File No: STD/1693

September 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro-

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

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

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

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1693	Henkel Australia Pty Ltd	1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro-	Yes	≤ 5 tonnes per annum	Component of household cleaning products

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Skin sensitisation (Category 1B)	H317 - May cause an allergic skin reaction

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

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

Environmental Risk Assessment
On the basis of the low hazard and reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

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

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

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

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 chemical during reformulation processes:
  - Enclosed, automated processes, where possible
  - Local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during reformulation processes:
  - Avoid contact with skin
  - Avoid inhalation of aerosols

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:
  - Coveralls
  - Impervious gloves
  - 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 chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Storage

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

Disposal

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the final use concentration of the notified chemical exceeds 0.1% in household cleaning products;
- the finalised repeated dose toxicity report becomes available;

or

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Henkel Australia Pty Ltd (ABN: 82 001 302 996)
135-141 Canterbury Road
KILSYTH VIC 3137

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are not varied.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU (2010) and Korea (2011)

2. IDENTITY OF CHEMICAL

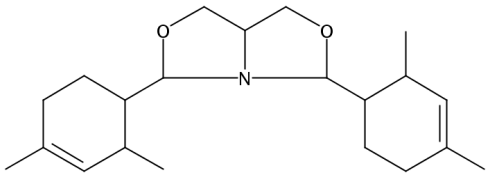
MARKETING NAME(S)
Sa57

CAS NUMBER
1196069-20-1

CHEMICAL NAME
1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro-

MOLECULAR FORMULA
$C_{21}H_{33}NO_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
331.49 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
100%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

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

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellow liquid

Property	*Value*	*Data Source/Justification*
Glass transition temperature	-29 °C	Measured
Boiling Point	305 °C at 102.7 kPa	Measured
Density	1,040 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.8×10^{-4} kPa at 20 °C	Measured
Water Solubility	4×10^{-4} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Unstable at pH 4 $t_{½}$ = 0.9 hours at 20 ºC, pH 7 $t_{½}$ = 11.9 hours at 20 ºC, pH 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.38 at 23 °C	Measured
Adsorption/Desorption	log Koc > 4.09	Measured
Dissociation Constant	Not determined	Does not contain dissociable functionality
Flash Point	172.5 °C at 101.3 kPa	Measured
Flammability	Not flammable	Measured
Autoignition Temperature	280 °C	Measured
Explosive Properties	Not thermally or mechanically sensitive	Measured
Oxidising Properties	Not oxidising	Measured

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

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

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

The notified chemical has a flash point of 172.5 ºC which is greater than 93 °C. Based on *Australian Standard AS1940* definitions for combustible liquid, the notified chemical may be considered as a Class C2 combustible liquid.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of fragrance formulations or finished household cleaning products.

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

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

PORT OF ENTRY
Sydney and Brisbane

TRANSPORTATION AND PACKAGING
The imported notified chemical or products containing it will be transported by road to the notifier's warehouse or customers' facilities for storage or reformulation. Fragrance formulations containing the notified chemical at ≤ 12% concentration will be imported and distributed in 200 L drums and 1,000 L intermediate bulk containers. End-use products containing the notified chemical at ≤ 0.1% concentration will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used in a variety of household cleaning products at ≤ 0.1% concentration.

OPERATION DESCRIPTION

Reformulation
The reformulation processes for incorporating the notified chemical into end-use products will likely vary depending on the specific type of household cleaning products formulated. This may involve both automated and manual processes including transferring and blending the notified chemical with other formulations. According to the notifier, a typical blending operation will be highly automated and occur in a fully enclosed/contained environment, followed by automated filling using sealed delivery systems into containers of various sizes.

End-use
Finished household cleaning products containing the notified chemical will be used by consumers and professional cleaners. The products may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines or open processes, and manually applied by sponge, mop, spray or brush followed by wiping or rinsing.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse	incidental	incidental
Mixer	4	5
Drum handling	4	5
Drum cleaning/washing	4	5
Maintenance	4	2
Quality control	2	5
Packaging	4	5
Professional end-use	8	365

EXPOSURE DETAILS

Transport and storage workers
Transport, storage and warehouse workers may come into contact with the notified chemical at ≤ 12% concentration only in the unlikely event of accidental rupture of containers.

Reformulation workers
During reformulation, dermal, ocular and possible inhalation exposure of workers to the notified chemical (at up to 12% concentration) may occur during weighing, transfer, blending, quality control analysis and cleaning/maintenance of equipment. Exposure is expected to be minimised through the use of local exhaust ventilation and enclosed and automated systems, and through the use of personal protective equipment (PPE) such as impervious gloves, safety glasses, protective clothing and respiratory protection.

Professional end users
Exposure to the notified chemical in end-use products (at ≤ 0.1 % concentration) may occur in professions where the services provided involve the use of cleaning products in the cleaning industry. The principal route of exposure is expected to be dermal, while ocular and inhalation exposures are also possible. Such professionals may use PPE

to minimise repeated or prolonged exposure and ensure that good hygiene practices are in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be repeated exposure of the public to the notified chemical at ≤ 0.1% concentration through the use of a wide range of household cleaning products. The principal route of exposure will be dermal, while ocular and inhalation exposures are also possible, particularly if products are applied by spray.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – rat	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation – *in vitro* human skin model test	non-irritating
Eye irritation – *in vitro* HET CAM	non-irritating
Skin sensitisation – mouse local lymph node assay	evidence of sensitisation (EC3 = 26.1%)
Repeat dose oral toxicity – rat, 28 days	NOAEL > 1000 mg/kg bw/day*
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration test	non genotoxic
Genotoxicity – *in vitro* mammalian cell gene mutation test	genotoxic
Genotoxicity – combined *in vivo* mammalian micronucleus and mammalian alkaline comet test	non genotoxic

* Based on the draft study report

Toxicokinetics

No data on toxicokinetics for the notified chemical was provided. For dermal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption (ECHA, 2017). Dermal uptake is likely to be moderate to high if the water solubility is between 100-10,000 mg/L and the partition coefficient (log Pow) values between 1 and 4 (ECHA, 2017). Based on the low molecular weight (331.49 g/mol), water solubility (0.4 mg/L) and partition coefficient (log Pow = 3.38 at 23 °C) of the notified chemical, absorption across biological membranes may occur.

