File No.: LTD/2125

February 2020

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

KIDE-5

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 Agriculture, Water and the Environment.

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
LTD/2125	Epson Australia Pty Ltd	KIDE-5	ND*	< 0.1 tonne per annum	Component of inkjet printing ink

*Not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

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 its low environmental hazard and use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in inkjet printing ink:
  - Avoid skin contact
  - Clean up spills promptly

- Service personnel should wear impervious gloves and ensure adequate ventilation is present when removing spent printer cartridges containing the notified chemical and during routine maintenance and repairs.

  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.

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 are 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 importation volume exceeds one tonne per annum notified chemical;
- the notified chemical is intended to be introduced at concentrations greater than 5%;
- the notified chemical is imported in any form other than as a component of sealed ink bottles of capacity 200 mL or less;
- the notified chemical is introduced in inks for home use;
- additional information has become available as to potential for genotoxicity or carcinogenicity of the notified chemical;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of inkjet printing ink, 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 a product containing 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)
Epson Australia Pty Ltd (ABN: 91 002 625 783)
3 Talavera Rd
NORTH RYDE NSW 2113

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

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, spectral 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 for boiling point, adsorption/desorption, dissociation constant and flash point.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
KIDE-5

MOLECULAR WEIGHT
> 1,000 g/mol

ANALYTICAL DATA
Reference NMR, IR, LC/MS and IC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: orange powder

Property	*Value*	*Data Source/Justification*
Melting Point	> 400 °C	Measured. Discolouration occurred on heating, indicating that decomposition may have occurred
Boiling Point	> 400 °C	Did not boil below this temperature
Density	1,652 kg/m^3 at 20 °C	Measured
Vapour Pressure	< 1.8 × 10^{-8} kPa at 25 °C	Calculated
Water Solubility	> 50 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	No hydrolysis at pH 4, 7 or 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 2.6 at 25 °C	Measured

Property	*Value*	*Data Source/Justification*
Adsorption/Desorption	Not determined	Some sorption to soil is expected from electrostatic mechanisms, however this is expected to be limited due to the high water solubility
Dissociation Constant	Strongest pKa(Acid): -0.8 ± 0.5 Strongest pKa(Base): 4.3 ± 1.3	Calculated using ACD/Labs I-Lab 2.0
Particle Size	Volume weighted mean = 984 µm Median (d50) = 889 µm Respirable fraction (< 10 µm) = 0.79%	Measured (imported in liquid solution)
Solid Flammability	Not highly flammable	Measured
Autoignition Temperature	281.7 °C	Measured
Explosive Properties	Considered negative	Measured
Oxidising Properties	Considered negative	Based on the chemical structure and oxygen balance values

DISCUSSION OF PROPERTIES
For 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.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported as a component of finished inkjet printer ink at a concentration of ≤ 5 %.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

PORT OF ENTRY
The main ports of each state

IDENTITY OF MANUFACTURER/RECIPIENTS
Epson Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by sea as a component of inkjet printing ink in 120 mL plastic bottles, and will not be reformulated or repackaged within Australia. The ink bottles will be transported by road to the notifier's warehouse for further distribution.

USE
The notified chemical will be used as a component of inkjet printing ink for office and commercial use at a concentration of ≤ 5%. No home use of the ink containing the notified chemical is expected.

OPERATION DESCRIPTION
The 120 mL ink bottles containing the notified chemical will be handled by service technicians and office workers. An end-user will remove the screw cap of the bottle and attach a supplied decanting nozzle to it. The ink will be decanted from the bottle into the ink tank within the printer. Emptied bottles will be re-sealed and disposed of in accordance with relevant Commonwealth, state, territory and local government legislation.

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 warehousing	2 – 4	150
Service technicians	1	200
Office workers	8	200

EXPOSURE DETAILS

Transport and Storage

Transport and storage workers will handle the notified chemical at ≤ 5% concentration in sealed bottles. These workers may come into contact with the notified chemicals only in the unlikely event of an accident when the packaging is breached. These workers are expected to wear coveralls and safety boots during handling.

End use

Service technicians, printing operators and office workers may come into contact with the ink containing the notified chemical. Dermal or possibly incidental ocular exposure to the notified chemical at ≤ 5% concentration may occur during operations including replacing spent ink bottles, transferring the ink from ink bottles to the tank of printers, and cleaning or maintaining printers. However, the exposure is expected to be infrequent or incidental, given the containment of the notified chemical within purposely designed ink bottles and the provision of safe use instructions. Occasional dermal exposure during printing may also occur if the printed pages are handled when wet, or if the ink-stained parts of the printer are touched.

There is also potential for incidental dermal and ocular exposure if ink leaks are discovered during maintenance. Exposure would be minimised through the proposed use of gloves by service technicians.

Once the ink dries, the notified chemical will be bound to the matrix of the substrates and is not expected to be available for exposure.

Inhalation exposure to the notified chemical is not expected, given the expected low vapour pressure of the chemical and the low likelihood of aerosols being released from the printers.

6.1.2. Public Exposure

The inkjet printer inks containing the notified chemical will not be made available to the general public for home use. Therefore, direct public exposure is unlikely to occur.

