File No: STD/1516

August 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

NT-45

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:

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

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1516	Canon Australia Pty Ltd Hewlett Packard Australia Pty Ltd	NT-45	No	≤ 300 tonnes per annum	Component of toner

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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

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

Environmental risk assessment
The notified chemical is not expected to pose an unreasonable risk to the aquatic environment based on the low ecotoxicity and assessed use pattern.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure when handling the notified chemical during end use:
  - Avoid contact with eyes

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

- 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

- Disposal should be in accordance with Australian, state, territory and local government laws. Landfilling is a disposal option frequently used for industrial chemicals.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent 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(2) of the Act; if
- the function or use of the chemical has changed from component of toner, 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.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

This notification has been conducted under the cooperative arrangement with USA. The health and environmental hazard assessment components of the USA report were provided to NICNAS and, where appropriate, used in this assessment report. The other elements of the risk assessment and recommendations on safe use of the notified chemical were carried out by NICNAS.

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Canon Australia Pty Ltd (ABN: 66 005 002 951)
Building A, The Park Estate, 5 Talavera Road
MACQUARIE PARK NSW 2113

Hewlett Packard Australia Pty Ltd (ABN: 74 004 394 763)
353 Burwood Hwy
FOREST HILL VIC 3131

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: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, use details and import volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: flash point and acute inhalation toxicity

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA (2013)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
NT-45

MOLECULAR WEIGHT
500-1000 Da

ANALYTICAL DATA
Reference IR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	67.6-74.0 °C	Measured
Boiling Point	> 400 °C at 100.8 kPa	Measured
Density	1000 kg/m^3 at 22 °C	Measured
Vapour Pressure	< 1.3 × 10^{-3} kPa at 25 °C	Measured

Water Solubility	< 3.22 x 10^{-4} g/L 20 °C	Measured, OECD 105, column elution method
Hydrolysis as a Function of pH	Not determined	The notified chemical contains functional groups that are expected to hydrolyse slowly in the environmental pH range (4-9).
Partition Coefficient (n-octanol/water)	log Pow > 6.5	Measured, OECD 117, HPLC method
Adsorption/Desorption	log K_{oc} > 5.63	Measured, OECD 121, HPLC method
Dissociation Constant	Not determined	No dissociable functionality
Particle Size	Inhalable fraction (< 100 μm): 0.134%	Measured
Flash Point	Not determined	Solid
Flammability	Not highly flammable	Measured
Autoignition Temperature	No self-ignition below the melting temperature	Measured
Explosive Properties	Predicted negative	Estimated based on chemical structure
Oxidising Properties	Predicted negative	Estimated based on chemical structure

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured or reformulated in Australia. The notified chemical will be imported as a component of a finished product, in closed toner cartridges at < 20% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 100	< 300	< 300	< 300	< 300

PORT OF ENTRY
To be decided

IDENTITY OF RECIPIENTS
Canon Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The finished product (containing the notified chemical at < 20%) will be contained in sealed plastic cartridges (0.2-4 L). These cartridges will be packed in boxes, stacked on pallets and distributed within Australia by road or rail.

USE
The notified chemical will be used as component of toner for electro-photocopying machine or electro-photographic printer at < 20% concentration.

OPERATION DESCRIPTION
There will be no manufacture, reformulation or repackaging of the notified chemical in Australia.

End-users (including service technicians, office workers and the general public) will remove the cartridge from the packaging and place the cartridge into the photocopier or printer. The cartridge will be disposed of when empty.

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)*
Import/waterside	< 8	10-50
Storage and transport	< 8	10-50
Office workers	10 seconds	2
Service technicians	1	200

EXPOSURE DETAILS

Waterside, storage and transport workers may come into contact with the notified chemical, as a component of toner (< 20%), only in the unlikely event of an accident.