Acute Toxicity

The notified chemical was of low acute oral toxicity when tested in rats.

Irritation

According to the results of *in vitro* assays, the notified chemical is not classified as a skin or eye irritant.

Sensitisation

The notified chemical was a skin sensitiser in a mouse local lymph node assay. When tested up to 100% concentration, the sensitisation potency (expressed as an EC3) was calculated to be 26.1%, warranting a classification as Category 1B skin sensitisation. The GHS cut-off for products/mixtures containing Category 1B skin sensitisers is > 1% concentration.

Repeated Dose Toxicity

In a repeated dose oral (gavage) toxicity study the notified chemical was administered to rats at 100, 300 and 1000 mg/kg bw/day for 4 weeks.

There were several mean clinical chemistry parameters that were different to the control means and some were statistically significant. However, these findings were not considered by the study authors to be of toxicological relevance as all mean values for these parameters were within the range of historical control data and there were no histopathological findings that correlated to the statistically significant parameters.

The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day (the highest dose tested) by the study authors, as all treatment-related changes were considered to be either of no toxicological relevance or non-adverse.

Mutagenicity/Genotoxicity
The notified chemical showed negative results in a bacterial reverse mutation assay, and was not clastogenic in an *in vitro* chromosome aberration test although an increased number of polyploidy cells were observed at the highest dose in the absence of metabolic activation. The notified chemical was clastogenic in an *in vitro* mammalian cell gene mutation test with Chinese hamster V79 cells. In a combined *in vivo* mammalian micronucleus and mammalian alkaline comet test the notified chemical was not clastogenic or aneugenic. Based on the negative results in the *in vivo* assay, the notified chemical is expected to be non-genotoxic.

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

Hazard Classification	*Hazard Statement*
Skin sensitisation (Category 1B)	H317 - May cause an allergic skin reaction

6.3. Human Health Risk Characterisation
The notified chemical is a weak skin sensitiser. Formulations containing the notified chemical at > 1% concentration may pose risks of skin sensitisation if exposed to it dermally. Therefore, control measures are required to mitigate possible adverse health effects to the workers who may come into contact with the notified chemical.

6.3.1. Occupational Health and Safety

Transport, Storage and Reformulation
Exposure of workers to the notified chemical (at ≤ 12% concentration) may occur during transport and blending operations. During reformulation, worker exposure will be limited through the use of engineering controls (such as enclosed, automated systems and local exhaust ventilation) and appropriate PPE (eye/skin protection and respiratory protection if inhalation exposure may occur), as stated by the notifier. Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

End-Use
Workers involved in professions where the services provided involve the use of household cleaning products in the cleaning industry may be exposed to the notified chemical at ≤ 0.1% concentration. PPE may be employed by workers to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the risk to such workers is expected to be of a similar or lesser extent than that for consumers using various cleaning products containing the notified chemical. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health
Members of the public will experience widespread and frequent exposure to the notified chemical at ≤ 0.1% concentration through use of household cleaning products. The main route of exposure is expected to be dermal and inhalation, with some potential for accidental ocular or oral exposure.

The notified chemical is a weak skin sensitiser. However, risk of skin sensitisation is not expected at the proposed low concentrations (≤ 0.1%) in end-use products.

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported as a fragrance ingredient for reformulation into finished laundry and household cleaning products. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and leaks. In the event of spills, the product containing the notified

chemical is expected to be collected with adsorbents and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve highly automated blending operations within a fully enclosed environment. Therefore, significant release of the notified chemical to the environment is not expected. The process will be followed by automated filling of the formulated products into containers of various sizes suitable for use. Wastes containing the notified chemical generated during reformulation include equipment wash water, residues in empty import containers and spilt materials. The notifier estimates that up to 0.2% of the import volume of the notified chemical may be released from reformulation and cleaning operations. Any wash waters resulting from the blending and cleaning operations are likely to be discharged to an on-site wastewater treatment plant before being discharged to sewer. Empty import containers are expected to be recycled or disposed of through licensed waste management services.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemical is expected to be released to sewer across Australia as a result of its use in laundry and household cleaning products. A small proportion of the notified chemical is expected to be disposed of to landfill as residues in empty end-use containers.

RELEASE OF CHEMICAL FROM DISPOSAL

A small proportion of the notified chemical may remain in end-use containers. Wastes and residues of the notified chemical in empty containers are likely either to share the fate of the container and be disposed of to landfill, or to be released to sewer when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Following its use, the majority of the notified chemical is expected to enter the sewer system through its use as a fragrance ingredient in laundry and household cleaning products before potential release to surface waters nationwide. The notified chemical is hydrolytically unstable in environmental conditions and will decompose during use. The notified chemical is not considered to be readily biodegradable (14% in 28 days) but ultimately biodegrades to form water and oxides of carbon and nitrogen. Based on its instability and log Pow of < 4.2, the notified chemical is also not expected to bioaccumulate. For the details of the environmental fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A predicted environmental concentration (PEC) worst case scenario has been calculated. It was assumed that 100% of the annual import quantity of the notified chemical is released to the sewer from its use in laundry and household cleaning products over 365 days/year, with no removal of the notified chemical by sewage treatment plant (STP) processes. The extent to which the notified chemical is removed from the effluent in STP processes based on the properties of the notified chemical has not been considered for the worst-case scenario:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	5,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	5,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	13.70	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River:	2.81	µg/L
PEC – Ocean:	0.28	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 2.81 µg/L may potentially result in a soil concentration of approximately 0.019 mg/kg. Assuming accumulation

of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 0.094 mg/kg and 0.19 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LL50 > 110 mg/L (WAF)	Not harmful to fish up to the limit of its water solubility
Daphnia Toxicity	48 h EC50 = 1.11 mg/L* (Study 1) 48 h EL50 > 110 mg/L (WAF) (Study 2)	Toxic to aquatic invertebrates Not harmful to aquatic invertebrates up to the limit of water solubility
Algal Toxicity	72 h EC50 > 2.2 mg/L	Not harmful to algae up to the limit of its water solubility
Respiration Inhibition Activated Sludge	3 h EC50 > 1000 mg/L	Not inhibitory to microbial activity up to the limit of its water solubility

WAF: Water accommodated fraction
* Above the limit of water solubility

Based on the above ecotoxicological data, the notified chemical is not expected to be acutely toxic to the limit of water solubility (see section C.2.2.). Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic toxicities. It must be reiterated that the notified chemical is hydrolytically unstable, which can lead to experimental problems in tests on aquatic toxicity as it is difficult to ensure defined and stable test substance concentrations.