Members of the public may come into contact with printed materials. However, once the ink is dried, the notified chemical will be bound to the matrix of the substrates and is not expected to be available for exposure.

6.2. Human Health Effects Assessment

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

Endpoint	***Result and Assessment Conclusion***
Acute oral toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation – *in vitro* reconstructed human epidermis test (Episkin)	non-irritating
Skin irritation – rabbit	non-irritating
Eye irritation – *in vitro* isolated chicken eye test	non-irritating
Eye irritation – rabbit	non-irritating
Skin sensitisation – *in chemico* DPRA test	positive
Skin sensitisation – *in vitro* ARE-Nrf2 luciferase test	negative
Skin sensitisation – mouse local lymph node assay	no evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic

Endpoint	*Result and Assessment Conclusion*
Genotoxicity – *in vitro* mammalian chromosome aberration test	genotoxic
Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test	non genotoxic

Toxicokinetics, metabolism and distribution
Absorption of the notified chemical through the skin may occur, as the measured log Pow value of 2.6 favours dermal absorption, particularly if water solubility is high (ECHA, 2017). However, the chemical's relatively high molecular weight (> 1,000 g/mol) would reduce the potential for absorption.

The notified chemical is an azo dye. Bacterial skin microflora have been reported to be able to break down azo dyes into smaller species, which may be more readily absorbed, through azo reduction (SCCNFP, 2002).

Acute Toxicity
The notified chemical was found to have low acute oral and dermal toxicity in rats. No information was submitted on acute inhalation toxicity.

Irritation
The notified chemical was non-irritating to the skin and eyes in *in vitro* and *in vivo* studies.

Sensitisation
One *in chemico* and one *in vitro* cell based assay were conducted to evaluate the skin sensitisation potential of the notified chemical. The tests are part of Integrated Approach to Testing and Assessment (IATA) which address specific events of the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation (OECD, 2012). The tests are thus considered relevant for assessment of the skin sensitisation potential of the notified chemical, along with other supporting information.

The *in chemico* direct peptide reactivity assay (DPRA) aims to address the first key event (molecular initiation) of the AOP by measuring the interaction of the notified chemical with cysteine and lysine, small synthetic peptides representing the nucleophilic centres in skin proteins. The ARE-Nrf2 Luciferase Assay aims to address the second key event (keratinocyte activation) of the AOP by measuring the expression of a report luciferase gene under the control of a promoter from the antioxidant response element (ARE), a responding gene known to be upregulated by contact sensitisers.

The notified chemical showed positive responses in one of the two tests (DPRA), suggesting potential for skin sensitisation. According to the OECD test guidelines (TG 442c and 442d), the suite of tests based on the AOP may not detect pre-haptens (chemicals that become sensitisers following auto-oxidation) and pro-haptens (chemicals requiring enzymatic activation to become sensitisers). Therefore, the negative result in the ARE-Nrf2 Luciferase assay may not reflect the actual skin sensitisation potential of the notified chemical.

The notified chemical was not found to be a sensitiser when tested at up to 25% concentration in a local lymph node assay.

Overall on the basis of the available information, while the notified chemical is not expected to skin sensitising, this cannot be completely ruled out given the positive DPRA result on the notified chemical.

Repeated Dose Toxicity
No repeated dose toxicity studies were provided for the notified chemical.

Mutagenicity/Genotoxicity
The results of a bacterial reverse mutation test performed according to OECD Guideline 471 were negative in the presence and absence of metabolic activation. However, the test guideline states that using a reductive metabolic activation system may be more appropriate for the detection of mutagenicity of azo dyes, as it is recognised that the standard procedure may not be sufficiently sensitive for these chemicals. According to the OECD Guideline 471, modified tests, such as that of Prival and Mitchell (1982), utilise a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out. This modified test is thought to yield a greater detection of mutagenic azo dyes. As such, bacterial mutagenicity potential of the notified chemical cannot be ruled out on the basis of the study performed.

In an *in vitro* chromosomal aberration study, when Chinese hamster lung fibroblasts (CHL/IU cells) were exposed to the notified chemical in the absence of metabolic activation, dose-related and statistically significant increases of structural chromosome aberrations were observed at dose levels of 15.6 and 31.3 µg/mL after 24 h exposure, but not after exposure for 6 hours.

The notified chemical was also studied in an *in vivo* mouse micronucleus assay through the oral route at dose levels up to 2,000 mg/kg bw/day and the results were negative under the conditions of the test. However, as the notified chemical did not cause signs of toxicity at the highest dose tested, it was not possible to determine whether the test substance had reached the bone marrow of the test animals.

Overall on the basis of the available information, while the notified chemical is not expected to be clastogenic *in vivo*, this cannot be completely ruled out given the positive chromosome aberration test result on the notified chemical, and the lack of verification that the chemical reached the bone marrow in the *in vivo* micronucleus test.