Service technicians may be exposed to the toner containing < 20% notified chemical during repair and cleaning of photocopiers and printers. Due to the low volatility of the notified chemical and the large particle size, inhalation exposure is not likely and dermal exposure is expected to be the main potential route of exposure. Exposure to the notified chemical may occur while changing cartridges if the toner is inadvertently handled.

Office workers may be exposed to the toner when replacing the cartridge in machines but the extent of exposure is predicted to be very low. Instructions on how to replace the cartridges safely are included with the cartridge. Occasional dermal exposure during use of the printer may occur if the printed pages were handled inadvertently before the toner had dried, or if toner-stained parts of the printer were touched. Once the toner dries, the notified chemical will be bonded to the paper as part of the print, and therefore not expected to be bioavailable.

6.1.2. Public Exposure

Dermal exposure of the public to toner containing the notified chemical (at < 20%) is expected to be similar, though less frequent, than that described above for office workers.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-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 = 1,000 mg/kg bw/day (male) NOAEL = 300-1,000 mg/kg bw/day (female)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted. Absorption across biological membranes is expected to be limited by the relatively high molecular weight (> 500 Da) and high log Kow.

Acute toxicity
In studies conducted in rats the notified chemical was found to have low acute oral and dermal toxicity.

As the dust generation properties of the notified chemical were shown to be low, it was considered impossible to generate a suitable test atmosphere from the notified chemical in its original form or as a liquid formulation, for use in an inhalation study.

Irritation
Based on studies conducted in rabbits the notified chemical was non-irritating to the skin and slightly irritating to eyes.

Sensitisation
The notified chemical at concentrations up to 10% (the highest attainable dose) in a mouse Local Lymph Node Assay showed no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation.

Repeated dose toxicity
A 28 day repeat dose study by oral gavage was conducted in rats with the notified chemical at dose levels of 30, 300 and 1000 mg/kg bw/day. The No Observed adverse Effect Level (NOAEL) was established as 1000 mg /kg bw/day for males, based on no changes observed at the highest dose tested. As the liver weight changes observed in the female at 1,000 mg/kg were of borderline significance, the NOAEL based on this was likely to be between 300 and 1,000 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation study and was not clastogenic in an *in vitro* mammalian chromosome aberration test.

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
There will be no reformulation or repackaging of the notified chemical in Australia. As such, only printer service technicians and office workers will be potentially exposed to the notified chemical at < 20% concentration in finished toner products on an infrequent basis when changing cartridges, removing waste boxes or during printer maintenance. Gloves may be worn by service technicians if performing regular printer maintenance operations. Based on the toxicity studies provided, exposure to the notified chemical under the proposed use scenario is not expected to result in adverse health effects.

Therefore, based on the information available, the risk to workers associated with use of the notified chemical at < 20% concentration in finished toner products is not considered to be unreasonable.

6.3.2. Public Health
Exposure of members of the public to the notified chemical during printing processes is not expected as the finished toner product containing the notified chemical is contained in a cartridge. Following printing onto paper (or other substrates), the notified chemical is not expected to be bioavailable. Therefore, under the proposed usage conditions, the risk to the public is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The toner powder containing the notified chemical will be imported into Australia in closed cartridges. Environmental release of the notified chemical is unlikely to occur during importation, storage and transportation as cartridges are designed to minimise release.

RELEASE OF CHEMICAL FROM USE
When used as a toner ingredient, the majority of the toner containing the notified chemical is expected to be applied to different paper or other media and fixed on the surface of the substrates. During printing, it is estimated < 2% of the total import volume of the notified chemical may be released to the environment as a result of toner waste. Collected waste toner residues are expected to be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL
Following its use, most of the notified chemical is anticipated to share the fate of printed articles and be disposed of to landfill or subjected to paper recycling processes. Approximately half of the amount of used paper is expected to be recycled. Limited amounts of the notified chemical contained in empty cartridges are expected to be disposed of to landfill.

7.1.2. Environmental Fate

A ready biodegradation study was provided (OECD 301 B) for the notified chemical. The test showed that the notified chemical degraded 54% after 28 days indicating that the chemical is not readily biodegradable.