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated, since the notified chemical is not harmful to aquatic life up to the limit of its solubility in water.

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) for the aquatic compartment has not been calculated as a PNEC value is not available. However, the notified chemical is hydrolytically unstable in environmental conditions and is not expected to bioaccumulate. Whilst the notified chemical is not readily biodegradable, it is considered to be ultimately biodegradable. Therefore, on the basis of the low hazard and assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point Glass transition temperature (amorphous components) at -29 °C

Method OECD TG 102 Melting Point/Melting Range
Remarks Determined by differential scanning calorimetry
Test Facility Henkel (2011a)

Boiling Point 305 °C at 102.7 kPa

Method OECD TG 103 Boiling Point
Remarks Determined by differential scanning calorimetry
Test Facility Henkel (2011b)

Density 1,040 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids
Remarks Determined using a oscillating densitimeter
Test Facility Henkel (2011c)

Vapour Pressure 1.8×10^{-4} kPa 20 °C

Method OECD TG 104 Vapour Pressure
Remarks Grain-Watson estimation
Test Facility Henkel (2011d)

Water Solubility 4×10^{-4} g/L at 20 °C

Method OECD TG 105 Water Solubility
EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks Column Elution Method
Test Facility Henkel (2010)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH

pH	*T (°C)*	*$t_{½}$ hours*
4	ND	ND
7	20	0.9
9	20	11.9

ND: The half-life period could not be determined due to the fast hydrolysis. The test item could not be detected.

Remarks GC-MS
Test Facility Henkel (2013)

Partition Coefficient (n-octanol/water) log Pow = 3.38 at 23 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method
Test Facility Henkel (2011e)

Adsorption/Desorption log Koc > 4.09

Method OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks HPLC method
Test Facility Henkel (2014)

Flash Point 172.5 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Closed cup method
Test Facility	Henkel (2011f)

Flammability Not flammable when contact with water

Method	EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water)
Remarks	Maximum gas generation rate < 1 L $kg^{-1}h^{-1}$
Test Facility	Henkel (2011g)

Autoignition Temperature 280 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility	Henkel (2011h)

Explosive Properties Not thermally or mechanically sensitive

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Friction sensitivity was not tested because the test item is a liquid.
Test Facility	Henkel (2011i)

Oxidizing Properties Not oxidising

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks	Mean pressure rise time of test mixture (19669 ms) was greater than that of reference item (3697 ms).
Test Facility	Henkel (2011j)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method
- Species/Strain: Rat/RccHan:WIST (SPF)
- Vehicle: Polyethylene glycol (PEG) 300
- Remarks – Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	2,000	0/3
2	3 M	2,000	0/3

- LD50: > 2,000 mg/kg bw
- Signs of Toxicity: No clinical signs of toxicity were noted.
- Effects in Organs: No macroscopic findings were recorded at necropsy.
- Remarks – Results: The body weight of the animals was within the range commonly recorded for this strain and age.

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

TEST FACILITY: Harlan (2010a)

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

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method
EPISKIN™ Reconstructed Human Epidermis Model
- Vehicle: None
- Remarks – Method: In a preliminary test the test substance was shown not to directly reduce MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide].

The test substance (10 µL) was applied to the tissues in triplicate. Following exposure period of 15 minutes (at 37 °C), the tissues were rinsed and incubated for 42 hours, before being treated with MTT and then incubated at 37 °C for 3 hours.

Negative and positive controls were run in parallel with the test substance:
- Negative control: deionised water
- Positive control: 5% sodium dodecyl sulphate in deionised water

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.896	100	4.4
Test substance	0.935	104.3	5.1
Positive control	0.085	9.5	2.6

OD = optical density; SD = standard deviation

- Remarks – Results: The relative mean viability of the tissues treated with the test substance was > 50% (predicted as non-irritating according to the test guideline).

CONCLUSION	Based on the relative mean tissue viability of > 50%, the notified chemical is not classified as a skin irritant according to the GHS criteria.
TEST FACILITY	Harlan (2010b)

B.3. Eye Irritation – *In Vitro* HET-CAM Test

TEST SUBSTANCE	Notified chemical
METHOD	Similar to the method described by Luepke N.P. and Kemper F.H. (1986)
Vehicle	None
Remarks – Method	Embryonic hens eggs (HETs) were incubated for 8 days at 37.5 °C and rotated to prevent an attachment of the embryo to one side of the egg. On the 9th day, the chorioallantoic membrane (CAM) was exposed by removing the outer shell and shell membrane. Neat test substance was tested on 3 eggs. Each CAM was exposed to 300 µL of test substance and observed for 5 minutes. Negative control was 0.9% physiological sodium chloride solution and positive controls were 1% solution of sodium dodecyl sulphate (SDS) and 0.1 N sodium hydroxide (NaOH). A dilution of Texapon ASV Spezial with 5% active substance (AS) was used as a reference item because of its known eye irritating properties.

