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March 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Ethane, 1-ethoxy-2-(2-methoxyethoxy)-

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
STD/1541	Mimaki Australia Pty Ltd	Ethane, 1-ethoxy-2-(2-methoxyethoxy)-	Yes	≤ 30 tonnes per annum	Component of inkjet printing inks

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is 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. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Flammable Liquids Category 4	H227 – Combustible liquid.
Reproductive Toxicity Category 2 Suspected Human Reproductive Toxicant	H361 – Suspected of damaging fertility or the unborn child.

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R62/63: Possible risk of impaired fertility/Possible risk of harm to the unborn child

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

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Flammable Liquids Category 4: H227 – Combustible liquid.
  - Reproductive Toxicity Category 2 Suspected Human Reproductive Toxicant: H361 – Suspected of damaging fertility or the unborn child.

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

- The notifier should comply with relevant State and Territory OHS labelling requirements.

- NICNAS will refer the notified chemical to Safe Work Australia, for their consideration.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced in the ink cartridges, during printing processes:
  - Local exhaust ventilation or other mechanical ventilation (as specified in Safe Work Australia Guidance Control guidance sheet *P39 Wide-format inkjet printing with solvent-borne inks*) if inhalation exposure may occur.

- 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 the ink cartridges, during printing processes:
  - Avoid contact with skin and eyes
  - Avoid breathing in vapours
  - Compliance with Safe Work Australia Guidance Control guidance sheet *P39 Wide-format inkjet printing with solvent-borne inks*

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced in the cartridges:
  - Coveralls
  - Disposable gloves if dermal exposure to the ink may occur
  - Respiratory protection if engineering controls are inadequate to control inhalation exposure

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A person conducting a business or undertaking at a workplace should take appropriate measures to avoid the occurrence of concentrations of the notified chemical in air that are within the explosive limits.

- A copy of the (M)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 for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

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

Storage

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

Emergency procedures

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

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 notified chemical is imported in any form other than as a component of printing ink at up to 85% concentration in sealed cartridges or foil bags;
- the ink containing the notified chemical is used for printing in other than commercial/industrial facilities.

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 inks, 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 notified chemical and a product containing the notified chemical provided by the notifier were 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
Mimaki Australia Pty Ltd (ABN: 55 162 136 076)
Unit 14, 38-46 South Street
RYDALMERE, NSW 2116

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: analytical data, degree of purity, impurities, additives/adjuvants, use details and import volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: all physico-chemical endpoints except for autoignition temperature and water solubility

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2006), China (2013), European Union (1990), Korea (2004), Switzerland (1999) and USA (2014)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
SS21 Ink Series (Products containing the notified chemical at concentrations ≤ 85%)

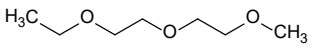
CHEMICAL NAME
Ethane, 1-ethoxy-2-(2-methoxyethoxy)-

OTHER NAME(S)
HISOLVE EDM

CAS NUMBER
1002-67-1

MOLECULAR FORMULA
$C_7H_{16}O_3$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
148.2 Da

ANALYTICAL DATA
Reference NMR, FTIR, HPLC-UV and UV-Visible spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless Liquid

Property	Value	Data Source/Justification
Melting Point	-72 °C	(M)SDS
Boiling Point	176 °C	(M)SDS
Density	923 kg/m^3 at 20 °C	(M)SDS
Vapour Pressure	0.13 kPa at 25 °C	Calculated using Antoine Equation
Water Solubility	> 1 × 10^3 g/L at 25 °C	Measured
Hydrolysis as a Function of pH	Not determined	Not expected to be hydrolysed in the environmental pH range (4 - 9)
Partition Coefficient (n-octanol/water)	log Pow = 0.009 at 25 °C	Calculated (KOCWIN v1.68)
Adsorption/Desorption	log K_{oc} = 0.66 at 25 °C	Calculated (KOCWIN v2.00)
Dissociation Constant	Not determined	Does not contain dissociable functional groups
Flash Point (closed cup)	68.5 °C	(M)SDS
Explosion Limits	Upper: 33.0% Lower: 2.5%	Measured
Autoignition Temperature	169 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidative properties
Surface Tension	26.8 mN/m at 25 °C	(M)SDS
Viscosity	1.2 mPa.s at 20 °C	(M)SDS

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. Based on the SDS of the notified chemical, it may react intensively with strong oxidising agents.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is 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. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Flammable Liquids Category 4	H227 – Combustible liquid

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. It will be imported as a component (≤ 85% concentration) of a series of inks for commercial ink-jet printers in sealed cartridges (220 or 440 mL/cartridge) or foil bags (2 L/bag). The neat form of the notified chemical will not be imported and reformulated. Imported inks containing the notified chemical will not be repackaged.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 30	≤ 30	≤ 30	≤ 30	≤ 30

PORT OF ENTRY
Sydney, Melbourne, Perth and Brisbane

TRANSPORTATION AND PACKAGING

The inks containing the notified chemical up to 85% will be packed in 220 mL or 440 mL sealed cartridges or 2 L foil bags. The packed inks will be transported by road throughout Australia.

USE

The notified chemical will be used as the solvent for an inkjet ink system for commercial digital printing. The printing substrates are expected to be the following:

Substrate	*Percentage of Use (%)*
Vinyl	60
Canvas	15
Paper	15
Shade Cloth	5
Others	5
Total	**100**

OPERATION DESCRIPTION

The cartridges and foil bags containing the notified chemical at up to 85% concentration will be delivered to the end-user printing sites in the same packaging in which they are imported. The sealed cartridges (maximum 440 mL in capacity) or foil bags (maximum 2 L in capacity) will be handled by service technicians or workers in accordance with the instructions provided with the packages. Based on the ink system, 9 cartridges with different colours may be installed in each printer and 9 foil bags with respective colours may be connected to the system to supply extra inks. It is expected that there will be two printers at each site. Cleaning and maintenance of the printers are expected to be carried out by the service technicians and workers. Empty cartridges and foil bags will be disposed of to landfill.

During printing, the inks containing the notified chemical up to 85% concentration will be transferred from the cartridges and the foil bags to the printing heads. The printing processes are expected to be fully automated. During the printing process, much of the notified chemical is expected to be volatilised. Small proportions of the chemical may be trapped in the printing substrates and volatilise slowly after printing. The notifier stated that at some sites there will be local exhaust ventilation in the printing areas above the printers; however, local exhaust ventilation is not expected to be used at all printing sites.

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)*
Storage and transport personnel (inks)	4	50
Printer operators	2	50
Service technicians	6	200
Storage and transport (printed media)	1	50

EXPOSURE DETAILS

Transport and storage

Transport and storage workers (for both inks and printed media) are unlikely to be exposed to the notified chemical except in the event of an accidental release of the wet inks.

End-use

Under the proposed use scenario, print workers and service technicians may have the potential for dermal and ocular exposure to the inks containing the notified chemical at up to 85% concentration during ink cartridge replacement, foil bag connection and printer maintenance/cleaning. As the ink packages are purposely designed to enclose the inks, and the printing processes are automated, the potential for dermal and ocular exposure to the notified chemical during printing is expected to be limited. Workers handling articles before the inks have completely dried may also come into minor dermal contact with the notified chemical in liquid form up to 85%

concentration. The notifier stated that, service technicians and printer operators are required to wear impervious gloves and safety glasses during the operations to minimise the potential for exposure.

Repeated inhalation exposure of printer operators to the notified chemical may occur during and immediately after the printing process, as a large proportion of the chemical is expected to be volatilised during printing. The extent of inhalation exposure during printing will be reduced at those sites with local exhaust ventilation over the printing machine. Further vapour may be released slowly from the print matrix, contributing to worker inhalation exposure in the printing and printed media storage areas.

In a typical use scenario provided by the notifier, assuming an average air space of 1,500 m^3 for a print workshop with a general ventilation airflow rate of 3 air changes/hour and with two printers using a total of 200 mL inks per day, the average air concentration of the notified chemical may reach 17.4 mg/m^3. Printer operators with an average body weight of 60 kg, assumed air inhalation rate of 23 m^3/day and exposure duration of 2 hours a day may inhale the notified chemical in a level of 0.56 mg/kg bw/day. It is acknowledged that there is a paucity of relevant exposure data for printing operators and that variables such as workshop dimensions and number of printers per site highly influence the estimated dose. The pattern of worker exposure is also highly variable and uncertain. For example, workers working in proximity of the printers while the printing processes are still occurring may be exposed to significantly higher than average level of the notified chemical, if local exhaust ventilation above the printing head is absent or insufficient.

Once the inks are dried, the notified chemical is expected to have evaporated and will not be available for further exposure.

6.1.2. Public Exposure

The inks in the cartridges and foil bags containing the notified chemical at up to 85% concentration are intended for use in commercial settings only and will not be sold to the public. The public may come into contact with dried inks on the substrates after the printing. However, once the inks are dried, the notified chemical is expected to be evaporated and will not be available for exposure to the public.