The notified chemical will be imported into Australia as an ingredient of toner in sealed cartridges, which will be distributed to customers for direct use for paper printing. It is assumed that 50% of the printed paper will end up in landfill and the rest will undergo paper recycling processes. During recycling processes, waste paper is repulped using a variety of chemical agents, which, amongst other things, enhance detachment of toners from the fibres. Very little of the notified chemical is expected to partition to the supernatant water, due to its low solubility in water. Based on its low solubility in water and high molecular weight, the notified chemical is not expected to cross biological membranes and is therefore not likely to bioaccumulate. Most of the notified chemical will reach landfill as a result of disposal of used paper or sludge waste from paper recycling. In landfill the notified chemical will be slowly degraded, eventually forming water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical will be used as ingredient of toner for electro-photocopying machines or electro-photographic printers. It is conservatively assumed that 100% of the total import volume of the notified chemical will be used as toner for printing on papers. Of this, it is assumed that 50% of the total import volume of notified chemical may be released to sewer from recycling processes. A predicted environmental concentration (PEC) for the worst case scenario has been calculated on the assumptions the recycling processes occurs only on working days, which is 260 days per annum. It is conservatively assumed that none of the notified chemical will be removed during waste water processing at sewage treatment plants (STPs).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	300,000	kg/year
Proportion expected to be released to sewer	50%	
Annual quantity of chemical released to sewer	150,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	576.92	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:	127.57	µg/L
PEC - Ocean:	12.76	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 127 µg/L may potentially result in a soil concentration of approximately 0.85 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 4.2 mg/kg and 8.5 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EC50 > 100 mg/L (nominal)	Not harmful to fish
Fish Toxicity	14 d study NOEC = 100 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L (nominal)	Not harmful to aquatic invertebrates
Algal Toxicity	72 h E_rC50 > 100 mg/L (nominal)	Not harmful to algae
Inhibition of Bacterial Respiration	NOEC (3 h) = 1000 mg/L	Not expected to inhibit bacterial respiration

Based on the above endpoints the notified chemical is not considered to be harmful to aquatic organisms up to the limit of water solubility. The notified chemical is not considered to be harmful to aquatic organisms under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). Therefore, the notified chemical is not formally classified under the GHS for acute hazard. Based on its measured chronic toxicity and biodegradability, the notified chemical is not formally classified under the GHS for chronic hazard.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) was not calculated since the notified chemical is not considered to be harmful up to the limit of solubility.

7.3. Environmental Risk Assessment

The risk quotient (RQ = PEC/PNEC) was not calculated since the PNEC was not available.
The notified chemical is not expected to pose an unreasonable risk to the aquatic environment based on the low ecotoxicity and assessed use pattern.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 67.6-74.0 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Different scanning calorimetry was used.
Test Facility Harlan Laboratories Ltd (2013a)

Boiling Point > 400 °C at 100.8 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Different scanning calorimetry was used.
Test Facility Harlan Laboratories Ltd (2013a)

Density 1000 kg/m^3 at 22.0 ± 0.5 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks A gas comparison pycnometer was used.
Test Facility Harlan Laboratories Ltd (2013a)

Vapour Pressure < 1.3×10^{-3} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks A vapour pressure balance was used.
Test Facility Harlan Laboratories Ltd (2012a)

Particle Size The test substance was considered to be essentially non-inhalable by study authors.

Method European Commission Guidance Document EUR 20268 'Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substance' of 2002.

Range (μm)	*Mass (%)*
< 100	0.134

Remarks The proportion of test substance passing through a 100 μm sieve was determined using an Inclyno sieve shaker for approximately 30 minutes.
Test Facility Harlan Laboratories Ltd (2013a)

Flammability Not highly flammable as it failed to ignite in the preliminary screening test.