RESULTS

Test material	*Mean Time till Haemorrhage (s)*	*Mean Time till Lysis (s)*	*Mean Time till Coagulation (s)*	*Mean Irritancy Index*
Negative control	301	301	301	0.00
Test substance	301	301	301	0.00
Positive Control - NaOH	10	47.7	17.7	19.26
Positive Control - SDS	13.7	30	301	11.11
Reference Item	19.7	40	301	10.78

Remarks – Results	No irritating effects were observed during 5 min incubation with the test substance. The calculated irritancy mean index is 0.00. The positive controls, negative control and the reference item gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not considered an eye irritant under the conditions of the test.
TEST FACILITY	Harlan (2010c)

B.4. Skin Sensitisation – LLNA

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse//CBA/Ca
Vehicle	Acetone/olive oil (4:1)
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.
Remarks – Method	No significant protocol deviations

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	4 F	272.6	-
25	4 F	757.9	2.78
50	4 F	2094	7.68
100	4 F	4484	16.45

EC3 26.1%

Remarks – Results There were no deaths or signs of systemic toxicity observed in the test or control animals. The body weights of the animals were within the range commonly recorded for animals of the strain and age.

CONCLUSION There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY Harlan (2010d)

B.5. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents

Species/Strain Rat/Wistar

Route of Administration Oral – gavage

Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: none

Vehicle Corn oil

Remarks – Method Functional observations were not conducted in female animals in week 4 in the 300 and 1,000 mg/kg bw/day dose groups.
No other significant protocol deviations.
Only the draft report without individual findings was provided.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5 per sex	0	0/10
Low Dose	5 per sex	100	0/10
Mid Dose	5 per sex	300	0/10
High Dose	5 per sex	1,000	0/10

Mortality and Time to Death

There were no unscheduled deaths.

Clinical Observations

Moving the bedding was noted in all animals treated at 300 mg/kg bw/day and 1000 mg/kg bw/day. Slight to moderate salivation was noted for nearly all animals treated at 300 mg/kg bw/day or 1000 mg/kg bw/day on several days. These findings were not considered by the study authors to be a systemic adverse effect or of toxicological relevance as there were considered to be a sign of a local reaction to the test substance. No clinical signs of toxicity were noted.

In treated animals, no effects were observed due to the treatment in any of the parameters of the functional observation battery when compared with the controls. There were no biologically relevant differences in body temperature between the groups.

There were no body weight gain changes due to the treatment. However, male animals at 1,000 mg/kg bw/day showed a 27% reduction in mean body weight compared to control mean weight during the first week of treatment. All animals showed weight gain during the study.

There were no changes in food intake due to the treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology and Blood Coagulation

No effects on haematological parameters and coagulation parameters were noted in treated animals with the exception of a severely and statistically significantly increased mean eosinophils count in females treated at 300 mg/kg bw/day. The effect was not seen in male animals and was not seen at 1,000 mg/kg bw/day in females and hence was considered to be incidental.

Clinical Chemistry

Statistical significance was found for slightly decreased mean values for creatinine in males treated at 300 mg/kg bw/day or 1,000 mg/kg bw/day, for aspartate aminotransferase in males treated at 100 mg/kg bw/day or 300 mg/kg bw/, for total cholesterol in males treated at 300 mg/kg bw/day and urea in males treated at 300 mg/kg bw/day or 1,000 mg/kg bw/day. Glucose was slightly and statistically significantly decreased for females treated at 300 mg/kg bw/day or 1000 mg/kg bw/day and a moderate statistical significant increase was noted for total bile acids in females treated at 1,000 mg/kg bw/day. These findings were not considered by the study authors to be of toxicological relevance as all mean values for these parameters were within the range of historical control data and there were no histopathological findings that correlated to the statistically significant parameters. There were no statistically significant changes were reported at 100 mg/kg bw/day.

Urinalysis

No statistically significant changes were noted in urinary parameters between dose groups and control group.

Effects in Organs

At necropsy, the thymus was enlarged in a male animal treated at 100 mg/kg bw/day. The thymus was brown coloured and axillary and mesenterial lymph nodes were red in a male animal treated at 1,000 mg/kg bw/day. The thymus showed red foci in a female animal treated at 300 mg/kg bw/day. The uterus was seen in two females each treated at 100 and 300 mg/kg bw/day. Histopathological showed no differences compared to the control animals.

In animals treated at 1,000 mg/kg bw/day mean relative liver weight showed a statistically significant increase (males: 19.06% above control, females: 30.06% above control). No histopathological effects were reported.

Absolute mean brain weight was slightly and statistically significantly increased in males treated at 300 mg/kg bw/day. The finding was not considered by the study authors to be test substance-related as no dose response was seen and histopathological evaluation showed no effects related to treatment.

There was a minimal increase of hyaline inclusions in tubular epithelia in the kidneys of males treated at 1,000 mg/kg. This finding was male rat specific and was due to deposition of α2-microglobulin.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 1,000 mg/kg bw/day by the study authors, considering that all treatment-related changes observed were either of no toxicological relevance or non-adverse.

TEST FACILITY	BSL (2018)

B.6. Genotoxicity – Bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation procedure/Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in	Test 1

Main Test	a) With metabolic activation: 3 – 5000 µg/plate b) Without metabolic activation: : 3 – 5000 µg/plate Test 2 a) With metabolic activation: 10 – 5000 µg/plate b) Without metabolic activation: : 10 – 5000 µg/plate
Vehicle	Dimethylformamide
Remarks – Method	The dose selection for Test 2 was based on the toxicity observed in the preliminary test (also reported as Test 1) carried out at 3 – 5000 µg/plate. Positive controls: With metabolic activation: 2-aminoanthracene Without metabolic activation: sodium azide (TA1535, TA100); 4-nitro-o-phenylene-diamine (TA1537, TA98); methyl methane sulfonate (WP2uvrA)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	≥ 1000	negative
Test 2		≥ 5000	≥ 1000	negative
Present				
Test 1	≥ 5000	> 5000	≥ 1000	negative
Test 2		≥ 5000	≥ 1000	negative

Remarks – Results	No significant increases in the frequency of revertant colonies were observed for any of the bacterial strains, at any concentration tested, either with or without metabolic activation. The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Harlan (2010e)