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 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 5.14 mg/L/4 hour; low toxicity
Rabbit, skin irritation	Slightly irritating
Rabbit, eye irritation	Slightly irritating
Mouse, skin sensitisation – Local lymph node assay	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 250 mg/kg bw/day
Rat, repeat dose oral toxicity combined with reproductive / developmental toxicity screening test – 42 days*	NOAEL = 250 mg/kg bw/day for both repeat dose and reproductive/developmental toxicity
Mutagenicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test	Non genotoxic

* This study was submitted to NICNAS through a separate new chemical notification. The study results are relevant for, and have been used to support this risk assessment.

Toxicokinetics, metabolism and distribution

The notified chemical has a molecular weight < 500, is highly volatile at ambient atmospheric conditions, and is highly water soluble. Based on its chemical and physical characteristics and information on a similar chemical, diethylene glycol dimethyl ether (CAS 111-96-6), it is expected that the notified chemical would be readily absorbed through biomembranes via all routes, distributed within the body and metabolised. It was also reported that dermal absorption of glycol ethers in both liquid and vapour form is very high (CICAD, 2002).

Acute toxicity
Acute toxicity studies on the notified chemical suggest that the notified chemical is of low toxicity via the oral, dermal and inhalation routes. In the acute oral toxicity study, although no mortality at the dose level of 2,000 mg/kg bw was observed, staggering gait after the dosing was recorded in the test animals. The acute dermal toxicity study recorded a transient slight body weight loss in the animals treated with the notified chemical. In the acute inhalation study, irregular respiration was observed in all test animals following exposure to the notified chemical, with one male exhibiting hypoactivity. Body weight loss was also noted in all animals treated with the notified chemical in the acute inhalation study.

Irritation and sensitisation
Skin irritation and eye irritation studies were provided for the notified chemical. The notified chemical was tested in neat form (100%) in the studies. The results showed that the chemical is slightly irritating to the eye and skin. However, in both studies, the irritation scores were below the GHS criteria for classification.

In a mouse local lymph node assay (LLNA) using non-radioactive method, the notified chemical was tested up to 100% concentration and did not show evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation.

Repeated dose toxicity
Two repeated dose oral toxicity studies were made available for the notified chemical. A 28-day study submitted by the notifier was based on a 14-day repeated oral toxicity range finding study. Doses in the main study were 250, 500 and 1,000 mg/kg bw/day (and 2 week recovery groups administered 0 or 1,000 mg/kg bw/day)

A 42-day combined repeated dose oral toxicity study with reproductive/developmental toxicity screening test conducted to the OECD TG 422 was separately submitted to NICNAS in a different notification (Appendix B.9). In this study the doses tested were 50, 250 and 1,000 mg/kg bw/day (and 2 week recovery groups administered 0 or 1,000 mg/kg bw/day).

All three studies used a Sprague-Dawley strain of rat, with animals dosed by gavage. Combined, the 28-day and 42-day studies provide data for a dose response characterisation with dose levels of 0, 50, 250, 500 and 1,000 mg/kg bw/day. Additionally, a direct comparison can be made between the studies at 250 and 1,000 mg/kg bw/day and at 1,000 mg/kg bw/day following a 2 week recovery period.

Mortality
In the 28-day repeated dose oral toxicity study, at the dose level of 1,000 mg/kg bw/day, 2 of 10 males were found dead during the dosing period. The cause of deaths was not established by histopathology examination. The clinical sign seen in these 2 animals was limited to a decrease in spontaneous motor activity on Day 9 only. No deaths occurred in the separately submitted 42-day repeated dose oral toxicity study combined with reproductive/developmental screening test during the 6-week dosing period, including the dose level of 1,000 mg/kg bw/day. An absence of treatment-related clinical signs of toxicity and effects on body weight in the animals of both studies, and the fact that no deaths were seen in the 42-day study suggest that the deaths in the 28-day study were unlikely to be related to the test material.

Clinical observations
In the 28-day repeated dose oral toxicity study, at dose levels of 500 or 1,000 mg/kg bw/day, the notified chemical induced a few sporadic behavioural changes in males during the treatment. These behavioural findings were not observed in the separately submitted 42-day study. Thus, an evaluation of all the available data does not provide robust evidence of treatment-related clinical signs of toxicity or a neurotoxicity potential for the notified chemical.

No treatment-related effect was seen on body weight or food consumption in either of two available repeated dose studies up to the dose level of 1,000 mg/kg bw/day, including recovery groups.

Laboratory findings
Statistically significant decreases were seen in alkaline phosphatase, sodium and chloride in both the 28-day and 42-day studies at 1,000 mg/kg bw/day. The alkaline phosphatase changes were also seen in the 28-day study at the dose levels of 250 and 500 mg/kg bw/day, and may possibly be reflective of an adverse effect on metabolic function. These changes were considered to be treatment-related and potentially toxicologically significant at 500 and 1,000 mg/kg bw/day. The decreases in sodium and chloride were slight and absent in animals at the end of recovery period. Therefore, these findings are not considered toxicologically significant but potentially

treatment-related. No treatment-related toxicologically significant changes in clinical chemistry parameters occurred at the next dose level of 250 mg/kg bw/day in either the 28-day or the 42-day study.

Statistically significant changes were seen in haematology parameters, in the 28-day study: reduction in haemoglobin concentration and reticulocyte count at 500 and 1,000 mg/kg bw/day, and increase in monocytes at 1,000 mg/kg bw/day. In the 42-day study, statistically significant changes were seen only at 1,000 mg/kg bw/day: decrease in mean corpuscular haemoglobin concentration, eosinophil count and ratio, and increase in monocyte ratio. An evaluation of all the available data therefore provides robust evidence of a treatment-related and toxicologically significant increase in monocytes, eosinophils and eosinophil ratio at 1,000 mg/kg bw/day. No treatment-related toxicologically significant changes in haematology parameters were considered to occur at the next dose level of 500 mg/kg bw/day.

No treatment-related effect was seen on urinalysis parameters in either study, up to the highest dose level of 1,000 mg/kg bw/day, including the recovery groups.

Effects on non-reproductive organs

Changes in spleen and liver (increase), adrenal and thymus (decrease) weight were consistently seen in the 28-day study at the dose level of 1,000 mg/kg bw/day. Liver weight increase was also seen in animals treated at 500 mg/kg bw/day. In the 42-day study, statistically significant changes in liver (increase) and thymus (decrease) weight were reported at the dose level of 1,000 mg/kg bw/day. While the liver weight changes were associated with microscopic changes, the thymus weight changes were associated with macroscopic and microscopic changes. The liver and thymus weight changes are considered potentially treatment-related and toxicologically significant. The adrenal weight findings in the 28-day study were seen in the absence of macroscopic and microscopic changes and are not considered toxicologically significant. The spleen weight findings in the 28-day study were seen in the presence of macroscopic and microscopic changes, but the findings were absent in the 42-day study, suggesting that the findings were likely not related to the test material. No treatment-related toxicologically significant changes in organ weights were considered to occur at the next dose level of 250 mg/kg bw/day.

The limited finding of minimal periacinar hepatocyte hypertrophy in the liver of 1 animal of the recovery group administered at 1,000 mg/kg bw/day in the 28-day study was not seen in the separately submitted 42-day study. Thus, it is not considered to be toxicologically significant but it may potentially be treatment-related.

At the end of the recovery period, no adverse treatment effects on the non-reproductive organs were seen in males administered at 1,000 mg/kg bw/day.

Reproductive/developmental toxicity

Reproductive toxicity

In both the 28-day and 42-day studies, reproductive/developmental toxicity-related findings were observed in the high dose treatment group of 1,000 mg/kg bw/day. Statistically significant reductions in epididymis and testis weights of the males were associated with histopathological changes in the reproductive organs including degeneration/necrosis of spermatocyte/spermatid, decrease of sperm and appearance of cell debris in the duct of the epididymides, some of which were of increased degree and incidence at the end of the recovery period. Reversibility of organ weight reductions was not recorded in either study, following a 2 week recovery period. These changes are considered treatment-related and toxicologically significant.

No treatment-related macroscopic effect was seen on male sex organs in either study, up to and including 1,000 mg/kg bw/day. Similarly, no treatment-related macroscopic effect on male sex organs was seen in 1,000 mg/kg bw/day recovery animals in either study.

Sperm analysis was carried out in the 28-day study at the end of the dosing period. The following effects seen in males administered at 1,000 mg/kg bw/day were considered treatment-related and toxicologically significant:

- Motility and count: a decrease in motility of spermatozoa and an increase in immobile spermatozoa which were associated with a decrease in percentage mobile spermatozoa.