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks It was determined by measuring the burning rate of the test substance prepared as a pile of set dimensions.
Test Facility Harlan Laboratories Ltd (2012b)

Autoignition Temperature Not to have a relative self-ignition temperature below its melting temperature.

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Test Facility Harlan Laboratories Ltd (2012b)

Explosive Properties Predicted negative

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.

Remarks	The oxygen balance of the test substance was calculated to be -307, more negative than -200, therefore it was not considered to pose an explosive risk.
Test Facility	Harlan Laboratories Ltd (2012b)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).
Remarks	There are no structural alerts within the chemical structure of the test substance.
Test Facility	Harlan Laboratories Ltd (2012b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 420 Acute Oral Toxicity - Fixed Dose Method.
EC Directive92/69/EEC B.1bis Acute Toxicity (Oral) Fixed Dose Method.

Species/Strain	Rat/Wistar (RccHan:WIST)
Vehicle	Arachis oil BP
Remarks - Method	No protocol deviations.

RESULTS

Sighting Study

Dose mg/kg bw	*Administered*	*Evident Toxicity*	*Mortality*
2000	1 F	none	0

Main Study

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

Discriminating Dose	> 2000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were observed.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	All animals showed expected body weight gains during the study.

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

TEST FACILITY: Harlan Laboratories Ltd (2012c)

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) – Limit Test.

Species/Strain	Rat/Wistar (RccHan:WIST)
Vehicle	Moistened with Arachis oil BP
Type of dressing	Semi-occlusive.
Remarks - Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	There were no signs of dermal irritation.
Signs of Toxicity - Systemic	Red/brown staining around the snout was noted in one male animal, two and four hours after dosing. There were no signs of systemic toxicity in other animals.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks - Results	Animals showed expected body weight gains during the study, except for one male and one female animal which showed body weight loss or no gain in body weight during the first week but expected gain in body weight during the second week.

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

TEST FACILITY Harlan Laboratories Ltd (2012d)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	2 M
Vehicle	Moistened with distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	The test substance was ground before use. The two animals were tested concurrently.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Erythema/Eschar	0	0	1	< 24 hours	0
Oedema	0	0	1	< 1 hour	0

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

Remarks - Results Very slight erythema was observed at both treated skin sites immediately and one hour after patch removal. Very slight oedema was also observed at one treated skin site immediately after patch removal. Both treated skin sites appeared normal at the 24-hour observation.

Both animals showed expected body weight gain during the study.

CONCLUSION The notified chemical is non-irritating to the skin.

TEST FACILITY Harlan Laboratories Ltd (2012e)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	2 M
Observation Period	72 hours
Remarks - Method	Analgesia was not used. The test substance was ground before use.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness	0	0.3	2	< 48 hours	0
Conjunctiva: chemosis	0	0	1	< 24 hours	0
Conjunctiva: discharge	0	0	1	< 24 hours	0
Corneal opacity	0	0	0	-	0

Iridial inflammation	0	0	0	-	0

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

Remarks - Results

No corneal or iridial effects were noted during the study.

Moderate conjunctival irritation was noted in one treated eye with minimal conjunctival irritation noted in other treated eye one hour after treatment. Minimal conjunctival irritation was noted in one treated eye at the 24-hour observation.

One treated eye appeared normal at the 24-hour observation and the other treated eye appeared normal at the 48-hour observation.

One animal showed slight body weight loss and the other animal showed expected body weight gain during the study.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Harlan Laboratories Ltd (2012f)

B.5. 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/Ca

Vehicle: Propylene glycol

Remarks - Method: No protocol deviations. Concentrations for the main study were chosen on the basis of a preliminary test using 10% of the notified chemical, which was the maximum attainable concentration in propylene glycol. The positive control study was not concurrent.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	964.49	-
2.5	1534.61	1.59
5	1396.93	1.45
10	1855.81	1.92
Positive Control (Phenylacetaldehyde)		
2.5%	not reported	6.48

Remarks - Results

No toxicity, irritation or increase >25% in ear thickness was seen in the preliminary test.