Carcinogenicity
Azo dyes are a concern for their potential induction of carcinogenicity. Liver azo reductase enzymes reductively cleave the molecule into component amines. This reduction may contribute to the carcinogenicity of many azo dyes. The notified chemical is not expected to be reductively cleaved to release any of the aromatic amines classified as carcinogens in the EU and identified in the REACH list of 22 aromatic amines in Annex XVII Appendix 8 (European Commission, 2006). In the absence of a carcinogenicity study, the potential for the notified chemical to cause carcinogenic effects cannot be completely ruled out.

In addition, azo dyes are known to have impurities, including the presence of component arylamines (SCCNFP, 2002; Øllgaard, 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed and activated as carcinogens. As such, these impurities may possibly contribute to the carcinogenicity potential of the notified chemical. Specific amine impurities were not identified in the submission for the notified chemical.

Health Hazard Classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on available information, the potential for the notified chemical to cause genotoxic and carcinogenic effects cannot be ruled out. The notified chemical is not expected to be irritating or sensitising at the concentrations of use, and has low acute oral toxicity. Based on its physico-chemical properties, the chemical may have potential for dermal absorption, although it is expected to be limited by its high molecular weight; however, metabolism to smaller species could occur in the skin.

Dermal or possibly ocular exposure to workers may occur during printing, changing bottles, printer repair and contact with printed substrates before they have dried. This exposure is expected to be infrequent and only incidental in nature, given the containment of the notified chemical within small ink bottles and its concentration in the ink (≤ 5%). Inhalation exposure is not expected to occur.

Where more frequent contact may occur, e.g. to printer technicians, any exposure potential would be further controlled through use of personal protective equipment (PPE).

Once the ink dries, the notified chemical will be bound to the matrix of the substrates and is not expected to be available for exposure.

Overall, based on the limited exposure and expected low dermal absorption potential of the notified chemical, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health
The printing ink containing the notified chemical will not be directly available to the general public, but the public may come into contact with printed substrates containing the notified chemical. However, once the inks are dried, the notified chemical is expected to be bound to the matrix of the substrates and will not be available for exposure.

Therefore, based on the proposed use patterns, the risk of the notified chemical to the public is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured in Australia. The notified chemical is to be imported as a part of a finished product and any potential release will be from accidental spills during transport or storage.

RELEASE OF CHEMICAL FROM USE
The notified chemical is to be used in small scale, commercial paper printing operations and will be bound to paper substrates once dried. Release of the notified chemical may occur from leakage of ink during use or refilling of ink containers. Any releases are expected to be collected and disposed of to landfill. No do-it-yourself (DIY) use of the notified chemical is anticipated.

RELEASE OF CHEMICAL FROM DISPOSAL
Most of the notified chemical is expected to share the fate of the printed substrates to which it has been applied, either subjected to substrate recycling processes, or being disposed of to landfill at the end of their useful lives. As estimated by the notifier, printing on paper accounts for the majority of the import volume of the notified chemical. According to the recent Australian National Waste Report (Blue Environment Ltd., 2018), 60% of the waste paper treated with the notified chemical is expected to be recycled domestically.

Empty containers containing the notified chemical are also expected to be disposed of either by recycling or to landfill. Recycled empty containers may be manufactured into low grade plastics, but will eventually be sent to landfill for disposal.

7.1.2. Environmental Fate
As a result of its use pattern, most of the notified chemical is expected to share the fate of the substrates to which it has been applied, either subjected to the paper recycling processes, or being disposed of to landfill at the end of their useful lives. Empty containers containing the notified chemical may be recycled or disposed of to landfill. In landfill, the notified chemical will be present as cured solids and will be neither bioavailable nor mobile.

During paper recycling processes, waste paper is repulped using a variety of chemical treatments which, amongst other things, enhance ink detachment from the fibres. Waste water from paper recycling processes containing the notified chemical is expected to be treated at an on-site wastewater treatment plant before potential release to sewers or surface waters.

The notified chemical is not expected to bioaccumulate based on its low log Pow and high molecular weight (MW > 1,000 g/mol), but is not readily biodegradable (1% after 28 days; OECD TG 301 C). The notified chemical is expected to eventually degrade via biotic and abiotic processes to form water, oxides of carbon, nitrogen, sulfur, chlorine and sodium salts. For the details of the environmental fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)
A Predicted Environmental Concentration (PEC) has not been calculated as the very low import volume and wide dispersal from disposal into surface waters from recycling of paper substrates containing the notified chemical will lead to minimal exposure of the notified chemical to the aquatic environment.

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	LC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity	EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	ErC50 > 99.4 mg/L	Not harmful to algal growth

Inhibition of Bacterial Respiration	IC50 > 1000 mg/L	Not harmful to bacterial respiration

Based on the above ecotoxicological endpoints for the notified chemical, it is not expected to be harmful to aquatic organisms. Therefore, the notified chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* for acute or chronic toxicity (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration (PNEC) was not calculated as the notified chemical is not considered harmful to aquatic species.