- Morphology: a statistically significant ($p < 0.05$) decrease in the percentage of normal spermatozoa and a statistically significant ($p < 0.05$) increase in percentage spermatozoa with head anomaly which

were associated with an increase (not statistically significant) in percentage of isolated head in the sample.

- Numeration in epididymis: a statistically significant ($p < 0.01$) lower epididymis tail weight and a decrease (not statistically significant) in the number of spermatozoa in the tail of the left epididymis.

Overall, a comparison of the data between the two available studies shows no significant qualitative differences in effects on male reproductive organs of the test material. Both studies provide no evidence of treatment-related macroscopic changes in male sex organs. However, both studies demonstrated a reduction in testis and epididymis weight along with microscopic changes in these organs at the dose level of 1,000 mg/kg bw/day.

There were no effects seen at the next dose levels of 500 or 250 mg/kg bw/day in the 28-day or the 42-day study, respectively.

Developmental toxicity

In the 42-day study, conducted to the OECD TG 422, prolongation of gestation period and decrease of delivery index were noted in females treated at the dose level of 1,000 mg/kg bw/day. In offspring, a decrease of litter number and birth index, and increase of number of stillborn were observed that were significantly different from the parameters of the vehicle control. Other adverse effects seen in the high dose group were total litter loss in 5 females and at post-natal Day 4 a reduced viability index and low body weights of offspring. The No Observed Adverse Effect Level (NOAEL) was established as 250 mg/kg bw/day in this study for both the repeated dose toxicity and the reproductive/developmental toxicity, based on the effects observed at the dose level of 1,000 mg/kg bw/day.

Further considerations

The 42-day study was evaluated by NICNAS, as part of a separate notification for the same chemical. The evaluation concluded that the observed findings in male reproductive organs at the dose level of 1,000 mg/kg bw/day could not be considered as a secondary, non-specific consequence of other toxic effects. The observed deaths in the 28-day study, sporadic clinical signs, limited changes in haematology parameters, change in a single clinical chemistry parameter, organ weight changes in the liver and thymus, macroscopic changes in the thymus, and microscopic changes in the liver and thymus, are also not considered to provide evidence of significant generalized toxicity for which the observed findings in male reproductive organs can be considered as secondary, non-specific consequence.

The 28-day study submitted by the notifier produced the same findings as in the separately submitted 42-day study regarding the male reproductive organs at the dose level of 1,000 mg/kg bw/day, such as decreases in absolute and relative testis and epididymis weight and microscopic changes in these organs. These effects on the male reproductive organs in both studies showed no reversibility after a 2 week recovery period and were consistent with the reproductive/developmental hazards seen with some short chain glycol ethers of this chemical class. Therefore, the concern that the notified chemical is a potential hazard for reproductive toxicity cannot be currently dismissed based on the available data. Additionally, this concern is supported by the fact that certain glycol ethers of this chemical class are considered as reproductive toxicants. *Safe Work Australia* (SWA) has listed a structurally very similar chemical, diethylene glycol dimethyl ether (CAS No. 111-96-6), as a reproductive Category 2 toxicant in the *Hazardous Substances Information System* (HSIS, web link - http://hsis.safeworkaustralia.gov.au/).

Recently, the US EPA has issued a Significant New Use Rule (SNUR, US EPA, 2014) on 7 commercially available ethylene glycol ethers regarding reproductive hazard concerns for this group of chemicals. According to the US EPA, based on both toxicity data and structure-activity relationships, ethylene glycol ethers that consist of 1, 2 or 3 glycol ether groups and terminal alkyl groups of 1 to 4 carbons can be anticipated to cause developmental and reproductive toxicity and/or haemolytic toxicity. Based on the structure of the notified chemical, it characteristically falls into the same category of ethylene glycol ethers for which the US EPA raised concerns. According to the US EPA SNUR documents, ethylene glycol ethers have been shown to cause damage to reproductive organs as well as toxicity to blood and blood forming organs. Exposure to this group of chemicals can pose risks to consumers, workers and children because of potential birth defects due to damage of reproductive organs.

Mutagenicity/Genotoxicity

The notified chemical is not expected to be mutagenic or genotoxic as a reverse mutation assay and an *in vivo* mammalian erythrocyte micronucleus test on the notified chemical did not reveal evidence of genotoxicity.

Health hazard classification
Evaluation of the 42-day study concluded that the observed findings in male reproductive organs at 1,000 mg/kg bw/day were not considered a secondary non-specific consequence of other toxic effects. The study also raised concerns for developmental toxicity at the highest dose tested of 1,000 mg/kg bw/day. According to the GHS (Section 3.7.2.5.3), findings on male reproductive organs in repeat dose studies can be considered sufficient to justify classification for reproductive toxicity in the absence of a one or two generation test.

The systemic findings in the 28-day study, excluding effects on the male reproductive organs, are also not considered to constitute marked toxicity such that the observed effects on male reproductive organs may be considered a secondary, non-specific consequence of such. Supportive evidence includes observed deaths at 1,000 mg/kg bw/day being unlikely to be related to the test material; no significant changes in bodyweight and food consumption; sporadic clinical signs of toxicity; limited (and generally non-reproducible) changes in haematology parameters; change in a single clinical chemistry parameter; and limited macroscopic changes and changes to organ weight. Furthermore, there were observed changes in male reproductive organs, including histopathological changes, which were still evident after a 2 week recovery period.

The 28-day and 42-day studies produced the same findings at 1,000 mg/kg bw/day: decreases in absolute and relative (to bw) testis and epididymis weight, and microscopic changes in these organs. Both studies also showed no reversibility of effects on testis and epididymis weight and microscopic changes in these organs after a 2 week recovery period. Additionally, sperm analysis in the epididymis was undertaken in the 28-day study with treatment-related effects being noted on motility, count, morphology and numeration in the epididymis at 1,000 mg/kg bw/day.

Overall, based on the findings observed in the two repeated dose studies, which are not considered as a secondary non-specific consequence of other toxic effects, it is concluded that the full safety of the notified chemical has not currently been established, with respect to reproduction and development. Classification as a Category 2 reproductive toxicant under the GHS is therefore justified in this instance, for the notified chemical.

Based on above considerations, the notified chemical is recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Reproductive Toxicity Category 2 Suspected Human Reproductive Toxicant	H361 – Suspected of damaging fertility or the unborn child.

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R62/63: Possible risk of impaired fertility/Possible risk of harm to the unborn child

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the study reports provided, the notified chemical is slightly irritating to the skin and eye. However, the enclosed nature of the ink packaging containing the notified chemical at a concentration ≤ 85% and computerised automatic printing processes would limit the dermal/ocular exposure and thus the irritation effects. Dermal and ocular exposure could also occur to the vapour of the notified chemical released during and after the printing. The notifier stated in the submission that service technicians and printer operators are required to wear protective gloves during operations to minimise the potential for exposure.

The major concern for long-term exposure to the notified chemical is the possibility of adverse reproductive and/or developmental outcomes to workers carrying out printing and associated processes. Since the notified chemical is expected to volatilise during printing and drying, repeated inhalation exposure for workers is expected to be the largest contributor to the daily systemic uptake of the chemical. Based on the NOAEL of 250 mg/kg bw/day established in the repeated oral toxicity study for the notified chemical and the daily average inhalation uptake of 0.56 mg/kg bw/day in a typical use scenario (see Section 6.1.1), a margin of exposure (MoE) can then be calculated as approximately 449. A MoE value ≥ 100 is considered to be acceptable for the proposed use scenario. However, as mentioned in Section 6.1.1, there is a paucity of relevant exposure data for

printing operators. The worker inhalation exposure scenario is also uncertain. It is acknowledged that these factors highly influence the calculated MoE. Therefore, given the hazard profile of the notified chemical, risk controls (i.e., engineering, work practices and personal protective equipment) should be implemented and their effectiveness monitored.

Under the conditions of use provided by the notifier regarding the product composition, usage and scenarios of use, the risk to printing workers from the use of the notified chemical is not expected to be unreasonable. However, changes in use parameters such as higher usage of the ink containing the notified chemical, longer daily worker exposure, higher concentrations of the notified chemical in the inks, or printing in poorly ventilated or small areas may significantly increase the inhalation exposure and thus the risk. The notifier has stated that local exhaust ventilation will be present over the printing machines at some sites but not others. At sites where local exhaust ventilation is absent or insufficient, workers working in proximity of the printers with printing processes continuously undertaken may be exposed to highly concentrated vapour of the notified chemical. Therefore controls to reduce inhalation exposure during printing and while the printed media are drying would further reduce exposure and risk. These could include local enhanced ventilation and safe work practices. The Safe Work Australia (SWA) guidance document, *P39 –Wide-format inkjet printing with solvent-borne inks*, recommends providing a good standard of general ventilation, and that ventilation equipment is maintained and working effectively. In order to provide fresh air, powered wall- or window-mounted fans, with five to ten air changes per hour and a through draught, are recommended in this guidance document. Safe work practices and use of appropriate PPE would further reduce exposure.