In the main test no mortalities and or signs of systemic toxicity were noted in the test or control animals, and body weight changes were comparable

The results show that the test substance elicited stimulation indices < 3.

The positive control gave satisfactory responses confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY Harlan Laboratories Ltd (2012g)

B.6. Repeat dose toxicity

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/Wistar (RccHan:WIST)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	Suspension in Arachis oil BP
Remarks - Method	No protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0
low dose	5 per sex	30	0
mid dose	5 per sex	300	0
high dose	5 per sex	1000	0

Mortality and Time to Death
There were no unscheduled deaths.

Clinical Observations
No clinical signs were observed. No treatment-related effects were detected for behaviour assessment, in functional performance tests or in sensory reactivity scores. No adverse effects on body weight change, dietary intake, food conversion efficiency or water consumption were detected.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were no treatment-related adverse changed detected for haematological parameters or blood chemical parameters.

Hematology was normal except for statistically significant increase ($p < 0.05$) in clotting time in high dose females. This was not considered significant because the observed values were within the historical control range and there were no other indicators to support an effect on blood coagulation.

A statistically significant increase ($p < 0.01$) in plasma potassium levels was detected in 1,000 mg/kg bw/day females in comparison with controls. However, there was no supporting evidence to suggest this finding was associated with test substance toxicity and on this basis the elevation in this isolated parameter was considered by the study authors to be fortuitous and of no toxicological importance.

Macroscopic abnormalities were confined to one female treated with 1,000 mg/kg bw/day which at terminal sacrifice was observed to have gross lesions in the thoracic cavity involving the presence of fluid and white fibrous adhesions adjoining the heart, lungs and thymus, with the hearth also noted to have a pale appearance. This isolated nature of this finding was suggested by study authors that it was associated with a partial mal-dose which led to the congestive changes.

No treatment-related differences in the stage of oestrus cycle were detected in high dose group females comparing with controls.

Effects in Organs
A statistically significant reduction in liver weights, both absolute and relative to terminal body weights were recorded for females treated with 1,000 mg/kg bw/day, compared to controls. As the individual values were within the historical range for this parameter, the finding was considered by study authors to be of no toxicological significance in the absence of any supporting histopathological correlates.

No treatment-related microscopic abnormalities were detected.

CONCLUSION
The No Observed Effect Level (NOEL) was established by study authors as 300 mg/kg bw/day for females in this study, based on a minor change observed in liver weight in females treated at 1,000 mg/kg bw/day and macroscopic abnormalities observed for one female treated at 1,000 mg/kg bw/day. The No Observed Adverse Effect Level (NOAEL) was established as 1,000 mg/kg bw/day for males based on no changes observed at the highest dose tested.

Accepting the NOEL for oral exposure in the 28 day study in female rats is 300 mg/kg bw/day was considered to have some uncertainty as the liver weight changes observed in the female at 1,000 mg/kg were of borderline significance. The NOAEL for liver weight changes in the female was likely to be between 300 and 1,000 mg/kg bw/day.

TEST FACILITY Harlan Laboratories Ltd (2013b)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 0, 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Tetrahydrofuran (THF)
Remarks - Method	No protocol deviations. In order to maintain the solubility in THF, the solution had to be kept at 40^0C during dosing. A preliminary test was carried out using two strains.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5,000			
Test 1		> 5,000	≥ 500	negative
Test 2		> 5,000	≥ 500	negative
Present	> 5,000			
Test 1		> 5,000	≥ 500	negative
Test 2		> 5,000	≥ 500	negative

Remarks - Results
No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation.

The positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

The test substance caused no visible reduction in the growth of the bacterial background lawn at any dose level and was therefore tested up to the maximum recommended dose level of 5000 µg/plate. A test substance precipitate (particulate in appearance) was noted at and above 500 µg/plate, this observation did not prevent the scoring of revertant colonies.