B.7. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
Species/Strain	Human
Cell Type/Cell Line	Peripheral lymphocytes
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat livers
Vehicle	Dimethyl sulfoxide
Remarks – Method	The dose selection for the main experiments was based on toxicity in a dose range-finding study carried out at 1 – 1000 µg/mL. Vehicle and positive controls (mitomycin C and cyclophosphamide) were run concurrently with the notified chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10*, 33*, 100*	3 h	24 h
Test 2	3*, 10*, 33*, 100*, 150*, 200*, 250*, 300*	24 h	24 h
Test 3	0.3*, 1*, 3*, 10*, 33*, 100*, 200*	48 h	48 h
Present			

Test 1	10*, 33*, 100*	3h	24 h
Test 2	10*, 33*, 100*	3h	48 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1		> 100	≥ 100	negative
Test 2	≥ 333	≥ 300	≥ 100	negative
Test 3	≥ 100	≥ 200	≥ 100	negative
Present				
Test 1		> 100	≥ 100	negative
Test 2		≥ 100	≥ 100	negative

Remarks – Results

No effects on the number of cells with endoreduplicated chromosomes were observed both in the absence and presence of metabolism activation, indicating that there was no disturbance to cell cycle progression. However, the test substance increased the number of polyploid cells in the absence of metabolism activation, at the 48 h continuous exposure time at the highest concentration tested. This indicates that the test substance has potential to disturb mitotic processes.

In both main tests, no statistically significant increases in the frequency of cells with structural or numerical chromosome aberrations were observed in the presence or absence of metabolic activation.

The positive and negative controls gave a satisfactory response confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to human peripheral lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY

WIL (2013a)

B.8. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test

Species/Strain: Chinese hamster

Cell Type/Cell Line: V79

Metabolic Activation System: S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver

Vehicle: Dimethyl sulfoxide

Remarks – Method: No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0.1*, 0.3*, 1, 3*, 6.6*, 10*, 12.5*, 16*, 20, 25	4 h	6 days	6 days
Present				
Test 1	0.3, 1, 3*, 10*, 33*, 100*, 130*, 160*, 200*, 250*	4 h	6 days	6 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	≥ 33	≥ 16	> 25	positive
Present				
Test 1	≥ 333	≥ 250	≥ 100	positive

Remarks – Results: The test substance induced 6.8- and 5.6-fold increases in the mutant frequency at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in the absence and presence of metabolism activation, respectively. Although the observed increases were not concentration-related, the increases were outside the historical control data range and more than 3-fold the control values.

CONCLUSION: The notified chemical was clastogenic to Chinese hamster V79 cells treated *in vitro* under the conditions of the test.

TEST FACILITY: WIL (2013b)

B.9. Genotoxicity – *In Vivo* Mammalian Micronucleus and Mammalian Alkaline Comet Tests

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test
OECD TG 489 *In Vivo* Mammalian Alkaline Comet Assay

Species/Strain: Rat/Wistar WI (Han) (SPF)
Route of Administration: Oral – gavage
Vehicle: Corn oil
Remarks – Method: The dose selection for the main experiments was based on toxicity in a dose range-finding study carried out at 2000 mg/kg bw.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	5 M	0	75-76
II (low dose)	5 M	500	75-76
III (mid dose)	5 M	1000	75-76
IV (high dose)	5 M	2000	75-76
V (positive control, EMS, comet assay)	5 M	200	75-76
VI (positive control, CP, micronucleus assay)	5 M	20	75-76

EMS = ethyl methane sulfonate; CP = cyclophosphamide

RESULTS

Doses Producing Toxicity: The animals treated at 500 mg/kg bw and the animals of the negative and positive control groups showed no treatment related clinical signs of toxicity or mortality. The animals treated at 1000 mg/kg bw showed no treatment related clinical signs with exception of 3 animals showing lethargy after 72 hours.

Clinical findings in the group treated at 2000 mg/kg bw included lethargy and slight ataxia. The severity of the effects was considered by the study authors to be slight.

Genotoxic Effects: No increase in the mean frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of animals treated with the test substance compared to the vehicle control group.

	No statistically significant increase in the mean Tail Intensity (%) was observed in liver and glandular stomach cells of test substance-treated animals at any of the dose levels tested compared to the vehicle control group in the comet assay.
Remarks – Results	The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic or aneugenic under the conditions of the *in vivo* mammalian micronucleus and mammalian alkaline comet tests.
TEST FACILITY	Charles River (2018)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated Sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BSB/BOD Sensor System
Remarks – Method	The study was carried out in-line with the recommended test guidelines and GLP. No deviations were recorded. Nitrification was considered because the test item contained nitrogen and can influence the degree of biodegradation.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0	1	41
14	4	14	75
28	14	28	82

Remarks – Results	All validity criteria for the control and test conditions were satisfied. The reference item sodium benzoate was sufficiently degraded to 75% after 14 days and to 82% after 28 days of incubation. The oxygen demand in the abiotic control was zero, therefore no correction of the test item degradation rates were required. The degradation rate of the test substance did not reach 60% within the 10-day window and after 28 days of incubation when no nitrification is considered.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	IBACON (2010)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static
Species	Rare minnow (*Gobiocypris rarus)*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	145 mg $CaCO_3$/L
Analytical Monitoring	UPLC-PDA
Remarks – Method	No deviations to the method were recorded. A range finding test was conducted using five fish per test concentrations of 1, 10 and 100 mg/L, as well as a control (also five fish). No mortalities were observed in any of the test concentrations. A limit test was performed using a Water Accommodated Fraction (WAF) of the test substance based on the results of the range finding test. The WAF (loading rate 110 mg/L) was prepared by dispersing the solid test item in water and stirring for 24 hours after which the solution was allowed to settle for one hour. The aqueous phase was decanted and used directly without filtration. The concentration of the

notified chemical was measured at the start of the study and daily thereafter, until study termination. A positive control was also run as a separate test using potassium dichromate as the reference item.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*
Nominal	*Measured*		*96 h*
Control	-	21	0
110 (WAF)	2.71	21	0