While the notified chemical is classified as a flammable liquid Class 4, under the proposed use scenario it is not likely that the air concentration of the notified chemical would reach the lower explosion limit of 2.5% if effective engineering controls (ventilation) are in place to reduce the inhalation exposure of workers. Therefore, if these controls are in place, the notified chemical is not expected to pose a flammability hazard in the workplace.

Overall, provided that adequate workplace controls are in place to reduce exposure to the inks containing the notified chemical, the risk to workers is not considered unreasonable.

6.3.2. Public Health

The products containing the notified chemical will not be sold to the public. The public may have contact with dry printed materials. However, as the notified chemical is used as a solvent of the ink, it is expected to be evaporated during the printing and not to be available for exposure after drying. Therefore, based on the use scenario, the risk of the notified chemical to the health of 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 or reformulated in Australia. It will be imported in sealed cartridges contained by foil bags (concentration of up to 80%). The foil bags will be packed within a cardboard box. The ink cartridges and bags are designed to prevent leakage and will not be opened during transport. Therefore there is assumed to be no release of the notified chemical to the environment from these activities.

RELEASE OF CHEMICAL FROM USE

The ink cartridges and foil bags are designed to prevent leakage and will not be opened during use, installation or replacement. Therefore, there will be no release of the notified chemical to the environment from these activities under normal conditions. However, if leakage or spillage occurs, the ink containing the notified chemical is expected to be contained with absorbent materials and will be disposed of to landfill. The residual ink containing the notified chemical is expected to be disposed of to landfill. The applied notified chemical is semi-volatile (VP = 0.1 kPa at 20°C) and the majority of the notified chemical is expected to be evaporated from the ink matrix during heat-drying before the substrate leaves the printer.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of the notified chemical is expected to be released to the atmosphere during drying. However, some chemical will remain within the dried ink matrix and will be disposed of to landfill and is expected to remain

associated with the substrate to which it has been applied. Of the notified chemical applied to paper, 50% is expected to be recycled. During recycling processes, waste paper is repulped using a variety of chemical agents which, amongst other things, enhances detachment of ink from the fibres.

7.1.2. Environmental Fate

The majority of the notified chemical is expected to be released to the atmosphere. The notified chemical is semi-volatile with a vapour pressure of 0.1 kPa at 25 °C. Thus, the notified chemical's potential for persistence in air and long range transport was assessed using AOP Program (v1.92) within the US EPA EpiSuite. This estimates the half-life of the notified chemical in air, based on a 12 hour day, as 3.66 h, which indicates that the notified chemical is expected to react rapidly with OH-radicals and therefore will not have the potential for long-range transport.

Notified chemical trapped in the ink matrices is expected to be disposed of to landfill with the substrate to which it is applied. The notified chemical is not readily biodegradable. Given the high water solubility and low log Koc, the notified chemical may leach from landfill and enter surface waters. A small proportion of the notified chemical may be released to sewer during paper recycling. Given the high water solubility and low log Koc, the notified chemical is not expected to partition to sludge during waste water treatment processes in sewage treatment plants (STPs). Therefore, the notified chemical is expected to remain in waste water and be released to aquatic environments.

Based on its high water solubility and low log Pow, the notified chemical is not expected to bioaccumulate. Ultimately, the notified chemical is expected to degrade via biotic and abiotic processes in the atmosphere and surface waters to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

Based on its use in printing, it is conservatively assumed that 100% of the total import volume of the notified chemical was used in paper printing. Further, it is assumed that 50% of the paper products containing the notified chemical will be recycled and 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 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	30,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	15,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	57.69	kg/day
Water use	200.0	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:	12.76	µg/L
PEC - Ocean:	1.28	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 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 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 12.75 µg/L may potentially result in a soil concentration of approximately 0.085 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 0.4252 mg/kg and 0.85 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
Fish	LC50 (96 h) > 200 mg/L	Not harmful to fish
Daphnia	EC50 (48 h) > 100 mg/L	Not harmful to aquatic invertebrates
Algae	EC50 (96 h) > 100 mg/L	Not harmful to algae

The notified chemical is not formally classified under the Globally Harmonised System of Classification of Chemicals (GHS; United Nations, 2009) due to a lack of aquatic toxicity.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species (Algae, E_rC50) for the notified chemical. Acute ecotoxicity endpoints for aquatic species from three trophic levels are available. Therefore, an assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Endpoint (Lower Limit)	100	mg/L
Assessment Factor	100	
PNEC:	1,000	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient values have been calculated as follows:

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	12.76	1,000	0.013
Q - Ocean:	1.28	1,000	0.001

The Risk Quotients (Q = PEC/PNEC) for a conservative discharge scenario have been calculated to be << 1 for the river and ocean compartments. The notified chemical is not expected to be bioaccumulative and is expected to slowly degrade in the environment. Based on the short half-life of the notified chemical in air, it is not expected to pose an unacceptable risk in this compartment. 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

Vapour Pressure 0.13 kPa at 25 °C (calculated)

Method	Antoine Equation: Log P = A - B / (T+C) P: vapour pressure in mmHg; T: temperature in °C A = 7.281; B = 1678.922 and C = 205.560 (chemical specific constants)
Remarks	The vapour pressure for the notified chemical was calculated as 1.6 mmHg at 25 °C which is equivalent to 0.13 kPa.
Test Facility	Toho Chemical Industry Co., Ltd. (No study report provided)

Water Solubility > 1,000 g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method
Test Facility	Sumika Chemical Analysis Service, Ltd (2014)

Explosion Limits Upper: 33.0%
Lower: 2.5%

Method	Measured using an Explosion Limit Measurement Device (no details provided)
Remarks	Sample gas was taken at 130 and 160 °C for the measurement of lower limit and upper limit respectively.
Test Facility	Material Hazard Laboratory, Japan Carlit Co., Ltd. (No study report provided)

Autoignition Temperature 169 °C

Method	Measured using ASTM-type Ignition Point Measurement Device (no details provided)
Remarks	Measurement conditions: Temperature – 23 °C Humidity – 41% Atmospheric pressure – 101.1 kPa
Test Facility	Material Hazard Laboratory, Japan Carlit Co., Ltd. (No study report provided)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Council Regulation No 440/2008 B.1 Acute Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley – CRL: OFA (SD)
Vehicle	Water for irrigation
Remarks - Method	No significant deviations of the protocol were noted. Animals were treated with the notified chemical at 2,000 mg/kg bw in a volume of 10 mL/kg bw diluted with the vehicle. Three animals were treated in the first step, followed by another three animals in the second step. Clinical observations, functional and neurobehavioural tests were performed on Days 1, 7 and 14.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	6 F	2,000	0/6

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Staggering gait was observed in 3 of the 6 test animals 4 hours after the dosing (all from the same group). No other clinical signs or significant body weight changes were observed.
Effects in Organs	No gross findings were noted at necropsy.
Remarks - Results	A single dose of the notified chemical at 2,000 mg/kg bw via the oral route did not induce significant sign of toxicity in female rats.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	CERB (2014a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/ Sprague-Dawley – CRL: OFA (SD)
Vehicle	None. The test substance was administered undiluted.
Type of dressing	Semi-occlusive.
Remarks - Method	No significant deviations of the protocol were noted. Test animals were treated with the notified chemical at a dose of 2,000 mg/kg bw in a volume of 2.17 mL/kg bw based on the density of the chemical. As well as the routine in-cage clinical observations, a functional and neurobehavioural examination was carried out on Days 2, 7 and 14.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 M/5 F	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	A red area was observed on the muscle under the treatment site in 1 of the 10 test animals.
Signs of Toxicity - Systemic	A transient slight loss of body weight was noted between Day 1 and Day 2 in both females and males. Passive response to finger approach was

	observed in 1 of the 10 test animals.
Effects in Organs	No significant macroscopic findings were noted at the necropsy.
Remarks - Results	A single dermal application of the notified chemical at a level of 2,000 mg/kg bw did not cause mortality, significant clinical signs or dermal changes in rats.

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

TEST FACILITY CERB (2014b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation).

Species/Strain	Rat/Sprague-Dawley derived, albino
Vehicle	Air
Method of Exposure	Nose only exposure.
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	2.62 μm (MMAD)
Remarks - Method	No significant deviations of the protocol were noted. Procedures and aerosolisation conditions were established through pre-test trials.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 M/5 F	35.55	5.14	0/10

LC50	> 5.14 mg/L/4 hours
Signs of Toxicity	Irregular respiration was observed in all test animals following the exposure with one male exhibiting hypoactivity. All animals recovered by Day 4 after the exposure. Body weight loss was noted in 9/10 test animals by Day 1, but had been re-gained by Day 7, and normal weight gain continued to Day 14.
Effects in Organs	No gross abnormalities were noted at the necropsy.
Remarks - Results	

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY Product Safety Labs (2014)

B.4. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	Three (males)
Vehicle	None
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	The purity of the test substance was not specified. After the 4 h application period, any residual test material was removed by gentle swabbing with 74% industrial methylated spirits.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	*2*	*3*			
Erythema/Eschar	2.0	1.7	1.3	2	< 7days	0[#]
Oedema	1.0	1.0	0.3	2	< 7days	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
# Slight desquamation was present.

