a test item nominal concentration of 100 mg/L. For both there were two replicates of 10 *Daphnia* each. The measured geometric mean of the test item was 0.0363 mg/L. No immobilisation of *Daphnia* was observed.
The definitive test was performed using a negative control and a nominal test item concentration of 100 mg/L. For both there were four replicates of five *Daphnia* each. The measured geometric mean of the test item was 0.0295 mg/L.

A positive control test with a reference substance, potassium dichromate, was performed using four concentrations: 0.625, 1.00, 1.6, and 2.56 mg/L.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
0	-	20	0	0
100	0.0295	20	0	0

EC50 > 0.0295 mg/L at 48 hours *[acute]*
NOEC (or LOEC) ≥ 0.0295 mg/L at 48 hours *[acute]*
Remarks - Results The validity criteria for the test were met. The dissolved oxygen concentrations after 24 h for the control and 100 mg/L were 8.10 and 8.53 mg/L, respectively.

CONCLUSION The notified chemical (STD 1640) is not toxic to the limits of water solubility.

TEST FACILITY Dr U. Noack-Laboratorien, (2015b)

C.2.4. Acute toxicity to aquatic invertebrates (STD 1641)

TEST SUBSTANCE	Notified chemical (STD 1641)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction test
Species	*Daphnia magna*
Exposure Period	48 h
Auxiliary Solvent	
Water Hardness	Total hardness 218 mg $CaCO_3$/L
Analytical Monitoring	
Remarks – Method	In a preliminary test four replicates, each replicate having five *Daphnia*, were used for each of four mixtures of a nominal concentration of 10 mg/L and one of a nominal concentration of 5 mg/L. Different separation techniques (centrifugation, prolonged exposure to dissolving, filtration) were tested to establish the best method to remove undissolved particles. Filtration was the most efficient method. A fourth sample was filtered and diluted 1 in 10. The 5 mg/L sample was also filtered. Immobilisation of *Daphnia* for the centrifugation, prolonged exposure to dissolving, and filtration techniques was 30%, 15% and 15%, respectively, while it was 5% for each of the 1 in 10 filtered dilution and filtered 5 mg/L sample. No immobilisation was observed for the negative control. *Daphnia* in the centrifuged and prolonged exposure experiments were covered with particles that could adversely affect their survival. Based on these results, the filtered 5 mg/L sample was used for the definitive test. The concentration in the definitive limit test was nominally 5 mg/L. This concentration and a negative control were tested in eight replicates, each replicate having five *Daphnia*. Daphnia were observed for immobilisation at 24 and 48 h. A positive control test with a reference substance, (potassium dichromate), was performed less than 1 month prior to this study.

RESULTS

Concentration mg/L		*Number of Daphnia*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0		40	0	1
5		40	0	2

EC50	> 5 mg/L
NOEC (or LOEC)	≥ 5 mg/L
Remarks - Results	The validity criteria for the test were met. In the definitive test, the dissolved oxygen concentrations for the control and 5 mg/L were 8.35 and 8.26 mg/L, respectively. The EC50 of the reference substance was calculated as 0.57 mg/L.
CONCLUSION	The notified chemical is not toxic to daphnia to the limit of its water solubility.
TEST FACILITY	Institute of Industrial Organic Chemistry Branch Pszczyna, (2015)

C.2.5. Chronic toxicity to aquatic invertebrates (Analogue 3)

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 211 *Daphnia magna* reproduction test (semi-static)
Species	*Daphnia magna*

Exposure Period	21 d
Auxiliary Solvent	None reported
Water Hardness	140 – 150 Total hardness mg/L
Analytical Monitoring	
Remarks – Method	Nominal concentrations of the test item were 0 (negative control), 0.01, 0.032, 0.1, 0.3, and 1.0 mg/L. These corresponded to mean measured concentrations of 0, 0.00952, 0.0267, 0.189, 0.308 and 0.986 mg/L, respectively. The highest test concentrations are above the water solubility. For each concentration, 10 *Daphnia* were used, the respective solutions being changed three times a week, for a total time period of 21 d. Test item concentrations from the fresh media and old media were analytically verified via HPLC at regular intervals.