7.3. Environmental Risk Assessment

A risk quotient was not calculated as the PEC is expected to be minimal and the notified chemical is not considered harmful to aquatic species. Therefore, on the basis of low environmental hazard and use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Boling Point > 400 °C

Method OECD TG 102 Melting Point/Melting Range (1995)
Remarks The method used was capillary tube in a metal block. There was no melting point from 30 to 400 °C. The appearance of the sample changed from orange powder to brown, then black as the temperature increased. The study authors also considered that no boiling point would occur in the range tested.
Test Facility CERI (2018a)

Density 1,652 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids (2012)
Remarks Pycnometer was used.
Test Facility CERI (2018b)

Vapour Pressure < 1.8 × 10^{-8} kPa at 25 °C
< 2.06 × 10^{-8} kPa at 80 °C (preliminary test)

Method OECD TG 104 Vapour Pressure (2006)
Remarks The gas saturation method was used because the result of preliminary test was less than 10 Pa. The test substance was not detected on the chromatogram. Therefore, the vapour pressure was calculated from the limit of determination.
Test Facility CERI (2018c)

Water Solubility > 50 g/L at 20 °C

Method OECD TG 105 Water Solubility
Remarks Flask Method
Test Facility CERI (2018d)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH

pH	*T (°C)*	*t½*
4	50	N/A
7	50	N/A
9	50	N/A

Remarks Only a preliminary test was conducted as the test substance did not hydrolyse at any pH after 5 days (> 90% of the initial concentration of test substance remained after 5 days). The test substance is hydrolytically stable at pH 4.0, 7.0 and 9.0.
Test Facility CERI (2018e)

Partition Coefficient (n-octanol/water) log Pow = 2.6 at 25 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks HPLC Method
Test Facility CERI (2018f)

Particle Size Volume weighted mean = 984 µm
Medium (d50) = 889 µm

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions (2007) ISO 13320:2009 "Particle Size Analysis – Laser Diffraction Methods", 2009 and CIPAC MT 187

Range (μm)	*Volume (%)*
< 1,950	90
< 889	50
< 115	10
< 10	0.79

Remarks The analysis was conducted initially using a visual microscope and was later undertaken using a Laser Diffraction Particle Size Analyser (Malvern Mastersizer).
Test Facility DEKRA (2018a)

Solid Flammability Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Remarks The test substance was formed into a powder train. It was ignited by a gas flame and the burning rate was determined. Two parallel flammability tests were performed. In the preliminary test the test substance could not be ignited. Based on these results, no main test was performed.
Test Facility Citoxlab (2018a)

Autoignition Temperature 281.7 °C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Remarks The test substance was placed in a wire-mesh cube in an oven at room temperature. The oven was heated from about 30 °C to a maximum of 400 °C at a rate of 0.5° C/min. The self-ignition temperature was the minimum oven temperature at which a certain volume of a test substance would ignite under defined conditions. Three replicate tests were performed.
Test Facility Citoxlab (2018b)

Explosive Properties Considered negative

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Results from the BAM Fallhammer, BAM Friction Negative and Koenen Tube tests were negative.
Test Facility DEKRA (2018b)

Oxidizing Properties Considered negative

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids) was not required.
Remarks Based on the chemical structure and an oxygen balance calculation, the chemical was not expected to cause oxidation.
Test Facility CSR (2018)

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 (2001)
Species/Strain	Rat/Crl:CD(SD)
Vehicle	Water
Remarks – Method	No protocol deviations

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity	There were no mortalities during the study. No abnormalities were noted except for soft stool for all animals one day after the test substance administration. The effect resolved 2 days after the administration.
Effects in Organs	No abnormal macroscopic findings were noted.
Remarks – Results	Animals gained weight as expected.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	CERI (2018g)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test (1987)
Species/Strain	Rat/Crl:CD(SD)
Vehicle	Water
Type of dressing	Occlusive
Remarks – Method	No protocol deviations

RESULTS

Number and Sex of Animals	*Dose (mg/kg bw)*	*Mortality*
5 per sex	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	No local effects were reported.
Signs of Toxicity – Systemic	There were no mortalities during the study. No abnormalities were noted except for slightly decreased spontaneous locomotion in all animals just after the application until 3 hours after the test substance administration. The effect resolved 1 day after the administration. Further tests on control animals indicated that the effect was possibly caused by compression from the elastic adhesive bandage.
Effects in Organs	No abnormal macroscopic findings were noted.
Remarks – Results	Animals gained weight as expected.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	CERI (2018h)

B.3. Skin Irritation – *In Vitro* Reconstructed Human Epidermis Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2015) using the Episkin model.
Vehicle	None
Remarks – Method	The notified chemical was applied as wetted powder, within the recommended dose range.

