File No: LTD/1797

November 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Pilot Ink 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 the Environment.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also 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

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
LTD/1797	Pilot Pen Australia Pty Ltd	Chemical in Pilot Ink 5	No	< 1 tonne per annum	Component of ballpoint pen ink

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 for the Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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.

Environmental risk assessment
Based on the assumed low hazard and the assessed use pattern of the notified chemical, it is not expected to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A copy of the (M)SDS should be easily accessible to employees.

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.

Emergency procedures

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

Regulatory Obligations

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

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from being a component of ballpoint pen 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.

(Material) Safety Data Sheet
The (M)SDS of the products containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Pilot Pen Australia Pty Ltd (ABN: 37 144 701 502)
39 Enterprise Circuit
PRESTONS NSW 2170

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, analytical data, degree of purity, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, adsorption coefficient, dissociation constant, flash point and autoignition temperature.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Japan
US (2007)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Chemical in Pilot Ink 5

MOLECULAR WEIGHT
< 500 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: White powder

Property	Value	Data Source/Justification
Melting Point	63 °C	Measured
Boiling Point	Decomposes at > 325 °C	Measured
Density	1,141 kg/m^3 at 20 °C	Measured
Vapour Pressure	< 8.4 x 10^{-10} kPa at 20 °C	Measured
Water Solubility	< 6.0 x 10^{-6} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	t½ = 7.6 – 8.2 days	Analogue data
Partition Coefficient (n-octanol/water)	log Pow = > 6.5 at 25 °C	Measured
Adsorption/Desorption	log Koc = 3.17	Calculated using KOCWIN v 2.00 (US EPA, 2011)
Dissociation Constant	pKa = 25.6	Analogue data based on computational

Property	Value	Data Source/Justification
		study
Particle Size	Inhalable fraction (< 100 μm): 100% Respirable fraction (< 10 μm): 63.41% MMAD* = 8.678	Measured
Flash Point	215.1 °C	Calculated
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 402 °C	Estimated
Explosive Properties	Not expected to be explosive	Estimated based on chemical structure
Oxidising Properties	Not expected to oxidise	Estimated based on chemical structure

* MMAD = Mass Median Aerodynamic Diameter

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 for the 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 be imported into Australia as a component (< 10%) of ballpoint pen ink.

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

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

PORT OF ENTRY
Sydney (by air and sea)

IDENTITY OF RECIPIENTS
Pilot Pen Australia Pty Ltd (for distribution to wholesalers and then the general public)

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component (< 10%) of ballpoint pen ink. Pens containing the notified chemical are in cardboard cartons for transport and storage.

USE
The notified chemical will be used as a component (< 10%) of ballpoint pen ink for writing or highlighting on absorbent surfaces such as paper and cardboard.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of ballpoint pen ink in pens. Reformulation will not take place in Australia.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

EXPOSURE DETAILS
Waterside, storage and transport workers and salespeople may come into contact with the notified chemical, as component of ink (< 10%), only in the unlikely event of an accident.

Occasional dermal exposure to the notified chemical (at a concentration < 10%) during use of pens by office workers may occur if surfaces are handled before the ink containing the notified chemical has dried or if there is incidental contact with the tips of pens.

Once the ink has dried, the chemical is bonded to the absorbent surface (such as paper and cardboard), and dermal exposure to the notified chemical from contact with dried ink is not expected.

6.1.2. Public Exposure
Dermal exposure of the public to ink containing the notified chemical (at < 10%) is expected to be similar to that for office workers,

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*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation (in vitro)	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Genotoxicity – in vitro Mammalian Cell Gene Mutation Test (mouse)	non-clastogenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test (human lymphocytes)	non-clastogenic

Toxicokinetics.
The notified chemical is of low water solubility (6 x 10^{-6} g/L at 20 °C) and highly lipophilic (Log Pow > 6.5), hence dermal absorption is expected to be limited. Given the low molecular weight of the notified chemical, absorption across the gastrointestinal tract may occur.

Acute toxicity.
In acute toxicity studies conducted in rats, the notified chemical was found to be of low toxicity by the oral and dermal routes.

Irritation and sensitisation.
In a study conducted in rabbits the notified chemical was found to be slightly irritating to the eyes. An *in vitro* study conducted using a human skin model found the notified chemical to be non-irritating.

The notified chemical (at concentrations up to 20%) in a mouse Local Lymph Node Assay showed no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation.