RESULTS

Concentration (mg/L)		*Number of Daphnia*	*Mortality of adults*	*Number juveniles per parent*
Nominal	*Actual*		*21 d (%)*	*21 d*
0	0	10	0	60.8 ± 11.6
0.01	0.00952	10	0	52.8 ± 9.25
0.032	0.0267	10	10	47.2 ± 6.61
0.1	0.189	10	20	56.3 ± 11.0
0.3	0.308	10	30	51.0 ± 14.1
1.0	0.986	10	100	-

EC50	0.381 mg/L (measured)
NOEC (or LOEC)	0.189 mg/L (measured)

Remarks - Results	The validity criteria for the test were met. After 21 d, the average number of juveniles per parent in the negative control group was 60. 8, which is greater than the number of 60 required in the test guidelines. At any of the measured concentrations of the test item (except 0.986 mg/L), the average number of juveniles per parent was not statistically different from the control (one way analysis Bonferroni t-test p = 0.05). Mortality of the *Daphnia* in the control did not exceed 20% by the end of the test. The percentage of stillborn individuals or aborted eggs was ≤ 5 % in both the control and test item concentrations.
CONCLUSION	The analogue and by inference, the notified chemical is harmful to aquatic invertebrate with long lasting effects.
TEST FACILITY	Dr U. Noack-Laboratorien, (2008)

C.2.6. Chronic toxicity to aquatic invertebrates (Analogue 2)

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 211 *Daphnia magna* reproduction test (semi-static)
Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	
Water Hardness	140 – 150 Total hardness mg/L
Analytical Monitoring	
Remarks – Method	Based on an acute *Daphnia* study, the test was performed with the following nominal dilutions of the stock solution: 0.18, 0.45, 1.12, 2.8, and 7.0 mg/L. Ten *Daphnia* were used for each of the test concentrations and the negative control. Test solutions were exchanged three times per week. Analytical monitoring of the concentrations of the test items was carried out at five test intervals.

RESULTS

Concentration (mg/L) Nominal	*Mean reproductive output (from ten Daphnia)*	*Standard deviation*	*Percent reduction offspring*
0	104	7.7	0
0.18	104	14.2	0
0.45	106	14.1	- 1.6
1.12	100	17.2	4.2
2.8	104	16.0	0.6
7.0	92	11.6	11.6

EC50 > 7.0 mg/L (nominal)
NOEC / LOEC ≥ 7.0 mg/L (nominal)

Remarks - Results The validity criteria for the test were met. After 21 d, the average number of juveniles per parent in the negative control group was 104. Analytical monitoring revealed that all the test solutions were on average < 0.01 mg/L.

CONCLUSION The test item at the evaluated concentrations did not induce any statistically significant mortality or immobility of adult *Daphnia*. Effects on reproduction were only recorded at the nominal concentration of 7.0 mg/L.

TEST FACILITY Evonik (2009)

C.2.7. Algal growth inhibition test (STD 1641)

TEST SUBSTANCE Notified Chemical (STD 1641)

METHOD OECD TG 201 Alga, Growth Inhibition Test
EC Council Regulation No 440/2008 C.3 Algal Inhibition Test
Species *Scenedesmus subspicatus*
Exposure Period 72 hours
Concentration Range Nominal: 1 – 100 mg/L
Actual: 0.6 – 1.9 mg Total Organic carbon (TOC)/L
Auxiliary Solvent None
Water Hardness Not Reported
Analytical Monitoring TOC
Remarks - Method Test item nominal concentrations of 0, 1, 3, 10, 30, and 100 mg/L were used. Measured test item concentrations for the nominal 10 and 100 mg/L concentrations at 0 h were 0.6 and 0.8 mg/L, respectively, and after 72 h were 1.9 and 0.7 mg/L, respectively. The starting concentration of the algae was 1 x 10^4 cells/mL. Concentrations of the algae were measured at 24, 48 and 72 h.

RESULTS

Biomass *EbC50 mg/L at 72 h*	*Growth* *ErC50 mg/L at 72 h*
1.9	8.4

Remarks - Results The measured concentrations of the test item in water are much greater than the water solubility values (0.070, 0.073 mg/L). Validity criteria were met as the biomass increase in the negative control was 43 fold within a 72 h period),

CONCLUSION Increased concentration of the test item was found to inhibit the growth of the alga.

TEST FACILITY Henkel (2000b)

C.2.8. Inhibition of microbial activity (Analogue 3)

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10-10,000 mg/L Actual: Not determined
Remarks – Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. 3,5-Dichlorophenol was used as the reference control. The test was conducted within the temperature range of 20-23°C, just outside the recommended range of 18-22°C. This did not appear to materially affect the study.
RESULTS	
IC50	> 10,000 mg/L at 3 hours
NOEC	Not determined
Remarks – Results	All validity criteria for the test were satisfied. The EC50 for the reference substance was 15.2 mg/L and the coefficient of variation between replicates was 0%. The 3 h IC50 was determined to be > 10,000 mg/L, based on nominal concentrations.
CONCLUSION	The analogue is not inhibitory to microbial respiration.
TEST FACILITY	Evonik (2010)

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