WAF = Water Accommodated Fraction
LOQ = Limit of Quantitation

LC50	> 2.71 mg/L (measured, WAF) at 96 hours
NOEC	≥ 2.71 mg/L (measured, WAF) at 96 hours
Remarks – Results	All validity criteria were met. The mean measured concentration was 2.71 mg/L. The dissolved oxygen concentration in the test and control solutions was ≥ 74 % O_2 saturation at 23 °C. Although the concentration of the test substance decreased with time, all results were within 80% of the initial concentration indicating the notified chemical is sufficiently stable in water during the test period to use the static test protocol and the mean measured concentration. The notified chemical is rapidly hydrolysed in water ($t_{½}$ = 0.9 hours at 20°C, pH 7) so the measured concentration would be expected to decrease significantly over 96 hours. However, the measured concentration remained relatively constant over 96 hours and one possible explanation is the establishment of a steady-state system, where undissolved test substance (which is not removed by filtration when preparing the WAF) replenishes the notified chemical in solution which is removed by hydrolysis. No abnormal behaviour was observed in any of the treatments. The 24 hour LC50 of the positive control was 325 mg/L, which was within the acceptable range.
CONCLUSION	The notified chemical is not harmful to fish up to the limit of its water solubility.
TEST FACILITY	PEAPC (2016)

C.2.2. Acute Toxicity to Aquatic Invertebrates

PRELIMINARY COMMENTS	In the tests with *Daphnia*, Study 1 used ethanol as an auxiliary solvent to help solubilise the notified chemical. However, the results of pre-tests indicated that physical effects e.g. trapping of test organisms, potentially due to hydrophobic effects, was the cause of mortality. As a result, cages were used to ensure that the test item had no physical effect on the daphnids. In Study 2, the experimental design was changed, where the notifier used a WAF and a solubilising agent Tween 80 in a semi-static test. The concentration of the notified chemical could not be measured before solution renewal (24 hours). Both test results are valid and reliable, but represent different outcomes. However, further interpretation of the studies is required to determine relevance of the studies. Study 1 eliminated overestimation of toxicity from physical effects, by the use of cages. However, the use of a water miscible solvent can result in an interaction, which alters the toxic response (OECD 2019).

Study 2 showed no effects either physical or chemical, but again the use of an emulsifier may result in an interaction, which alters the toxic response (*ibid*). Therefore both studies cannot eliminate the potential effects of solvents/emulsifier.

However, for both studies the loading of the notified chemical in water was deliberately increased to above its water solubility, even though this could not be measured in Study 2. Study 2 demonstrated that even with overloading of the notified chemical there are no toxic effects to the limit of water solubility. For study 1 toxic effects are only apparent when the loading is in excess of the water solubility with the NOEC, being just above the water solubility of the notified chemical. Therefore the toxicity shown in study 1, is not relevant to environmental conditions where it exceeds the notified chemical's water solubility. Accordingly study 2 demonstrates that the notified chemical is not toxic to the limits of its water solubility and study 1 supports this evidence.

Therefore both tests demonstrate that there is no toxic effects at the limit of water solubility, of the notified chemical to *Daphnia.*

(STUDY 1)

TEST SUBSTANCE: Notified chemical

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

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Ethanol
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	GC-MS
Remarks – Method	Pre-experiments were performed to determine the solubility of the test item in test water and to select suitable methods for the preparation of a stock solution and the dosage of the test item into the test media. Due to the low water solubility and the degradation properties of the test item in water, the test item was diluted in ethanol. Since the test item formed undissolved droplets on the water surface of the aquaria, cages were introduced in the aquaria to ensure that the test item has no physical effect on the daphnids. Due to a calibration error that was discovered at the end of the test, the pumps delivered approximately 200 % of the desired test concentrations. However, as all results were based on measured concentrations, the increased dosage did not have an effect on the solvent control and the blank control. A positive control was also run as a separate test using potassium dichromate as the reference item.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Measured*		*24 h*	*48 h*
Control	Control	20	0	0
Solvent Control	Solvent Control	20	0	0
0.53	0.578	20	0	0
0.85	0.612	20	0	2
1.4	1.08	20	0	9
2.2	1.63	20	7	17
3.5	3.01	20	14	20

EC50 1.11 mg/L at 48 hours

NOEC	0.578 mg/L at 48 hours
Remarks – Results	Daphnia showed no immobilisation and no signs of stress in both controls and the dissolved oxygen was ≥ 8.9 mg/L; thus all the validity criteria were satisfied. The 24 hour EC50 of the positive control was 1.63 mg/L, which was within the acceptable range. In all of the test concentrations, oily droplets were observed on the water surface from test start to test end. The amount of droplets increased with increasing test concentrations, showing that the maximum water solubility under the test conditions was achieved. After 24 hours from the start of the test, the concentration of the test item was reduced to 40-70 % (except concentration 0.53 mg/L with 96 % recovery) of the nominal concentration before renewal. The EC50 was calculated by Probit analysis and the NOEC was determined directly from the raw data. It must be noted that at the highest nominal dose of 3.5 mg/L, all of the *Daphnia* were immobilised. However, this is at least 10 times the solubility limit of the test substance. At the lowest dose of 0.53 mg/L just above the water solubility limit, no *Daphnia* were immobilised which is probably a better and more practical representation of the toxicity.
CONCLUSION	The test substance is toxic to aquatic invertebrates.
TEST FACILITY	IBACON (2011a)

(STUDY 2)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi-Statich
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Tween 80 (Polyoxyethylene (20) sorbitan monooleate)
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	GC-MS
Remarks – Method	The test was carried out according to test guideline and GLP where no deviations were recorded. A limit test was performed using a water accommodated fraction (WAF) of the test substance at a nominal loading of 110 mg/L (as close as possible to saturation of the substance in water) and stirred for 24 hours in the dark at room temperature and allowed to settle for one hour. This stock solution was subsequently diluted to various concentrations. To avoid physical effects on daphnids due to the test substance's low water solubility (as observed in Study 1 above), a small amount (100 µL/L) of the nontoxic surfactant Tween 80 was added as a solubilising agent. However, because Tween 80 interferes with the analytical determination of the concentration of the test substance before and after 24 hours, an additional set of solutions and controls were tested on daphnids without Tween 80. A positive control was also run as a separate test using potassium dichromate as the reference item.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Measured*		*24 h*	*48 h*
Control	Control	20	0	0
Solvent Control	Solvent Control	20	0	0
6.875	ND	20	0	0
13.75	ND	20	0	0
27.5	ND	20	0	0
55	ND	20	0	0
110	ND	20	0	0

ND: Not Determined as Tween 80 interferes with GCMS analysis.