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

of the test.

TEST FACILITY Harlan Laboratories Ltd (2012h)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

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

Species/Strain Human
Cell Type/Cell Line Lymphocytes
Metabolic Activation System S9 fraction from phenobarbital/β-naphthoflavone induced rat liver, used at 2% in Test 1 and 1% in Test 2.
Vehicle Tetrahydrofuran (THF)
Remarks - Method No protocol deviations. Solubility and subsequent precipitation limited the concentration that could be tested.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 2.5*, 5, 10, 20*, 40*, 80*	4	24
Test 2	0*, 2.5, 5, 10*, 20, 40*, 80*	24	24
Present			
Test 1	0*, 2.5, 5*, 10, 20*, 40*, 80*	4	24
Test 2	0*, 2.5, 5, 10*, 20, 40*, 80*	4	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 19.53			
Test 1		≥ 80	≥ 5	negative
Test 2		> 80	≥ 20	negative
Present	≥ 78.13			
Test 1		≥ 80	≥ 10	negative
Test 2		> 80	≥ 20	negative

Remarks - Results

The test substance did not induce any statistically significant increases in the frequency of cells with aberrations or in the numbers of polyploid cells at any dose level either in the absence or presence of metabolic activation.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

In test 1, the precipitate affected some of the metaphases in both exposure groups (in the absence and presence of S9) at 80 μg/mL but it was considered acceptable to score this dose level.

The mitotic index data confirmed the qualitative observations in that some toxicity was observed at 80 μg/mL, and 21% and 27% mitotic inhibition was achieved in the absence and presence of S9 respectively. The toxicity in both exposure groups was marginally less than what was seen in the preliminary toxicity test.

In test 2, there was no reduction in mitotic index at the dose levels tested in either exposure group. The minor differences in toxicity seen in the

	different experiments may be due to the effects of precipitate on the slide making accurate mitotic index analysis difficult. It was also noted that the toxicity in both exposure groups was less than what was observed in the preliminary toxicity test. However, the aggregation of precipitate observed in the preliminary toxicity test at and above 78.13 µg/mL indicated maximum exposure to the cells was being achieved at this concentration and, therefore, it was considered that the test substance had been adequately tested over both experiments.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Harlan Laboratories Ltd (2013c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

Ecotoxicological Investigations

96-hour Acute Fish Toxicity Test

Rainbow trout (*Oncorhynchus mykiss*) were exposed to the test substance under semi-static conditions. Following a range-finding test, single replicates of seven *O. mykiss* were exposed to a dilution water control (dechlorinated, softened tap water) or the test substance at a nominal loading rate of 100 mg/L. Total organic carbon (TOC) analysis of the test preparations at 0 hours (fresh media) and 24 and 96 hours (old media) showed measured test concentrations of less than the limit of quantitation (LOQ) (1.0 mg C/L). To prepare the test solutions, 2100 mg test substance was added to the surface of 21 litres of dechlorinated tap water to give the 100 mg/L loading rate. After the addition of the test substance, the dechlorinated tap water was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a dimple at the water surface. At the start and end of each mixing period, and after the 1-hour settlement period, the 100 mg/L loading rate was observed to be a clear colourless water column with white flakes of the test substance floating on the surface. Over the course of the study, temperature = 14 – 15 °C, pH = 7.7 – 8.3, DO = 9.4 – 10.0 mg O_2/L, dilution water hardness = 140 mg/L as $CaC0_3$. A loading rate of 0.52 g fish/L was calculated. There were no mortalities or sub-lethal effects in the 7 fish exposed to the nominal loading rate of 100 mg/L.