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability (%)*
Negative control	0.819	100	5.3
Test substance	0.794	97	4.9
Positive control	0.032	3.8	1.7

OD = optical density; SD = standard deviation

Remarks – Results	The test substance did not interact with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) as there was no colour change after 3 hours of incubation in MTT working solution. Therefore the false estimation of viability could be excluded and additional controls and data calculations were not required. Two additional test substance-treated living tissues were used for the non-specific optical density evaluations due to colour of the test substance. The mean optical density measured at 570 nm of tissues was 0.008 and non-specific colour % was 1%. As the value is < 5%, addition data calculation was not conducted. The test substance was considered non-irritating as the relative mean viability (97%) was > 50%. The results for the positive and negative controls met the acceptability criteria for the test.
CONCLUSION	The notified chemical was considered non-irritating to the skin under the conditions of the test.
TEST FACILITY	Citoxlab (2018c)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (2015)
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks – Method	No protocol deviations

RESULTS

Remarks – Results	There were no deaths, test substance related effects on body weight or clinical signs observed. No effects on the skin were evident, with all erythema and oedema scores at 1 h, 24 h, 48 h and 72 h being zero.
CONCLUSION	The notified chemical is non-irritating to the skin.

TEST FACILITY Citoxlab (2019a)

B.5. Eye Irritation – *In Vitro* Isolated Chicken Eye Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 438 Method for Identifying Ocular Corrosives and Severe Irritants (2018)

Vehicle None. However, the test substance was grounded to fine powder for application.

Remarks – Method The purity of the test substance was reported as 95.3%. The test substance was directly administered to the isolated chicken eyes. The control eyes and test eyes were evaluated pre-treatment and at approximately 30, 75, 120, 180 and 240 minutes after the post-treatment rinse. Corneal thickness and corneal opacity were measured at all time points. Fluorescein retention was measured on two occasions, at base line (t = 0) and approximately 30 minutes after the post-treatment rinse.

Positive control: imidazole
Negative control: saline (0.9% w/w sodium chloride)

RESULTS

Test Substance-Experiment I

Observation	*Value*	*ICE Class*
Mean maximum corneal swelling at up to 75 min	0.0%	I
Mean maximum corneal swelling at up to 240 min	0.0%	I
Mean maximum corneal opacity	0.67	II
Mean fluorescein retention	0.67	II
Overall ICE Class	1 × I, 2 × II	

Test Substance-Experiment II

Observation	*Value*	*ICE Class*
Mean maximum corneal swelling at up to 75 min	0.0%	I
Mean maximum corneal swelling at up to 240 min	0.0%	I
Mean maximum corneal opacity	0.50	I
Mean fluorescein retention	0.33	I
Overall ICE Class	3 × I	

Positive Control-Experiment I

Observation	*Value*	*ICE Class*
Mean maximum corneal swelling at up to 75 min	10.7%	II
Mean maximum corneal swelling at up to 240 min	27.3%	III
Mean maximum corneal opacity	4.00	IV
Mean fluorescein retention	3.00	IV
Overall ICE Class	1 × III, 2 × IV	

Positive Control-Experiment II

Observation	*Value*	*ICE Class*
Mean maximum corneal swelling at up to 75 min	9.0%	II
Mean maximum corneal swelling at up to 240 min	25.5%	III
Mean maximum corneal opacity	4.00	IV
Mean fluorescein retention	3.00	IV
Overall ICE Class	1 × III, 2 × IV	

Negative Control-Experiment I and II

Observation	*Value*	*ICE Class*
Mean maximum corneal swelling at up to 75 min	0%	I
Mean maximum corneal swelling at up to 240 min	0%	I
Mean maximum corneal opacity	0.00	I

Observation	*Value*	*ICE Class*
Mean fluorescein retention	0.00	I
Overall ICE Class	3 × I	

Remarks – Results

The test substance and the positive control (imidazole) were stuck on the surface of the cornea after the post-treatment rinse. The test substance treated cornea surfaces were cleared at 30 minutes after the post-treatment rinse. The surface of the positive control treated cornea was not cleared 240 minutes after the post-treatment rinse.

There was no significant corneal effect for the test substance in both experiments. As the test substance was solid, experiment II was required to confirm the negative results from experiment I according to the recommendations of the OECD No. 438 guideline.

CONCLUSION

The notified chemical was not corrosive or a severe eye irritant under the conditions of the test. The notified chemical was considered to be a non-irritant and not subject to classification, on the basis of this study.

TEST FACILITY

Citoxlab (2019b)

B.6. Eye Irritation – Rabbit

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion (2017)

Species/Strain: Rabbit/New Zealand White

Number of Animals: 3 M

Observation Period: 72 hours

Remarks – Method: No 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	0	0	0	1	< 24 h	0
Conjunctiva – Chemosis	0	0	0	0	-	0
Conjunctiva – Discharge	0	0	0	1	< 24 h	0
Corneal Opacity	0	0	0	0	-	0
Iridial Inflammation	0	0	0	0	-	0

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

Remarks – Results

There were no deaths, test substance related effects on body weight or clinical signs observed.

One hour after the application, all rabbits showed conjunctival redness (score 1) and discharge (score 1). The fur around the eye was stained orange by the test substance. All these effects resolved within 24 hours. No other effects were seen on the eyes.

CONCLUSION

The notified chemical is non-irritating to the eyes.