Repeated dose toxicity
No repeated dose toxicity data is available on the notified chemical. Given the low potential for dermal absorption systemic toxicity by the dermal route is not expected.

Mutagenicity/Genotoxicity.
The notified chemical was found to be non-clastogenic in an *in vitro* mammalian cell gene mutation test (with L5178Y mouse lymphoma cells) and in an *in vitro* mammalian chromosome aberration test (human lymphocytes).

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is of low toxicity, presenting only as a very slight eye irritant. The notified chemical will be imported in finished products without reformulation or repackaging. Only transport, storage and retail workers may come into the contact with the notified chemical in the event of accidental rupture of packages. Therefore, the risk to the health of workers is not considered to be unreasonable.

6.3.2. Public Health
The risk to public health is expected to be similar to that for office workers. Therefore, the risk to public health 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 be imported as a component of pen inks. As manufacturing and reformulation will take place overseas, no release of the notified chemical is expected in Australia from these activities.

RELEASE OF CHEMICAL FROM USE
No release of ink contained in the pens is expected except in the case of an accident where damage to pens may occur. The notified chemical will be physically bound on applied substrate. Hence it is expected to be stable within an inert matrix on applied substrates.

RELEASE OF CHEMICAL FROM DISPOSAL
The majority of the notified chemical is expected to be applied on absorbent surfaces such as paper and cardboard. The notified chemical is expected to share the fate of the applied substrate which are expected to be disposed of to landfill at the end of their useful life, or be recycled after use. Hence, up to 50% of the total import volume of the notified chemical may be released to sewers as residues in recycling waste waters. Empty pens containing residues of the notified chemical are expected to be disposed of to landfill.

7.1.2. Environmental Fate
A measured ready biodegradability study conducted on the notified chemical indicated that it is readily biodegradable. Therefore the notified chemical is expected to be rapidly degradable and is not expected to persist in the environment. For the details of the environmental fate studies please refer to Appendix C.

Notified chemical present in pens will be applied on absorbent surfaces such as paper and cardboard. The notified chemical is expected to be disposed of to landfill along with papers or released to sewer in recycling wastewaters when papers are recycled. It is expected to have bioaccumulative potential based on the reported log P_{OW} of > 6.5. This is not considered to be a concern since the notified chemical showed a biodegradability of 89% in 28 days. Based on its low water solubility (< 6×10^{-6} g/L) and high partition coefficient (log P_{ow} > 6.5), the notified chemical is expected to partition to sludge during paper recycling and waste water treatment processes. The sludge is expected to be disposed of to landfill or applied to agricultural soils. In landfill, soils and water, the notified chemical is expected to degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)
Based on its use in pen inks, it is conservatively assumed that 100% of the total import volume of the notified chemical will be applied on absorbent surfaces such as paper and cardboard. Using a worst-case scenario, it is assumed that 50% of the paper products containing the notified chemical will be recycled and will be released to the sewer with no removal during recycling or STP processes. As the notified chemical is to be processed at

paper recycling facilities located throughout Australia, it is anticipated that such releases will occur on 260 days of the year into the Australian effluent volume. The resultant estimate for the predicted environmental concentration (PEC) in sewage effluent nationwide is summarised in the table below.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	500	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	1.92	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.43	µg/L
PEC - Ocean:	0.04	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 0.425 µg/L may potentially result in a soil concentration of approximately 0.0028 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 0.014 mg/kg and 0.028 mg/kg, respectively.

7.2. Environmental Effects Assessment

Ecotoxicological data were submitted for the notified chemical. Details of the studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Daphnia	EC50 (48 h) > 0.00404 mg/L*	Not harmful to aquatic invertebrates up to its water solubility limit
Algae	EC50 (72 h) > 0.00189 mg/L*	Not harmful to algae up to its water solubility limit

*Limit test

Based on the above reported endpoints for the notified chemical, it is not considered to be harmful to daphnia and algae. Therefore, the notified chemical is not harmful to aquatic organisms. Consequently, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified chemical has not been formally classified for acute and chronic toxicities.

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 expected to be harmful to aquatic life, up to the limit of its solubility, based on the studies provided by the notifier.