96-hour LL50 > 100 mg/L
NOEC ≥ 100 mg/L

48-hour Acute Daphnia Toxicity Test

Daphnia magna were exposed to the test substance (100% purity) under static conditions. Following a range-finding study, four replicates of five *D. magna* were exposed to a dilution water control (deionised reverse osmosis water) or the test substance at a nominal loading rate of 100 mg/L. Test substance was analysed in solution at 0 (fresh media) and 48 hours (old media) by total organic carbon (TOC) analysis (LOQ = 1.0 mg C/L). Given the background level of carbon in the control vessels and also the low level of carbon in the test vessels, it was considered that all the results were around the LOQ of the analytical method. To prepare the test solutions, an amount of the test substance (200 mg) was added to the surface of 2 L of reconstituted water to give the 100 mg/L loading rate. At the start of the mixing period, the 100 mg/L loading rate was observed to be a clear colourless water column with flakes of the test item on the surface. After 23 hours stirring and a 1-hour standing period, the 100 mg/L loading rate was observed to remain a clear colourless water column with flakes of the substance on the surface. Microscopic inspection of the preparation showed no micro-dispersions or undissolved test substance to be present. After siphoning and for the duration of the test, the 100 mg/L loading rate was observed to be a clear, colourless solution. Over the course of the study, temperature = 21°C, pH = 7.7 - 8.0, DO = 8.7 - 8.9 mg O_2/L, and dilution water hardness = 250 mg/L as $CaC0_3$. A loading rate of 25 daphnids/L was calculated. There was no immobilisation observed in the 20 daphnids exposed to the 100 mg/L loading rate for a period of 48 hours.

48-hour EL5O > 100 mg/L
48-hour NOEL = 100 mg/L

72-hour Algae Toxicity Test

Green algae (*Pseudokirchneriella subcapitata*) were exposed to the test substance under static conditions. Three replicates of *P. subcapitata* (5 x 103 cells/mL) were exposed in the test substance at a nominal loading rates of 1.0, 3.2, 10, 32 and 100 mg/L. Additionally, six replicates of. *P subcapitata* (5 x 103 cells/mL) were exposed to the culture medium (control) under the same conditions. Test substance was analysed via total organic carbon (TOC) analysis. The algae were illuminated with a light intensity of approximately 7000 lux with constant shaking at 150 rpm. To prepare the test solutions, amounts of the test substance (10, 32, 20, 64 and 200 mg) were each separately added to the surface of 10, 10, 2, 2 and 2 litres of culture medium to give the 1.0, 3.2, 10, 32 and 100 mg/L loading rates, respectively. The aqueous phase was removed by mid-depth siphoning (the first 75 - 100 mL discarded) to give the 1.0, 3.2, 10, 32 and 100 mg/L loading rates. Microscopic inspection of the preparations showed no micro-dispersions or undissolved test item to be present. An aliquot (500 mL) of each of the loading rates was separately inoculated with algal suspension (3.2 mL) to give the required test concentrations of 1.0, 3.2, 10, 32, and 100 mg/L. The test vessels were plugged with polyurethane foam bungs. Microscopic inspection of the preparations showed that there were no micro-dispersions of the test item present. At the start of the test, all control and test cultures were observed to be clear,

colourless solutions. After 72-hours, all the controls and test cultures were observed to be pale green dispersions. TOC analysis of the test preparations at 0 hours showed measured carbon concentrations to range from less than the LOQ, which was considered to be 1.0 mg C/L to 7.7 mg C/L. A decline in measured carbon concentration was observed at 72 hours in the range of less than the control to 2.6 mg C/L. Over the course of the study, temperature = 24 ± 1°C and pH = 7.6 - 8.4. Mean growth rate % inhibition at nominal loading rates of 1.0, 3.2, 10, 32 and 100 mg/L was 2, -1, -0, 0 and -5%, respectively. Mean yield % inhibition at nominal loading rates of 1.0, 3.2, 10, 32 and 100 mg/L was 9, -5, -2, -4 and -30%, respectively.