TEST FACILITY

Citoxlab (2019c)

B.7. Skin Sensitisation – *In Chemico* DPRA Test

TEST SUBSTANCE	Notified chemical
METHOD	Based on OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)
Vehicle	Water
Negative control	Acetonitrile
Positive control	Cinnamaldehyde
Remarks – Method	No protocol deviations. At the end of the incubation period, a visual inspection of all samples was performed prior to HPLC analysis. As precipitate and/or micelles and phase separation were noted with the positive control incubated with the cysteine and lysine peptides, these vials were centrifuged at 400 *g* for a period of 5 minutes at room temperature to force precipitate to the bottom of the vial. Therefore, only supernatants were injected into the HPLC/UV system. The vials for the other samples were directly transferred into the HPLC/UV system.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle Control	0.503 ± 0.001	0.490 ± 0.001
Negative Control	0.504 ± 0.002	0.407 ± 0.001
Test Substance	56.70 ± 8.79	100.00 ± 0.00
Positive Control	96.44 ± 018	54.47 ± 0.65

SD = Standard Deviation

Remarks – Results	The mean of the percentage cysteine and percentage lysine depletions was 78.35% for the test substance, placing it in the high reactivity category (42.47% - 100%). The positive and negative controls fulfilled all quality criteria confirming the validity of the test. The test substance did not co-elute with the cysteine or lysine peptides.
CONCLUSION	The test substance was considered to have high reactivity for peptide depletion under the conditions of the test, showing positive results in the first key event (molecular initiating) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.
TEST FACILITY	Citoxlab France (2019a)

B.8. Skin Sensitisation – *In Vitro* ARE-Nrf2 Luciferase Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 442d *In Vitro* Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation (2018) - The ARE-Nrf2 luciferase KeratinoSens™ test method (Appendix IA)
Vehicle	Water
Negative control	Dimethylsulphoxide
Positive control	Cinnamaldehyde
Remarks – Method	Solubility of the test substance in water at 200 mM (before dilution) was aided by sonication and heating at 60 °C for 10 minutes. In this form it was a homogeneous orange gel. No filtration was applied, in case the test substance was a suspension.

A minor deviation on the acceptance criteria was not considered by the study authors to affect the validity of the study. During the first run, the average $EC_{1.5}$ value for the positive control was not within two standard deviations of the historical mean but slightly above the upper limit (i.e., 14.82 instead of 2.8-13.9). However, this value was equivalent to the maximal $EC_{1.5}$ value for the historical data. All the other acceptance criteria of this run were reached and negative results for the test substance were homogenous between both runs.

RESULTS

Sample	*Concentration (µM)*	*Mean Cell viability (% ± SD)*	*Mean Luciferase Induction (% ± SD)*
Test substance			
Dose Level 1	0.98	100 ± 15	1.0 ± 0.0
Dose Level 2	1.95	85 ± 17	0.7 ± 0.1
Dose Level 3	3.91	74 ± 19	0.6 ± 0.0
Dose Level 4	7.81	71 ± 18	0.6 ± 0.1
Dose Level 5	15.63	69 ± 13	0.7 ± 0.2
Dose Level 6	31.25	70 ± 11	0.8 ± 0.1
Dose Level 7	62.5	70 ± 8	0.9 ± 0.0
Dose Level 8	125	76 ± 11	0.8 ± 0.1
Dose Level 9	250	75 ± 14	0.8 ± 0.1
Dose Level 10	500	77 ± 16	0.8 ± 0.1
Dose Level 11	1,000	77 ± 14	0.7 ±0.0
Dose Level 12	2,000	72 ± 16	0.6 ± 0.0
Positive Control			
Dose Level 1	4	103 ± 5	1.1 ± 0.0
Dose Level 2	8	104 ± 11	1.4 ± 0.2
Dose Level 3	16	106 ± 5	1.6 ± 0.1
Dose Level 4	32	113 ± 11	2.3 ± 0.5
Dose Level 5	64	107 ± 8	4.8 ± 0.6

SD = Standard Deviation

Remarks – Results

There was no precipitate/emulsion in any test substance-treated wells at the end of the 48-hour treatment.

There was no statistically significant induction of luciferase activity above the threshold of 1.5 fold (50% increase) when compared with the negative control, at any tested concentrations and in either run. As the I_{max} values were < 1.5, no $EC_{1.5}$ was calculated. There was no decrease in cell viability to below 70% in the first run. Therefore, neither IC_{30} nor IC_{50} was calculated.

In the second run, there was a decrease in cell viability to < 70% but > 50% at concentrations ≥ 3.91 µM with an IC_{30} at 2.47 µM. Since the cell viability was > 50% in this run, no IC_{50} was calculated.

No geometric mean IC_{30} was calculated since the cell viability in the first run was > 70%.

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

CONCLUSION

The test substance was negative in the second key event (keratinocytes response) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.