7.3. Environmental Risk Assessment

A risk quotient (PEC/PNEC) for the notified chemical was not calculated as a PNEC was not derived. Release of the notified chemical to the aquatic environment in ecotoxicologically significant quantities is not expected based on its reported use pattern. The notified chemical is not expected to be bioaccumulative and is expected to be readily biodegradable in the environment. Based on the assumed low hazard and the assessed use pattern of the notified chemical, it is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 63 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Determined by differential scanning calorimeter.
Test Facility NOTOX (2009a)

Boiling Point Decomposes without boiling at > 325 °C

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Determined by differential scanning calorimeter. An exothermic effect was observed from 325 °C which was attributed to reaction and/or decomposition of the test substance.
Test Facility NOTOX (2009a)

Density 1141 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Determined using a gas comparison stereopycnometer.
Test Facility NOTOX (2009a)

Vapour Pressure < 8.4 x 10^{-10} kPa at 20 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Determined using isothermal thermogravimetric effusion.
Test Facility NOTOX (2009a)

Water Solubility < 6.0 x 10^{-6} 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

The limit of detection (LOD) was calculated to be 0.0014 mg/L. When taking the dilution factor into account, this corresponds to LOD of < 6.0 x 10^{-6} g/L.
Test Facility NOTOX (2009)

Partition Coefficient (n-octanol/water) log Pow = > 6.5 at 25 °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 NOTOX (2009a)

Particle Size

Method The sample was analysed using the Malvern Mastersizer 2000 Laser Diffraction Analyser. The particle size was analysed between 0.02 µm and 2000 µm over 5 runs (to ensure repeatability of results). The test was conducted to BS ISO 13320-1:1999

Range (µm)	*Mass (%)*
< 2.687 µm	10
< 8.127 µm	50
< 16.547 µm	90

Remarks	The sample was observed to be a white crystalline powder with medium size particles and some clusters and lumps. Under 100 x magnification (optical microscope) the particles appeared to be irregular shaped crystals with particle size between 4 and 33 μm. At 400 x magnification (optical microscope) the sample was observed to be made up of agglomerated small particles. All particles fall into the inhalable fraction, 63.41% of the sample being in the thoracic fraction (< 10 μm)
Test Facility	Chilworth (2009)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks	The test substance melted on contact with flame. No propagation of combustion of the test substance was observed within the 4 minute test period.
Test Facility	Notox (2009a)

Autoignition Temperature > 402 °C

Method	Test substance was heated from 25 °C to 402 °C (the point where 70% weight loss was observed) with a rate of 20 °C per minute using a thermogravimetric analyser.
Remarks	No autoignition of test substance observed.
Test Facility	Notox (2009a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Crl:CD(SD)
Vehicle	0.5 w/v% CMC-Na solution containing 0.1 w/v% Tween 80
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	300	0/3
2	3 F	300	0/3
3	3 F	2000	0/3
4	3 F	2000	0/3

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no signs of systemic toxicity.
Effects in Organs	No abnormalities were detected in the organs.
Remarks - Results	All animals showed expected body weight gains

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	MCSI (2008a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/ Wistar/ Crl:WI (Han)
Vehicle	Propylene glycol
Type of dressing	Occlusive.
Remarks - Method	There were no deviations for the protocol.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Effects were observed within 2 to 48 hours. White staining was observed in all animals with 4/10 animals also exhibiting focal erythema (1/5 M and 3/5 F). All animals had recovered after 72 hours.
Signs of Toxicity - Systemic	All effects were observed within 2 to 48 hours. Hunched posture (observed after 48 hours) was observed in 7/10 animals with 2/7 animals exhibiting flat posture (observed at 2 and 4 hours after exposure). Toxicity affecting the nose and eyes was observed in 5/10 animals (chromodarcryorrhoea: 6/10 with 1/6 also exhibiting ptosis) was observed. All animals had recovered after 72 hours.
Effects in Organs	No abnormalities were detected in organs. However, 1F showed scab formation in the thoraco-dorsal region of the skin. This was considered to be incidental rather than an effect of exposure.
Remarks - Results	All animals showed expected body weight gains

CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	WIL (2013a)

B.3. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 431 In vitro Skin Corrosion - Human Skin Model Test EpiSkin™ Reconstituted Human Epidermis Model
Vehicle	Water
Remarks - Method	The test substance (10 mg in 10 µL) was applied to the tissues in triplicate. Following 15 minute exposure periods, the tissues were rinsed and then incubated at 37 °C for approximately 42 hours. Following treatment with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 0.3 mg/mL], the tissues were incubated at 37 °C for 3 hours. Positive (sodium dodecyl sulphate; 5%) and negative (phosphate buffered saline) controls were run in parallel with the test substance. The optical densities were determined at 570 nm.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*
Negative control	0.708	100
Test substance	0.714	101
Positive control	0.045	6

OD = optical density; SD = standard deviation

Remarks - Results	The relative mean viability of the test substance treated tissues (compared to the negative control) was 101% after a 15-minute exposure period. The positive and negative controls gave satisfactory results, confirming the validity of the test system. A mean tissue viability of > 50% is considered as non-irritating indicating the notified chemical is not an irritant.
CONCLUSION	The notified chemical is non-irritating to the skin under the conditions of the test.
TEST FACILITY	NOTOX (2009b)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	3 days
Remarks - Method	Observations were made at 1, 24, 48 and 72 hours following exposure to the test item.