72-bour EL50 (yield and growth rate) > 100 mg/L
72-hour NOEL (yield and growth rate) = 100 mg/L

Conclusion

All three studies are considered unacceptable. It is unclear why the TOC method of measuring the material in solution was used (it was not described as to why it was chosen over other methods) nor why there was so little recovery of the test material when measuring using this method. Therefore, the acute and chronic concern concentrations were derived from the modelled predicted values using ECOSAR v 1.11 (US EPA, 2009) for the ester category. The predicted values indicate that the acute and chronic concern concentration has no effects at saturation.

Chronic toxicity to fish

TEST SUBSTANCE	Notified chemical
Method	OECD TG 204 Fish, Prolonged Toxicity Test: 14-Day Study Semi - Static test.
Species	*Gobiocypris rarus* (Chinese Rare Minnow)
Exposure Period	14 day
Auxiliary Solvent	None
Water Hardness	165 mg $CaCO_3$/L
Analytical Monitoring	GPC
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. The test substance (100 mg/L) was mixed with water and stirred for 72 h in dark. This mixer was filtered through a 0.45 µm nitrocellulose membrane to give the saturated solution. This was used as the test solution.

RESULTS

Concentration mg/L Nominal	*Number of Fish*	*Mortality*			
		2 d	*8 d*	*10 d*	*14d*
Control	10	0	0	0	0
100	10	0	0	0	0

LL50	> 100 mg/L
NOEL	= 100 mg/L
Remarks – Results	The validity criteria for the test were met. The result is based on the nominal concentration. In the test, the body weights of the test fish increased in all groups. The specific growth rate of all treatment groups was not significantly different from that of the control. The analytical measurements showed that the measured concentrations of the test substance was lower than the limit of detection (LOD = 0.189 mg/L). No mortality occurred in treatments groups. The LC50 for *Gobiocypris rarus* exposed for 14 d was higher than the saturated solution of the test substance.
CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility.

TEST FACILITY	Bioassay (2014)

Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 100 and 1,000 mg/L
Remarks – Method	The test was conducted according to the guidelines above. No significant deviations from the test guidelines were reported.
Results	
IC50	> 1,000 mg/L
NOEC	1,000 mg/L
Remarks – Results	All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	Harlan (2012i)

BIBLIOGRAPHY

Bioassay(2014) [Notified Chemical]: Prolonged Toxicity Test (14-day Study) (Study Number R1303010647B, February, 2014). Shanghai, P.R China, Bioassay and Safety Assessment Laboratory (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012a) [Notified Chemical]: Determination of Vapour Pressure (Harlan Study Number 41203183, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012b) [Notified Chemical]: Determination of Hazardous Physico-Chemical Properties (Harlan Study Number 41203184, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012c) [Notified Chemical]: Acute Oral Toxicity in the Rat – Fixed Dose Method (Harlan Study Number 41203166, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012d) [Notified Chemical]: Acute Dermal Toxicity (Limit Test) in the Rat (Harlan Study Number 41203175, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012e) [Notified Chemical]: Acute Dermal Irritation in The Rabbit (Harlan Study Number 41203167, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012f) [Notified Chemical]: Acute Eye Irritation in The Rabbit (Harlan Study Number 41203168, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012g) [Notified Chemical]: Local Lymph Node Assay in the Mouse (Harlan Study Number 41203169, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012h) [Notified Chemical]: Reverse Mutation Assay "AMES Test" Using *Salmonella Typhimurium* and *Escherichia Coli* (Harlan Study Number 41203170, October, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2012i) [Notified Chemical]: Toxicity to Activated Sludge in a Respiration Test (Harlan Study Number 41203181, August, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2013a) [Notified Chemical]: Determination of General Physico Chemical Properties (Harlan Study Number 41203182, April, 2013). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2013b) [Notified Chemical]: Twenty-eight Day Repeated Dose Oral (Gavage) Toxicity Study in Rat (Harlan Study Number 41203177, April 2013). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories Ltd (2013c) [Notified Chemical]: Chromosome Aberration Test in Human Lymphocytes *in Vitro* (Harlan Study Number 41203179, January 2013). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

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