TEST FACILITY

Citoxlab France (2019b)

B.9. Skin Sensitisation – LLNA

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2010)
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	1% aqueous Pluronic® PE9200 solution
Preliminary study	Yes. Based on the results from a preliminary irritation/toxicity test in CBA/CaOlaHsd mice using two doses (2 animals per dose): 25% (w/v) and 10% (w/v) in 1% Pluronic, 25% (w/v) dose was selected as top dose for the main study.
Positive control	α-Hexylcinnamaldehyde (tested concurrently)
Remarks – Method	Doses for the main study were based on the solubility results in the preliminary study. Minor deviations from the study plan, not considered to have affected the outcome of the study, were fluctuations in temperature/humidity and the fact that the test substance formulations were prepared the day before application and were continuously stirred overnight to be suitable for application. Formulations were used within 24 hours of the start of their preparation. The first main study performed was considered to be invalid (both the negative and positive control values were out of Citoxlab Hungary Ltd.'s historical control range and the positive control substance did not produce a significant lymphoproliferative response), and was not reported. Therefore the second main study was conducted as agreed by the sponsor.

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	339.9	1.0
5	4 F	326.3	1.0
10	4 F	229.8	0.7
25	4 F	358.3	1.1
Positive Control			
25	4 F	1216.1	3.6

Remarks – Results	No deaths were noted. No signs of systemic toxicity or body weight change related to the test substance treatment were observed in the test or control animals. Test substance residue (some effects were minimal) was noted on the ears of the animals after the treatment on days 1-3 in half of animals in the 25% and 10% dose groups.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Citoxlab (2019d)

B.10. Genotoxicity – Bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test (1997) Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver

Concentration Range in Main Test	With and without metabolic activation: 0, 314, 628, 1,255, 2,510, 5,020 µg/plate
Vehicle	Water

Positive Controls

Strain	Without S9 (µg/plate)	Without S9 (µg/plate)
TA100	AF-2 (0.01)	B[*a*]P (5.0)
TA1535	NaN_3 (0.5)	2AA (2.0)
WP2 *uvrA*	AF-2 (0.01)	2AA (10.0)
TA98	AF-2 (0.1)	B[*a*]P (5.0)
TA1537	ICR-191 (1.0)	B[*a*]P (5.0)

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN_3: Sodium azide
ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine·2HC1
2AA: 2-Aminoanthracene
B[*a*]P: PBenzo[*a*]pyrene

Remarks – Method	No protocol deviations. The study report was translated. Doses were adjusted to account for the purity of 95.3% of the sample. The criteria set for increase of revertants was ×2 or higher.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5,020			
Test 1		> 5,020	> 5,020	negative
Test 2		> 5,020	> 5,020	negative
Present	> 5,020			
Test 1		> 5,020	> 5,020	negative
Test 2		> 5,020	> 5,020	negative

Remarks – Results	Neither an increase in the number of revertant colonies nor a dose-related response was recorded for any of the bacterial strains, either with or without metabolic activation. The positive controls produced satisfactory responses, confirming the activity of the S9-mix and the sensitivity of the bacterial strains.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BML (2017)

B.11. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (2016)
Species/Strain	Chinese hamster
Cell Type/Cell Line	Lung fibroblasts (CHL/IU cells)
Metabolic Activation System	S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Vehicle	Water
Positive Controls	Mitomycin C (MMC) without metabolic activation and Cyclophosphamide monohydrate (CPA) with metabolic activation
Remarks – Method	Doses were chosen on the basis of the cell growth inhibition test with the aim that Relative Population Doubling (RPD) or Relative Increase in Cell Count (RICC) compared to the control would be 40-50%. As the results of the 6 h treatments were negative, a 24 h treatment was conducted.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			

Test 1	0*, 125, 250, 500*, 1,000* and 2,000*	6 h	24 h
Test 2	0*, 7.81*, 15.6*, 31.3*, 62.5, 125 and 250	24 h	24 h
Present			
Test 1	0*, 500*, 1,000* and 2,000*	6 h	24 h

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Cell Inhibition Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 2,000	> 2,000	> 2,000	negative
Test 2	≥ 250	≥ 15.6	> 250	positive
Present				
Test 1	> 2,000	> 2,000	> 2,000	negative

Remarks – Results

The test substance did not induce numerical aberrations but induced structural aberrations.

The frequencies of cells with structural aberrations at all doses in the short-term (6 h) treatments in the presence and absence of S9 mix were within the range of the historical data of the negative control. The frequency of cells with structural aberrations at 15.6 and 31.3 µg/mL (the highest concentrations analysed) in the 24 h continuous treatment in the absence of S9 mix were outside the range of the historical data, showing dose-related and statistically significant increases. Therefore, structural aberration was considered positive.

The frequencies of numerical aberrant cells were within the range of the historical data under all the conditions tested.

The positive controls performed as expected, confirming the validity of the test system.

CONCLUSION

The notified chemical was clastogenic to Chinese hamster lung fibroblasts treated *in vitro* under the conditions of the test.