EL50	> 110 mg/L at 48 hours (95% Confidence Interval)
NOEL	≥ 110 mg/L at 48 hours (95% Confidence Interval)
Remarks – Results	*Daphnia* showed no immobilisation and no signs of stress in both controls and the dissolved oxygen was ≥ 8.3 mg/L; thus all the validity criteria were satisfied. The 24 hour EC50 of the positive control was 0.932 mg/L, which was within the acceptable range. The results of the parallel study run without Tween 80 for analytical determination demonstrated that the concentration at the nominal loading of 110 mg/L decreased by 42 % after 24 hours. The results using Tween 80 as a solubilising agent demonstrated that the test substance was not toxic to *Daphnia,* however, because those solutions could not be analysed by GCMS, the test substance remaining after 24 hours was unknown. The concentrations reported in the parallel test could be used as an approximate indication of the amount of test substance that remained.
CONCLUSION	The test substance is not harmful to aquatic invertebrates up to its water solubility limit.
TEST FACILITY	IBACON (2019)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation No 440/2008 C.3 Algal Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.1, 0.32, 1.0, 3.2 and 10 mg/L
Auxiliary Solvent	DMF
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	GC-MS
Remarks – Method	The study was carried out in-line with the recommended test guidelines and no deviations were recorded. A stock solution of the notified chemical was prepared by dissolving the solid material in DMF. The stock solution was diluted with DMF to prepare a series of solutions containing the notified chemical at different concentrations. Finally, test solutions were prepared by diluting these DMF solutions with water to give test media with a range of concentrations of the notified chemical but the same concentration of DMF (100 µL/L). The test encompassed seven treatment groups (5 dose rates/test item, a solvent control and a control) with three replicates per test concentration and six replicates for the controls.

RESULTS

Biomass		*Growth*	
EyC50 mg/L at 72 h	*NOEyC mg/L*	*ErC50 mg/L at 72 h*	*NOErC mg/L*
> 2.2*	0.7*	> 2.2*	0.7*

*Geometric mean measured concentrations

Remarks – Results: All validity criteria for the test were satisfied. The cell concentration of the control cultures increased by a factor of 173 after 72 hours Due to the low water solubility and the degradation properties of the test substance in water its concentration decreased below the LOQ after 24 hours at concentrations < 10 mg/L. The test substance could only be assessed at the highest nominal concentration of 10 mg/L, resulting in 22 % recovery after 72 h. In order to account for the decay of the test item, the effects assessment is conservatively based on this measured geometric mean applied to each concentration in the test. The 72-hour ErC50 and EyC50 were calculated by Probit analysis and the 72-hour NOECs were determined using the Bonferroni-Welch t-test. The 72-hour EC50 for the positive control was 1.64 mg/L based on the growth-rate.

CONCLUSION: The test substance is not harmful to algae up to the limit of its water solubility.

TEST FACILITY: IBACON (2011b)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE: Notified Chemical

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

Inoculum: Activated Sludge
Exposure Period: 3 hours
Concentration Range: Nominal: 10, 32, 100, 320 and 1000 mg/L
Remarks – Method: The test was carried out according to the test guidelines and no deviations were recorded. Six controls (pure water, synthetic sewage feed and inoculum, but without addition of the test item) were tested in parallel. The reference item 3,5-dichlorophenol was tested at the nominal test concentrations of 1, 4 and 16 mg/L (five replicates for each concentration). In parallel, a nitrification inhibitor N-allylthiourea (ATU) was tested with six controls at the same nominal concentrations of the test item and the reference item.

RESULTS
EC50: > 1000 mg/L
NOEC: 32 mg/L
Remarks – Results: All validity criteria for the inoculum control and reference items were met as recommended by the test guidelines. For the total respiration, in comparison to the inoculum controls, the total respiration rate of the activated sludge was not inhibited at the two lower test concentrations and only slightly inhibited for the other test concentrations. The inhibition was 4.4 % at 32 mg/L and 16.3 % at the highest test concentration of 1000 mg/L and as such, no 3-hour EC50 for total respiration could be established.

For respiration without nitrification, in comparison to the inoculum controls, the heterotroph respiration was constantly inhibited between 22 % and 28 % for all test item concentrations. A concentration related

	inhibiting effect could not be determined. For respiration based on nitrification, the respiration rates of the activated sludge was not inhibited by test item concentrations between 10 mg/L and 1000 mg/L (below 5 % inhibition for all test concentrations). The NOEC was determined to be above a test item concentration of 1000 mg/L.
CONCLUSION	The test substance is not harmful to bacterial respiration of activated sludge.
TEST FACILITY	IBACON (2018)

BIBLIOGRAPHY

BSL (2018) 28-Day Repeated Dose Oral Toxicity Study in Wistar Rats with Sa 57 (Study No. 1310706, July, 2018). Planegg, Germany, BSL Bioservice Scientific Laboratories Munich GmbH (Unpublished report submitted by the notifier).

Charles River (2018) A Combined Micronucleus and Alkaline Comet Test in the Rat with Sa 57 (Study No. 516562, April, 2018). DD 's-Hertogenbosch, The Netherlands, Charles River Laboratories Den Bosch BV (Unpublished report submitted by the notifier).

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance, June 2017, version 3.0. European Chemicals Agency, https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf.