Remarks - Results: Well defined erythema and slight oedema was noted at all treated skin sites after 1 hour after the patch removal. After 7 days, slight desquamation was observed on all treatment sites.

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

TEST FACILITY: Safepharm (1997a)

B.5. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion (2012).
EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3F
Observation Period: 8 days
Remarks - Method: No significant deviations of the protocol were noted. In 1 animal, corneal examinations at 24 h and 48 h were not confirmed by fluorescein application to the eye.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.3	1.0	1.0	2	< 8 days	0
Conjunctiva: chemosis	1.3	0.3	0.7	3	< 8 days	0
Conjunctiva: discharge	1.3	0.3	0.3	2	< 8 days	0
Corneal opacity	0.0	0.0	0.3	1	< 48 hours	0
Iridial inflammation	0.0	0.0	0.0	0	N/A	0

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

Remarks - Results: After the treatment with the test substance, eye irritation signs such as conjunctiva redness, discharge and chemosis were observed in all animals. These signs were recovered within 8 days.

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

TEST FACILITY: CERB (2014c)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain: Mouse/CBA/J Rj female
Vehicle: Acetone/olive oil (4:1 v/v)

Remarks - Method

Non-radioactive method was used. Recording of local reactions was made before each application and just before euthanasia. Ear thickness measurements were made before application on Days 1, 3 and 6. Lymphoproliferative response was measured by incorporation of 5-bromo-2´-deoxyuridine on Day 6. The results were elucidated using an Elisa system on Day 7.

Dose levels of 50%, 75% and 100% of the notified chemical were selected based on a preliminary study. DNCB at a concentration of 0.5% in the vehicle was used as a positive control.

RESULTS

Concentration (% w/w)	*Proliferative response (Cell count/lymph node) (× 10^6)*	*Cellularity Index (Test/Control Cell Count Ratio)*	*Stimulation Index (Test/Control OD* Ratio)*	*Increase in Ear Thickness (Day 1 to Day 6) (%)*
Test Substance				
0 (vehicle only)	3.4	1.0	1.0	32
50	4.2	1.2	1.1	17
75	4.8	1.4	1.2	20
100	4.9	1.4	1.3	25
Positive Control				
DNCB (0.5%)**	24.2	7.1	8.9	40

*Optical density
**2,4-dinitrochlorobenzene

Remarks - Results

Increases of cell count and ear thickness with dose levels of the notified chemical in the test animals were noted. The study authors considered that the changes might be due to the irritation properties of the test substance. However ear thickness also increased in the control group. An EC3 value cannot be calculated based on the stimulation index (SI) obtained in the study since the SI for the notified chemical at 100% was < 3.

CONCLUSION

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

TEST FACILITY

CERB (2014d)

B.7. Repeat dose oral toxicity – 14-day dose range finding study

TEST SUBSTANCE

Notified chemical

METHOD

Range finding study for subsequent OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents

Species/Strain: Rat/Sprague-Dawley Crl: OFA (SD)
Route of Administration: Oral – gavage
Exposure Information: Total exposure days: 14 days
Dose regimen: 7 days per week
Post-exposure observation period: None
Vehicle: Water for irrigation
Remarks - Method: The study was conducted to determine the tolerance and possible toxicity of the notified chemical in rats and to establish a suitable dose range for the subsequent 28-day repeated dose oral toxicity study (see Appendix B.8).

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	3 M/3 F	0	0/6
Low dose	3 M/3 F	500	0/6

Mid dose	3 M/3 F	1,000	0/6
High dose	3 M/3 F	2,000	1/6

Mortality and Time to Death

One female in the high dose group was in moribund condition on Day 5 and euthanised the same day. Before Day 5, this animal was found to have clinical signs such as reduced or absence of spontaneous locomotor activity, staggering gait or piloerection.

Clinical Observations

No clinical signs were noted in animals of the low dose group.

Reduced spontaneous locomotor activity (in 4 of 6 animals), staggering gait (in 2 of 6 animals) and piloerection (in 2 of 6 animals) were observed hours after the treatment in both males and females of the mid dose group between Day 2 and Day 5.

In the high dose group, clinical signs such as absence or reduced spontaneous locomotor activity (in 5 of 5 animals) were observed hours after treatment in both males and female. From Day 1 to Day 5, staggering gait was observed in 4 of 6 animals and piloerection was seen in 3 of 3 females.

From Day 7, males in all treatment groups including the low dose group showed a dose dependent body weight gain reduction associated with reduced food consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical chemistry, haematology and urinalysis were not performed in this dose range study.

Effects in Organs

Both males and females in the all treatment groups showed a dose-dependent relative liver weight increase, which might be associated with macroscopic changes in the liver. In high dose group, multiple punctate, nodule or area changes on the heart in 2 out of 3 males were noted and considered likely to be a dose-related effect. No major variation was observed in kidneys.

Remarks – Results

Since females treated with the notified chemical did not show changes in body weight gain in comparison with the control group, the study authors considered that it could suggest that males are more sensitive to the chemical.

CONCLUSION

Based on the results obtained, the study authors considered that the highest dose of the notified chemical should not exceed 1,000 mg/kg bw/day in the subsequent 28-day repeated dose oral toxicity study (see Appendix B.8 below).

TEST FACILITY CERB (2014e)

B.8. Repeat dose oral toxicity – 28-day study

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain	Rat/Sprague-Dawley Crl: OFA (SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Water
Remarks - Method	No significant deviations of the protocol were noted.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5 M/5 F	0	0/10
Low dose	5 M/5 F	250	0/10
Mid dose	5 M/5 F	500	0/10
High dose	5 M/5 F	1,000	1/10
Control recovery	5 M/5 F	0	0/10
High dose recovery	5 M/5 F	1,000	1/10

Mortality and Time to Death

At the treatment level of 1,000 mg/kg bw/day, 1/5 males from the high dose treatment group died on Day 28 and 1/5 males from the high dose recovery group died on Day 24 (i.e. during the dosing period). The only clinical sign of toxicity seen in these animals was a decrease in spontaneous motor activity on Day 9. Additionally, the animal from the high dose treatment group had decreased testes, multiple punctate on the left kidney, an enlarged liver and a decreased thymus, while the animal from the high dose recovery group had a white area on the spleen with adhesion, an enlarged liver and dark red thyroids/parathyroids. Neither had testicular or epididymal change. Other findings such as enlarged or dark red, mottled and swollen lungs could be regarded as agonal changes (i.e. associated with the death of the animal and unlikely to be treatment-related). The cause of death was not established by histopathology assessment and the deaths are therefore considered unlikely to be related to the test material.

Clinical Observations

At the dose level of 500 mg/kg bw/day, 3/5 males showed a decrease in abdominal tone on Day 7, 1/5 males showed a decrease in body tone on Day 25 and chromodacryorrhoea was observed in 1/5 males on Day 25. At 1,000 mg/kg bw/day, a decrease in spontaneous locomotor activity was observed in 2/5 males on Day 7 and 3/5 males on Day 9. One of the animals showing decreased motor activity on Day 7 also exhibited this on Day 14. Similarly, one of the animals showing decreased motor activity on Day 9 also exhibited this on Day 14. Additionally at 1,000 mg/kg bw/day, an absence of spontaneous locomotor activity in one animal was seen on Day 9 and decreased abdominal tone on Day 14 in the same animal. In the high dose recovery group administered at 1,000 mg/kg bw/day, decreased motor activity was seen in all animals on Day 9, with this finding also seen in one (and the same) animal on Days 10, 11, 12 and 16 (i.e. no findings were seen during the recovery period). All the clinical observations were sporadically seen over the dosing period with no dose response for the incidence of abdominal tone and chromodacryorrhoea.

No treatment-related effect was seen on body weight or food consumption in the study up to the dose level of 1,000 mg/kg bw/day, including recovery groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical chemistry

At the end of the dosing period, a statistically significant decrease in alkaline phosphatase was seen at dose levels of 250, 500 and 1,000 mg/kg bw/day (26%, 34% and 36% respectively, all with $p < 0.01$). No treatment-related statistically significant changes were seen at the end of the recovery period. These changes were seen in the presence of changes in liver weight and microscopic changes to the liver, and were considered treatment-related and potentially toxicologically significant at the dose levels of 500 and 1,000 mg/kg bw/day. No treatment-related toxicologically significant changes in clinical chemistry parameters occurred at the dose of 250 mg/kg bw/day.