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	0.3	0.3	0.3	1	< 48 h	0
Conjunctiva: chemosis	0	0	0	0	0	0
Conjunctiva: discharge	0	0	0	0	0	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results: Conjunctival redness was observed in all 3 animals. All treated eyes appeared normal at the 48-hour observation. All three animals exhibited conjunctival chemosis and discharge on dosing with the notified chemical, but these effects were resolved at the 24-hour observation. No iridial irritation or corneal opacity were observed

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

TEST FACILITY: WIL (2013b)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain: Mouse/CBA/JNCrlj

Vehicle: Acetone/olive oil 4:1

Remarks - Method: No significant protocol deviations. Positive (α-hexylcinnamaldehyde) and negative (acetone/olive oil 4:1) controls were run concurrently.

A preliminary screening study was conducted on two mice exposed to 25 µl of 1 w/v% and 20 w/v% of the suspended test substance, once daily for 2 consecutive days. No changes were observed in either of the animals. The maximum concentration was determined to be 20 w/v%.

Each group contained 6 animals.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	968.2	-
20	750.0	0.77
5	1013.3	1.05
1	911.0	0.94
Positive Control		
α-hexylcinnamaldehyde (25%)	7148.0	7.38

Remarks - Results: No deaths or abnormal clinical signs were observed in any animals. Body weight losses were observed in 5/30 animals on Day 3 and 1/30 animals on Day 6, The losses were not dose dependant. Slight body weight losses are spontaneously observed in female mice, and the authors concluded that the changes were not due to the test substance, and there were no effects in the evaluation in this study. All other animals showed steady growth, and the 5/30 animals that exhibited body weight loss at Day 3 recorded body weight gains equal to or higher than the body weights of Day 1.

None of the test substance groups exhibited a SI value ≥ 3.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY MCSI (2008b)

B.6. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse
Cell Type/Cell Line	L5178Y/TK$^{+/-}$ -3.7.2C/lymphoma cells
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Tetrahydrofuran
Remarks - Method	No significant protocol deviations.

Vehicle and positive controls were used in parallel with the test material. Positive controls: i) without S9: Methyl methanesulfonate (MMS); ii) with S9: Cyclophosphamide (CP).

A preliminary toxicity test (10 – 1000 µg/mL) was performed to determine the toxicity of the test substance. The preliminary toxicity test (10 – 1000 µg/mL) determined that the test substance was toxic to the L5178Y cells at a concentration of 1000 µg/mL after 24 hours of treatment. No toxicity was observed for cells treated for 3 hours.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0.03, 0.1, 0.3, 1, 3, 10, 33, 100	3 h	48 h
Test 2	0.03, 0.1, 0.3, 1, 3, 10, 33, 100	24 h	48 h
Present			
Test 1	0.03, 0.1, 0.3, 1, 3, 10, 33, 100	3 h	48 h
Test 2	0.03, 0.1, 0.3, 1, 3, 10, 33, 100	3 h	48 h

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 1000	> 100	≥ 33	Negative
Test 2		> 100	≥ 33	Negative
Present				
Test 1	> 1000	> 100	≥ 33	Negative
Test 2		> 100	≥ 33	Negative

Remarks - Results After 3 hour exposure, no toxicity was observed and all dose levels were evaluated in the absence and presence of S9-mix. After exposure for 24 hours, the relative total growth of the highest test concentration was 34% compared to the total growth of the negative controls, whereas the dose level of 33 µg/mL the relative total growth was 20%.

No significant increase in the mutation frequency was observed after exposure to the notified chemical either in the presence or absence of

metabolic activation. The numbers of small and large colonies in the exposed cell cultures were comparable to the number present in solvent controls.