Harlan (2010a) SAT 090073: Acute Oral Toxicity Study in Rats (Study No. 1310706, October, 2010). Füllinsdorf, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2010b): *In Vitro* Skin Irritation Test: Human Skin Model Test with SAT 090073 (Study No. 1310702, August, 2010). Rossdorf, Germany, Harlan Cytotest Cell Research GmbH (Unpublished report submitted by the notifier).

Harlan (2010c): *In Vitro* Eye Irritation Test: Hen's Egg Test – Chorioallantoic Membrane (HET-CAM Test) with SAT 090073 (Study No. 1310704, August, 2010). Rossdorf, Germany, Harlan Cytotest Cell Research GmbH (Unpublished report submitted by the notifier).

Harlan (2010d): Local Lymph Node Assay (LLNA) In Mice with SAT 090073 (Study No. 1310705, October, 2010). Rossdorf, Germany, Harlan Cytotest Cell Research GmbH (Unpublished report submitted by the notifier).

Harlan (2010e): *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with SAT 090073 (Study No. 1310701, May, 2010). Rossdorf, Germany, Harlan Cytotest Cell Research GmbH (Unpublished report submitted by the notifier).

Henkel (2010) Determination of Water Solubility (Study No. 09-18557-2/10-00457-2, January, 2010). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011a) Determination of the Melting Point/Melting Range of the Test Item 1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro- (Study No. 11-03655/1-1, March, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011b) Determination of the Boiling Point of the Test Item 1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro- (Study No. 11-03655/1-2, March, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011c) CAS 1196069-20-1 (Sa57): Determination of Relative Density (L 142 A/21-25 (A.3) (Study No. 11-03655/1-3, February, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011d) Determination of the Vapour Pressure of the Test Item 1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro- (Study No. 11-03655/1-4, March, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011e) Determination of Partition Coefficient (Study No. 11-03655/1, May, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011f) Determination of the Flash Point (EEC A.9.) of the Test Item 1*H*,3*H*,5*H*-Oxazolo[3,4-*c*]oxazole, 3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl)dihydro- (Study No. 11-03655/1-7, March, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011g) Determination of the Flammability (EEC A.12.) of the Test Item CAS 1196069-20-1 (Sa57) (Study No. 11-03655/1-8, April, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011h) Determination of the Auto Ignition Temperature (Liquids and Gases) (EEC A.15.) of the Test Item CAS 1196069-20-1 (Sa57) (Study No. 11-03655/1-10, April, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011i) Determination of the Explosive properties (EEC A.14.) of the Test Item CAS 1196069-20-1 (Sa57) (Study No. 11-03655/1-9, April, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2011j) Determination of the Oxidising properties (EEC A.21.) of the Test Item CAS 1196069-20-1 (Sa57) (Study No. 11-03655/1-11, March, 2011). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2013) Sa57 Hydrolysis as a function of pH according to OECD guideline for the testing of Chemicals 111 (Study No. 13-07169-3, November, 2013). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

Henkel (2014) Estimation of Adsorption Coefficient (Koc) of Sa57 on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (Study No. 13-07169-2/14-00330-2, April, 2014). Düsseldorf, Germany, Henkel AG & Co KGaA (Unpublished report submitted by the notifier).

IBACON (2010) Ready Biodegradability of Sa 57 (SAT No. 090073) in a Manometric Respiratory Test (Project No. 55413163, December, 2010). Rossdorf, Germany, Institut fur Biologische Analytik und Consulting IBACON GmbH (Unpublished report submitted by the notifier).

IBACON (2011a) Acute Toxicity of Sa 57 (SAT No. 090073) to *Daphnia magna* in a 48-hour Immobilisation Flow Through Test (Project No. 55411220, February, 2011). Rossdorf, Germany, Institut fur Biologische Analytik und Consulting IBACON GmbH (Unpublished report submitted by the notifier).

IBACON (2011b) Toxicity of Sa 57 (SAT No. 090073) to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test (Project No. 55411210, April, 2011). Rossdorf, Germany, Institut fur Biologische Analytik und Consulting IBACON GmbH (Unpublished report submitted by the notifier).

IBACON (2018) 1H, 3H, 5H-Oxazolo[3,4-c]oxazole,3,5-bis(2,4-dimethyl-3-cyclohexen-1-yl) dihydro-: Toxicity to Activated Sludge in a Respiration Inhibition Test (Study No. 130331171, June, 2018). Rossdorf, Germany, Institut fur Biologische Analytik und Consulting IBACON GmbH (Unpublished report submitted by the notifier).

IBACON (2019) Triplal O: Acute Toxicity to *Daphnia magna* in a Semi-Static 48-hour Immobilisation Test (Study No. 140881220, August, 2019). Rossdorf, Germany, Institut fur Biologische Analytik und Consulting IBACON GmbH (Unpublished report submitted by the notifier).

Luepke, N.P. and Kemper, F.H.. The Het-Cam test: “An Alternative to the Draize Eye test”. Food Chem. Toxicol. (1986) 24 (6/7): 495 – 496.

PEAPC (2016) Key Lab of Pesticide Environmental Assessment and Pollution Control, MEP (PEAPC), Report for Acute Toxicity Test to Fish (*Gobiocypris rarus*) of Sa57 (Study No.: S2015NC014-02 Report No.: R2015NC014-02, December, 2016). Nanjing, China, Nanjing Institute of Environmental Sciences, MEP (NIES) (Unpublished report submitted by the notifier).

OECD (2019) OECD Series on Testing and Assessment, No. 23, “Guidance Document on Aqueous-phase Aquatic Toxicity Testing of Difficult Test Chemicals”, 2nd Ed., February, 2019.

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html.

WIL (2013a) Evaluation of the Ability of Sa 57 to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes (With Repeat Experiment) (Study No. 503136, December, 2013). DD 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2013b) Evaluation of The Mutagenic Activity Of Sa 57 in an *In Vitro* Mammalian Cell Gene Mutation Test With V79 Chinese Hamster Cells (Study No. 503135, December, 2013). DD 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).