TEST FACILITY

CERI (2019a)

B.12. Genotoxicity – *In Vivo* Mammalian Erythrocyte Micronucleus Test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test (2016)

Species/Strain: Mice/Crl:CD1(ICR)

Route of Administration: Oral – gavage

Vehicle: Water

Remarks – Method: No analysis was carried out on plasma levels of the notified chemical, in order to confirm that exposure of the bone marrow occurred. Dosage was chosen on the basis of an acute oral toxicity study, where toxicity was not observed at 2,000 mg/kg body weight.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	5 M	0	24
II (low dose)	5 M	500	24
III (mid dose)	5 M	1,000	24
IV (high dose)	5 M	2,000	24
V (positive control, M)	5 M	2	24

M = mitomycin C

RESULTS	
Doses Producing Toxicity	There were no deaths, test substance related effects on body weight or clinical signs observed.
Genotoxic Effects	At the low dose only, there were statistically significant increases in the MNPCE/PCE (micronucleated polychromatic erythrocytes/polychromatic erythrocytes) ratio. However, the increase did not occur in a dose-related manner and was within the range of the historical data of the negative control. Therefore, the results were considered negative. No statistically significant differences in the PCE/TE (polychromatic erythrocytes/total erythrocytes) were recorded in any doses of the test substance group compared with the negative control group. There were no clinical signs after treatment that would indicate systemic exposure. Hence the exposure of the test substance to bone marrow cells was not confirmed. The study authors considered that the potential to induce micronuclei of the test substance had been adequately evaluated due to 2,000 mg/kg/day being the maximum dose recommended in the test guideline.
Remarks – Results	The positive control performed as expected, confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* mammalian erythrocyte micronucleus test.
TEST FACILITY	CERI (2019b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD, DOC and HPLC
Remarks – Method	As per OECD test guidelines, no deviations were noted. Aniline was used as a reference substance.

RESULTS

Test Substance		*Aniline*	
Day	*% Degradation**	*Day*	*% Degradation*
7	0	7	67
14	0	14	87
21	0	21	91
28	1	28	92

*Determined by BOD

Remarks – Results	All validity criteria were met. The reference compound reached 87% degradation at 14 days, dissolved oxygen was < 60 mg/L at day 28 in the reference test and pH was maintained between 6.2 and 7.5 across all samples.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	CERI (2018i)

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	*Oryzias latipes* (Japanese medaka)
Exposure Period	96 hours
Auxiliary Solvent	None
Analytical Monitoring	HPLC
Remarks – Method	As per OECD test guidelines. A limit test only was conducted. The study was conducted under shaded conditions. A positive control test was run less than 6 months prior to the primary test using copper (II) sulfate.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0
100	101.3	7	0	0	0	0	0

LC50 > 100 mg/L at 96 hours

NOEC	100 mg/L at 96 hours
Remarks – Results	All validity criteria were met. Dissolved oxygen was maintained at > 60% of the air saturation value and analytical measurement of the test concentrations were between 80 – 120% of the nominal values. The positive control showed a 96 hr LC50 of 0.33 mg/L which is within the expected value of 0.13 – 0.9 mg/L.
CONCLUSION	The notified chemical is not harmful to fish.
TEST FACILITY	CERI (2019c)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Analytical Monitoring	HPLC
Remarks – Method	As per OECD test guidelines. A limit test only was conducted. The study was conducted under shaded conditions. A positive control test was run less than 6 months prior to the primary test using potassium dichromate.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	20	0	0
100	98.8*	20	0	0

*Geometric mean of concentrations measured at the start and end of the study

EC50	> 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks – Results	All validity criteria were met. Dissolved oxygen was maintained between 8.7 and 8.9 mg/L, pH was maintained between 7.7 and 7.9 and temperature was maintained between 20.2 and 20.3 °C. The positive control showed a 48 h EC50 of 0.13 mg/L which is within the expected range of 0.10 – 0.35 mg/L.
CONCLUSION	The test substance is not harmful to daphnia.
TEST FACILITY	CERI (2019d)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.316 – 100 mg/L Actual: 0.273 – 99.4 mg/L
Auxiliary Solvent	None
Analytical Monitoring	HPLC

Remarks – Method	As per OECD test guidelines, no deviations were noted. A positive control test was run less than 6 months prior to the primary test using potassium dichromate.

RESULTS

Growth	
ErC50 (mg/L)	*NOEC (mg/L)*
> 99.4	2.93

Remarks – Results	All validity criteria were met. The control sample had a growth factor of 57, a mean coefficient of variation for section-by-section specific growth rates of 8.7% and the coefficient of variation of specific growth rates in replicate control cultures was 0.89%. Regarding the growth rate, Bartlett's test was done to determine the homogeneity of variance for the data. Then one-way analysis of variance and Dunnett's multiple comparison test was used to determine the significance of the difference between the control and exposed organisms. The NOEC was determined by the results of statistical analysis and cell condition. The positive control showed a 72 h ErC50 of 1.2 mg/L which is within the expected range of 0.61 – 1.4 mg/L.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	CERI (2019e)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE

METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Remarks – Method	As per OECD test guidelines, no deviations were noted. 3, 5-dichlorophenol was used as a reference substance.

RESULTS

IC50	> 1,000 mg/L
Remarks – Results	All validity criteria were met. The coefficient of variation in oxygen consumption between replicates was 5.8% and the average oxygen consumption per gram in the control sample was 39.7 mg O_2/g/h. The EC50 of 3, 5-dichlorophenol was 11 mg/L which is within the expected range of 2 – 25 mg/L.
CONCLUSION	The notified chemical is not inhibitory to microbial respiration.
TEST FACILITY	CERI (2018j)

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