Increases above the historical control data range were observed at dose levels of 0.1, 10 and 33 µg/mL in the absence of S9-mix after a 24 hour exposure period. However, the authors did not consider these increases to be biologically relevant as a dose-related response was not observed, precipitation was observed at 2/3 of the dose levels, the increases in mutation frequency were below that of the control and a < 1.4-fold increase was observed.

The positive and negative controls gave satisfactory responses confirming the viability of the test system.

No significant increase in the mutation frequency was observed at any dose level either with or without metabolic activation or exposure period.

CONCLUSION The notified chemical is not clastogenic to L5178Y mouse lymphoma cells treated in vitro under the conditions of the test.

TEST FACILITY WIL (2013c)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Tetrahydrofuran
Remarks - Method	No significant protocol deviations.

Vehicle and positive controls were used in parallel with the test material. Positive controls: i) without S9: Mitomycin C (MMC-C); ii) with S9: Cyclophosphamide (CP).

A preliminary toxicity study was performed to determine the toxicity of the test substance (3 hour exposure (with and without metabolic activation) at concentrations 3, 10 and 33 µg/mL; and 24 and 48 hour exposure (without metabolic activation) at concentrations 1 - 1000 µg/mL)

No toxicity was observed in the presence or absence of metabolic activation at any of the concentrations tested.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	3*, 10, 333, 1000*, 1250, 1500, 1750, 2000*	24 h	24 h
Test 2	3*, 10, 333, 1000*, 1250, 1500, 1750, 2000*	48 h	48 h
Present			
Test 1	3*, 10*, 33*	3 h	48 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic	*Test Substance Concentration (µg/mL) Resulting in:*

Activation	*Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 1000	> 2000	≥ 333	Negative
Test 2		> 2000	≥ 333	Negative
Present				
Test 1	> 1000	> 33	≥ 33	Negative

Remarks - Results

In the presence or absence of metabolic activation, there was no increase in the number of cells with chromosome aberrations, polyploid cells or cells with endoreduplicated chromosomes.

The positive and negative controls gave satisfactory responses confirming the viability of the test system.

CONCLUSION

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

TEST FACILITY

WIL (2013d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	Biodegradability Test of Chemical Substances by Microorganisms> (Yakushokuhatsu No. 1121002, Heisei 15.11.13 Seikyoku No. 2, Kanpokihatsu No. 031121002, November 21, 2003; the latest revision, April I, 2005).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biological Oxygen Demand (BOD)
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
28	89	28	79

Remarks - Results	All validity criteria for the test were satisfied. The reference compound, aniline, reached the 60% pass level by day 3 indicating the suitability of the inoculum. The degree of degradation of the notified chemical after the cultivation period was 89%. Therefore, the test substance is classified as readily biodegradable according to the test.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	MSCI (2006)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Semi - static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Dimethylformamide (DMF), 100 µL/L
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Liquid Chromatography Mass Spectrum (LC/MS)
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Test substance (100 mg) was mixed with 100 mL of dilution water. The mixture was stirred for 24 hours and filtered to give test concentration of 0.0053 mg/L.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Mean measured*		*24 h*	*48 h*
Control		20	0	0
Solvent control		20	0	0
100	0.00404*	20	0	0

*Limit test of the solubility in dilution water

EC50	> 0.00404 mg/L at 48 hours
NOEC	0.00404 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The EC50 values and the 95% confidence limits could not be determined statistically because the immobility of daphnids at the maximum concentration level was less than 50%. Therefore, the EC50 values have been indicated in the estimated concentration range.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates
TEST FACILITY	MSCI (2008c)

C.2.2. 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: 100 mg/L Actual: 0.02 mg/L
Auxiliary Solvent	
Water Hardness	Not reported
Analytical Monitoring	LC/MS
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Test substance (100 mg) was mixed with 100 mL of dilution water. The mixture was stirred for 48 hours and filtered to give test concentration of 0.022 mg/L.

RESULTS

Biomass		*Growth*	
E_yC50	*NOEC*	*ErC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
Not calculated	Not calculated	> 0.00189	0.00189

Remarks - Results	All validity criteria for the test were satisfied. The initial test concentration was 0.02 mg/L which reduced to 0.000051 mg/L at 72 hours. The time weighed average of measured concentration was calculated to be 0.00189 mg/L. This is attributed to high log Pow of the test substance (> 6.5) sticking to the glassware. The EC50 values and the 95% confidence limits could not be determined statistically because the growth inhibition at the maximum concentration level was less than 50% in the limit test. Therefore, the EC50 values have been indicated in the estimated concentration range.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	MSCI (2009)

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