Haematology

At the end of the dosing period, a statistically significant decrease was seen in haemoglobin concentration and reticulocyte count at 500 mg/kg bw/day (10%, $p < 0.01$ and 24%, $p < 0.05$ respectively) and 1,000 mg/kg bw/day (8%, $p < 0.01$ and 31%, $p < 0.01$ respectively). Additionally, a statistically significant increase was seen in monocytes at 1,000 mg/kg bw/day (62% $p < 0.01$). All other statistically significant findings were of low magnitude and seen in the absence of a dose response and considered likely incidental and not treatment-related. No statistically significant changes were seen at the end of the recovery period.

No treatment-related toxicologically significant changes in haematology parameters were noted to occur at the dose level of 500 mg/kg bw/day and below.

Urinalysis

No treatment-related effect was seen on urinalysis parameters in the study, up to the dose level of 1,000 mg/kg bw/day, including the recovery groups.

Effects in Organs

Non-Reproductive Organs

Organ weight
At the end of the dosing period, a statistically significant decrease was seen in absolute and relative (to body weight) adrenal (35%, $p < 0.01$ and 31%, $p < 0.01$ respectively) and thymus weight (61%, $p < 0.01$ and 58%, $p < 0.01$ respectively) at the dose level of 1,000 mg/kg bw/day. Relative (to body weight) organ weight only, a statistically significant increase was seen in liver weight at 500 and 1,000 mg/kg bw/day (18% and 23% respectively) along with a statistically significant increase in spleen weight (31%, $p < 0.05$) at 1,000 mg/kg bw/day. At the end of the recovery period, a statistically significant decrease in absolute and relative (to body weight) thymus weight (67% and 68% respectively) was seen at 1,000 mg/kg bw/day.

The liver weight changes were associated with microscopic findings at the dose levels of 500 and 1,000 mg/kg bw/day. The thymus weight changes were only associated with macroscopic and microscopic findings at 1,000 mg/kg bw/day. These organ weight changes are considered likely to be treatment-related and toxicologically significant. The adrenal weight findings were seen in the absence of macroscopic and microscopic changes and therefore are not considered toxicologically significant. The spleen weight findings, while seen in the presence of macroscopic and microscopic changes, are likely not related to the test material as suggested by the separately submitted 42-day study. No treatment-related toxicologically significant changes in organ weight were considered to occur at the dose of 250 mg/kg bw/day.

In the males of the recovery group administered 1,000 mg/kg bw/day, it is noted that the statistically increase in absolute and relative thymus weight was not associated with microscopic changes. Consequently, this finding is not considered toxicologically significant and is likely incidental. Therefore, it is considered that no adverse treatment-related effects on non-reproductive organs were seen in males administered 1,000 mg/kg bw/day at the end of the recovery period.

Macroscopic Findings
At the end of the dosing period, macroscopic changes were seen in the liver (multiple punctate or punctate in 2/4 males), thymus (reduced size in 1/4 males) and spleen (areas of macroscopic change in 3/4 males) at the dose level of 1,000 mg/kg bw/day, with microscopic changes also seen in these organs. The macroscopic changes in the liver and thymus in the presence of associated microscopic findings are considered potentially treatment-related and toxicologically significant. The macroscopic changes to the spleen are considered likely incidental and not toxicologically significant as suggested by the separately submitted 42-day study.

Other changes were seen in single animals and/or in the absence of a dose response and were not associated with microscopic changes. These changes were considered likely incidental and not treatment-related. The macroscopic findings seen in the sub-maxillary lymph nodes at the end of the recovery period at 1,000 mg/kg bw/day are also considered likely incidental and not treatment-related.

No treatment-related toxicologically significant macroscopic changes in the liver and thymus occurred at the next dose level of 500 mg/kg bw/day.

Microscopic Findings
At the end of the dosing period, microscopic changes were seen at 500 and 1,000 mg/kg bw/day dose levels in the liver (minimal – moderate periacinar hepatocyte hypertrophy in all males in each dose group with mean group score for severity of 1.60/4 and 2.00/4 respectively), thymus (minimal to moderate atrophy in 2/5 and 4/4 males respectively with corresponding mean group scores for severity of 0.60/4 and 2.75/4) and spleen (minimal – moderate focal peritonitis in 2/5 and 2/4 males with corresponding mean group scores for severity of 0.60/4 and 1.25/4). The microscopic changes in the liver and thymus are considered potentially treatment-related and toxicologically significant. The microscopic changes in the spleen are considered likely incidental.

While microscopic changes were also seen in the liver at 250 mg/kg bw/d (minimal periacinar hepatocyte hypertrophy in 2/5 males, with a mean group score for severity of 0.40/4), noting the low incidence and severity and the absence of changes in organ weight or clinical chemistry parameters reflective of liver toxicity, these findings are considered potentially treatment-related but not toxicologically significant.

At the end of the recovery period, at the dose level of 1,000 mg/kg bw/day, the only treatment-related finding

reported was minimal periacinar hepatocyte hypertrophy in the liver (in 1/5 animals equating to a mean group score for severity of 0.20/4). It is not considered to be toxicologically significant. Therefore, no adverse treatment effects were seen in males administered at 1,000 mg/kg bw/day at the end of the recovery period.

Reproductive Organs

Organ weight

At the end of the dosing period, a decrease was seen in absolute and relative testis and epididymis weight in males administered at 1,000 mg/kg bw/day. Although not statistically significant, the magnitude of these changes which were associated with microscopic changes in the testes and are considered toxicologically significant. Statistically significant decreases in absolute and relative testis and epididymis weight were seen in males administered 1,000 mg/kg bw/day at the end of the recovery period (see table below).

Organ	*Absolute weight reduction (%)*		*Reduction relative to body weight (%)*		*Reduction relative to brain weight (%)*	
	Day 29	*Day 43*	*Day 29*	*Day 43*	*Day 29*	*Day 43*
Testis	27	23**	19	25**	24	20**
Epididymis	18	21**	11	23**	16	19*

* $p \leq 0.05$, ** $p \leq 0.01$, when compared with the vehicle control group

No treatment-related toxicologically significant changes in male reproductive organ weights occurred at the dose of 500 mg/kg bw/day.

Macroscopic Findings

No treatment-related effect was seen on male sex organs in the study including recovery groups, up to the dose level of 1,000 mg/kg bw/day.

Sperm Analysis

At the end of the dosing period, the following effects seen in males administered at 1,000 mg/kg bw/day were considered treatment-related and toxicologically significant:

- Motility and count: a decrease in motility of spermatozoa (47 vs 80 in controls) and an increase in immobile spermatozoa (37 vs 24 in controls) which were associated with a decrease in percentage mobile spermatozoa (56.8 vs 76.3 in controls).

- Morphology: a statistically significant ($p < 0.05$) decrease in the percentage of normal spermatozoa (65.5 vs 89.0 in controls) and a statistically significant ($p < 0.05$) increase in percentage spermatozoa with head anomaly (17.5 vs 2.6 in controls) which were associated with an increase (not statistically significant) in percentage of isolated head in the sample (11.4 vs 3.4 in controls).

- Numeration in epididymis: a statistically significant ($p < 0.01$) lower epididymis tail weight (0.150 g vs 0.213 g in controls) and a decrease (not statistically significant) in the number of spermatozoa in the tail of the left epididymis (2.34 vs 3.78 in controls).

No sperm analysis at the end of the recovery period was reported.

Microscopic Findings

At the end of the dosing period, microscopic changes were seen at 1,000 mg/kg bw/day in the testes (tubular dilatation in 3/4 males, tubular degeneration in 2/4 males and intratubular spermatid debris in all males, with corresponding mean group scores for severity of 0.75/4 , 1.25/4 and 1.25/4 respectively) and epididymis (tubular dilatation in 1/4 males, free luminal degenerative spermatids in 2/4 males and hypospermia in 3/4 males, with corresponding mean group scores for severity of 0.25/4, 0.50/4 and 1.50/4 respectively). At the end of the recovery period, treatment-related findings were still seen in the animals treated at 1,000 mg/kg bw/day in the testes (minimal tubular dilatation in 1/5 males, minimal tubular degeneration in 1/5 males and minimal intratubular spermatid debris in 2/5 males, equating to mean group scores for severity of 0.20/4, 0.20/4 and 0.40/4 respectively) and epididymis (minimal to moderate free luminal degenerative spermatids in 4/5 males, equating to a mean group score for severity of 1.6/4).

No treatment-related toxicologically significant microscopic changes in male reproductive organs occurred at the dose of 500 mg/kg bw/day.

Remarks – Results

During the treatment period, 2 males at the dose level of 1,000 mg/kg bw/day died. The deaths could not be attributed to the systemic toxicity of the test material, as clinical signs seen in these animals were limited to a decrease in spontaneous motor activity on Day 9 only with no effect was seen on body weight, and the study authors stated that the reasons for these deaths could not be established. Both animals had no changes in the male reproductive organs.

The observed clinical signs of toxicity at 1,000 mg/kg bw/day were sporadically seen over the dosing period with no dose response for the incidence of abdominal tone and chromodacryorrhoea. At the dose level of 1,000 mg/kg bw/day, decreases were seen in haemoglobin concentration and reticulocyte count along with an increase in monocytes. It is noted that no effect was seen on the principal haematopoietic organ, the bone marrow. The decrease in alkaline phosphatase at 1,000 mg/kg bw/day was a marker of liver toxicity. While the toxicological significance of this decrease is unknown, it may possibly be reflective of an adverse effect on metabolic function.

At the dose level of 1,000 mg/kg bw/day, the only treatment-related and toxicological significant changes in organ weight were seen in liver and thymus. These findings were associated with macroscopic and microscopic changes in the liver and with microscopic changes in thymus. While the liver weight findings are considered adverse and treatment-related, the observed microscopic changes (hepatocyte hypertrophy) can be indicative of an adaptive effect. Consequently, noting the incidence and severity of the effects in the liver and thymus, which are only associated with a change in a single clinical chemistry parameter, it is considered unlikely that the findings in the liver and thymus would have a secondary specific effect on the changes seen in the male reproductive organs. Overall, the observed deaths, sporadic clinical signs of toxicity, limited changes in haematology parameters, change in a single clinical chemistry parameter, organ weight changes in the liver and thymus, macroscopic changes in the thymus, and microscopic changes in the liver and thymus, are not considered to provide evidence of significant generalized toxicity for which the observed findings in male reproductive organs can be considered to be a secondary non-specific consequence.

No effects on male reproductive organs were seen at the dose level of 500 mg/kg bw/day. At this dose level, the observed clinical signs of toxicity were sporadic with some showing no dose response. Increased liver weight with a decrease in alkaline phosphatase was seen at this dose level, along with hepatocyte hypertrophy in the liver and atrophy in the thymus that were considered treatment-related. However, as indicated above, these findings are not considered to provide evidence of significant generalized toxicity.

At the dose level of 1,000 mg/kg bw/day, the decrease of testis and epididymis weight was associated with multiple changes in the sexual organs in the males, including counts and morphology of spermatozoa, and microscopic findings. The study authors considered that the testicular or epididymal findings may demonstrate a general degenerative effect as well as a "blockage effect". Therefore, the observed changes in male reproductive organs at the dose level of 1,000 mg/kg bw/day, including histopathological changes, which were still evident after a 2 week recovery period, raise a concern that the test material is likely to impair reproductive function. In the absence of a one or two generation fertility study, the full safety of the chemical cannot be established.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 250 mg/kg bw/day in this study, based on the effects of the notified chemical on liver and thymus observed at the dose level of 500 mg/kg bw/day.

TEST FACILITY CERB (2014f)

B.9. Repeat dose oral toxicity – 42-day study combined with reproductive/developmental toxicity screening test

REMARKS NICNAS received this toxicological study report as a separate notification, which is considered relevant to the notified chemical. The summary of this study report is provided below for information.

TEST SUBSTANCE Notified chemical (99.98% in purity)

METHOD OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Species/Strain	Rat/Crl:CD (SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 42 days for males and satellite females (without mating); until Day 4 of lactation for females Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Water
Remarks - Method	Each group consisted of 12 males and 12 females. Five satellite females each were added to the high dose (1,000 mg/kg bw/day) group and the control group. Ten animals (5 males and 5 satellite females) from the high dose group were examined for recovery along with ten animals (5 males and 5 satellite females) from the control group.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	19 (7 M/12 F)	0	2/19
Low dose	24 (12 M/12 F)	50	0/24
Mid dose	24 (12 M/12 F)	250	2/24
High dose	19 (7 M/12 F)	1,000	7/19
Control recovery	10 (5 M/5 F)	0	0/10
High dose recovery	10 (5 M/5 F)	1,000	0/10

Mortality and Time to Death

All male animals survived the test until scheduled necropsy.

One female each in the control group and the high dose group, and 2 females in the mid dose group were found non-pregnant after mating. One female in the high dose group was found non-copulated and 1 female in the control group was found non-delivering. Above animals were terminated before the study was completed.

Due to total litter loss, 5 more unscheduled sacrifices were recorded in the females of the high dose group.

Clinical Observations

Decreased motor activity was noted in females of the high dose group.

Body Weight and Food Consumption

No significant difference of body weight was noted in males and females between the vehicle control and test groups throughout the dosing and recovery period, including female gestation and lactation period.

No treatment-related changes of food consumption were noted in the study. High values of food consumption were noted in females of the high dose recovery group on Day 50; however, the changes were not considered to be treatment-related since these changes were noted only at one measurement time point and no significant difference was noted in the body weight on Day 50.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology findings in the high dose group at the end of dosing included low values of platelets, white blood cells, neutrophils, basophils and large unstained cells in females and low mean corpuscular haemoglobin concentration (MCHC) in males. Changes in high dose group males at the end of recovery included a reduction in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and MCHC, but were not considered by the study authors to be treatment-related.

Some changes in clinical chemistry parameters after dosing and after recovery were noted by the study authors but not considered to be treatment-related.

Qualitative urinalysis parameters tested in males only did not differ from the controls.

Effects in Organs

At the end of dosing, significant increase of liver weight was noted in high dose females and males. Compared to the vehicle control group, the absolute and relative liver weight increases for the males in the group were 29.0% ($p < 0.05$) and 23.6% ($p < 0.01$) respectively. For the females, the absolute liver weight increase was

17.9% ($p < 0.05$); however, the relative liver weight increase of 12.5% was not statistically significant. Microscopic findings revealed associated minimal to mild hypertrophy of centrilobular hepatocyte. While no hypertrophy of hepatocyte was noted in the vehicle control, low dose (50 mg/kg bw/day) and mid dose (250 mg/kg bw/day) groups for both males and females, 3 of the 5 males in the high dose group were found to have minimal hepatocyte hypertrophy and 1 male was found to have mild hepatocyte hypertrophy. For females of the high dose group, 3 of the 5 animals were found to have minimal hepatocyte hypertrophy.

Significant decrease of epididymides weight was recorded in the males of the high dose group at the end of dosing. Compared to the vehicle control group, the absolute and relative epididymides weight decreases were 21.5% and 24.2% (both $p < 0.01$) respectively. The decreases in weights of epididymides were associated with microscopic findings including degeneration/necrosis of spermatocyte/spermatid, decrease of sperm and cell debris in the duct of the epididymis. In addition, significant reduction of relative thymus weights in males of the high dose group was also noted ($p < 0.05$).

At the end of the recovery period, relative liver weight of high dose males remained 12.27% higher than the vehicle control group ($p < 0.05$). For females of the high dose group, absolute and relative liver weights were not significantly different from the vehicle control after the recovery. Hepatocyte hypertrophy was not seen in the recovery animals.

The weights of epididymides and testes were statistically significantly reduced for the males of the high dose group after the recovery. Compared to the vehicle control group, the absolute and relative epididymides weight decreases were 31.3% and 29.6% (both $p < 0.01$) respectively. The absolute and relative testes weight decreases were 26.0% ($p < 0.01$) and 23.3% ($p < 0.05$) respectively.

Other organ weight changes in the high dose recovery group, including thyroid weight increase in females and heart weight increase in males, were considered by the study authors not to be treatment-related.

Reproductive/Developmental Effects

In male animals, the significant decreases in weights of epididymides and testes in the high dose group were associated with histopathological changes, both at the end of dosing and end of the recovery period. (Testis weight changes were statistically significant only after the recovery period). Microscopic findings included minimal to mild degeneration/necrosis of spermatocyte/spermatid, decrease of sperm and cell debris in the duct of the epididymis. The study authors noted that the degree and incidence of these changes were greater at the end of the recovery period than at the end of dosing. At the end of dosing, minimal spermatocyte/spermatid degeneration/necrosis was found in 2 of 5 males in the high dose group while mild spermatocyte/spermatid degeneration/necrosis was found in 1 of the 5 animals. After the recovery period, minimal spermatocyte/spermatid degeneration/necrosis was found in 3 of the 5 males in the high dose group while mild spermatocyte/spermatid degeneration/necrosis was found in 1 of the 5 animals. Similar situations were seen in the examinations of the epididymis of the high dose group with decrease of sperm and appearance of cell debris in the duct. The study authors considered that the changes in the testes were suggestive of effects on the spermatocytes/spermatids and the changes in the epididymis suggested degeneration of the seminiferous epithelium.

In female animals, prolongation of gestation period and decrease of delivery index were noted in the high dose group, with delivery index of 79.5% comparing to 91.9% in the vehicle control. Decrease of litter number and birth index and increase of number of stillborn were observed in the offspring of high dose females, with birth index of 70.5% comparing to 90.0% and stillborn rate of 11.2% comparing to 1.8% in the vehicle control. Total litter loss in 5 females of the high dose group was recorded, mostly due to cannibalism and loss of suckling. At postnatal Day 4, the offspring in the high dose had a lower viability index and body weight. The total viability index in this group was 48.2%, significantly lower than 98.7% in the vehicle control ($p < 0.01$). The study authors noted that there were no changes indicating implantation failure, however there was a decrease in delivery index and an effect on the maintenance of pregnancy.

The study authors suggested that although the causes of the changes in offspring remain unclear, it is possible that the test substance may have affected the offspring via milk.

No effect of the test substance was noted on mating or conception, despite the abnormalities seen in the testis and epididymis in males of the high dose group. The study authors suggested that this may be related to the maturation time of the sperm, and it is likely that the test substance may affect reproductive function if administered for a longer period before mating to cover a full spermatogenesis cycle.

Remarks – Results

The study authors considered the main treatment-related observations for repeated dose toxicity were changes in organ weights and histological changes, in liver, testes and epididymis. For reproductive toxicity, they considered the effects on dams and offspring to be the main treatment-related observations.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 250 mg/kg bw/day in this study for both the repeated dose toxicity and the reproductive toxicity, based on the adverse effects observed at the dose level of 1,000 mg/kg bw/day.

TEST FACILITY Provided in a separate notification

B.10. Genotoxicity – bacteria reverse mutation test

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 471 Bacterial Reverse Mutation Test
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2*uvr*A-
Metabolic Activation System	S9 mix (microsomal enzyme fraction of Aroclor 1254 induced rat liver homogenate)
Concentration Range in Main Test	a) With metabolic activation: 50 – 5,000 µg/plate b) Without metabolic activation: 50 – 5,000 µg/plate
Vehicle	Water
Remarks - Method	The purity of the test substance was not specified. Preliminary toxicity study was conducted on TA100 and WP2*uvr*A- without metabolic activation with test substance in the range of 50 to 5,000 µg/plate.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Preliminary Test	> 5,000	-	> 5,000	Negative
Main Test I	-	> 5,000	> 5,000	Negative
Main Test II	-	> 5,000	> 5,000	Negative
Present				
Main Test I	-	> 5,000	> 5,000	Negative
Main Test II	-	> 5,000	> 5,000	Negative

Remarks - Results Cytotoxicity and precipitation of the test substance were not observed with or without metabolic activation during the study.

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

TEST FACILITY Safepharm (1997b)

B.11. Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test (1997).

Species/Strain	Mouse/OF1
Route of Administration	Oral – gavage
Vehicle	Water
Remarks - Method	No significant deviations of the protocol were noted.

Dose levels in the main genotoxicity assay were selected based on a preliminary toxicity assay (2 males and 2 females tested at 2,000 mg/kg bw/day) followed by a confirmatory toxicity assay (5 males and 5 females tested at 2,000 mg/kg bw/day).

Except for the positive control which was only given 1 dose to the animals, doses of the test substance were administered once daily for 2 days. Sampling was carried out 24 h after the last dose.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day × 2 days)*	*Sacrifice Time after last dose (hours)*
I (vehicle control)	5 M/5 F	0	24 hours
II (low dose)	5 M/5 F	500	24 hours
III (mid dose)	5 M/5 F	1,000	24 hours
IV (high dose)	5 M/5 F	2,000	24 hours
V (positive control, CP)	5 M/5 F	50 (1 dose only)	24 hours

CP = cyclophosphamide, 1 dose only at 50 mg/kg bw, administered intraperitoneally

RESULTS

Doses Producing Toxicity	Statistically significant decreases in the ratio of PCE/NCE were observed in all treatment groups for females, and in mid dose and high dose groups for both sexes combined, suggesting the notified chemical reaching bone marrow of the test animals.
Genotoxic Effects	No statistically significant increase in the number of micronuclei was noted in all test groups.
Remarks - Results	

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* mammalian erythrocyte micronucleus test.

TEST FACILITY Institut Pasteur de Lille (2014)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	"*Test on degradation of chemicals by microbes and others*" specified in the "*Test methods for newly registered chemical substances and others*" (Pharmaceutical and Food Safety Bureau Notice No. 1121002 issued on Nov. 21 2003, Manufacturing Industries Bureau Notice No. 2 and Environmental Policy Bureau Notice No. 031121002 issued on 2003-11-13, Japan)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	TOC-VCPH carbon analyser
Remarks - Method	The test was carried out following the test guidelines and good laboratory practice (GLP). The test concentration was 100 mg/L of the notified chemical.

RESULTS

Test substance		Aniline	
Day	% Degradation	Day	% Degradation
7	-1	7	55
14	-1	14	71
21	-1	21	72
28	0	28	72

Remarks - Results	All test validity criteria were met. The notified chemical underwent no biodegradation after 28 days under test conditions.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Kurume Laboratory (2008a)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test.
Species	*Cyprinus carpio*
Exposure Period	Exposure: 28 days Depuration: 48 hours
Auxiliary Solvent	None reported
Concentration Range	Nominal: 0.01 mg/L and 0.001 mg/L
Analytical Monitoring	Gas chromatography mass spectrometric analysis
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Based on the results of the range finding test, the test was conducted at nominal concentrations of 0.01 and 0.001 mg/L notified chemical/L. No significant deviations to the test protocol were reported. The test sample was dissolved in deionised water to a test substance concentration of 1,000 mg/L as a stock solution. The required nominal concentrations of the test substance were prepared from this stock solution.

RESULTS

Bioconcentration Factor	The accumulation rate during exposure period was 0.77 or less in the first concentration group and 7.7 or less in the second concentration group.
Lipid content in test fish	The average lipid content in the test fish was as follows: Before test: 4.23% After test: 5.12%
Remarks - Results	The validity criteria for the test were met. It was not possible to calculate the accumulation rate in the steady state, because the test substance was not detected in all of the last three continuous analyses. However, because the accumulation rates were all less than 100, it was assumed that the steady state was established after 28 days.
CONCLUSION	Under the conditions of this test, the notified chemical is not considered to be bioaccumulative.
TEST FACILITY	Kurume Laboratory (2008b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	"*Acute toxicity test with fishes, Testing methods for industrial wastewater*" (JIS K0102-2008-71, Japan)
Species	*Oryzias latipes*
Exposure Period	96 hour
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	
Remarks – Method	Conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. Semi-batchwise (water exchange in every 24 hours) method was followed and the calculations were done with Doudoroff method. The test sample was dissolved in ion-exchange water to a test substance concentration of 1,000 mg/L, to give a concentrate solution. The test concentration of 200 mg/L was prepared with a control group.

RESULTS

Nominal Concentration (mg/L)	*Number of Fish*	*Mortality*			
		24 h	*48 h*	*72 h*	*96 h*
Control	10	0	0	0	0
200	10	0	0	0	0

LC50	> 200 mg/L at 96 hours
NOEC	Not Applicable
Remarks – Results	The validity criteria for the test were met.
CONCLUSION	The notified chemical is not harmful to fish.
TEST FACILITY	Kurume (2008b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours

Auxiliary Solvent	Not reported
Water Hardness	199.88 mg $CaCO_3$/L
Analytical Monitoring	GC Analysis
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.

RESULTS

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

EC50	> 100 mg/L at 48 hours
NOEC	Not Applicable
Remarks - Results	All validity criteria for the test were satisfied. The treatments concentrations were measured at the beginning and end of the test. The toxicity test was conducted as a limit test. The 48-hour LC50 was determined, based on the time-weighted mean of measured concentrations, by visual observations.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates

TEST FACILITY Chemex Environmental Int. Ltd., Cambridge, UK (2014a).

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: 100 mg/L
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	GC-MS
Remarks - Method	The test concentration of 100 mg/L was prepared by adding 0.1 g of sample into 1 litre of freshwater media.

RESULTS

Biomass		*Growth*	
EyC50 (mg/L at 72 h)	*NOEC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOEC (mg/L)*
> 100	100	> 100	100

Remarks - Results	The validity criteria for the test were met. The statistic t Tests indicated no significant difference between the control and 100 mg/L therefore the EC50 values (by yield) of notified chemical to *Pseudokirchneriella subcapitata* were estimated as > 100 mg/L at 48 hours and > 100 mg/L at 72 hours. The EC50 value (by growth rate) was estimated as > 100 mg/L from 0 to 48 hours and > 100 mg/L from 0 to 72 hours. The NOECr (0-72h) (growth rate) was estimated as 100 mg/L. The NOECy (0-72h) (yield) was estimated as 100 mg/L.

CONCLUSION The notified chemical is not harmful to algae

TEST FACILITY Chemex Environmental Int. Ltd., Cambridge, UK (2014b).

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