File No: LTD/1819

May 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2-Anthracenesulfonic acid, 1-amino-4-[[3-[[4-chloro-6-[[3-[[[2-(ethenylsulfonyl)ethyl]amino]carbonyl]phenyl]amino]-1,3,5-triazin-2-yl]amino]-2,4,6-trimethyl-5-sulfophenyl]amino]-9,10-dihydro-9,10-dioxo-, sodium salt (1:2)

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
LTD/1819	Huntsman Advanced Materials Pty Ltd Chemiplas Australia Pty Ltd	2-Anthracenesulfonic acid, 1-amino-4-[[3-[[4-chloro-6-[[3-[[[2-(ethenylsulfonyl)ethyl]amino]carbonyl]phenyl]amino]-1,3,5-triazin-2-yl]amino]-2,4,6-trimethyl-5-sulfophenyl]amino]-9,10-dihydro-9,10-dioxo-, sodium salt (1:2)	Yes	1 tonne per annum	Dye for textiles

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*
Eye irritation (Category 1)	H318 – Causes serious eye damage

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:

R41: Risk of serious damage to eyes

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 reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Eye irritation (Category 1): H318 – Causes serious eye damage

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.

- Due to the eye irritation properties of the notified chemical, the notifier should consider their obligations under the Australian Dangerous Goods Code.

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 product Eriofast Blue 3R:
  - Enclosed and automated processes, where possible
  - Adequate general ventilation and local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in the product Eriofast Blue 3R:
  - Avoid contact with eyes
  - Avoid breathing in any dust, mist or aerosol

- 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 product Eriofast Blue 3R:
  - Coveralls
  - Gloves
  - Goggles
  - Respiratory protection including organic vapour cartridges, if formation of dust, mist or aerosol is expected

  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

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

Emergency procedures

- Spills and/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 importation volume exceeds one tonne per annum notified chemical;
- the notified chemical is intended to be introduced in a solid form other than non–dusting powder/granule that may generate inhalable or respirable particles;
- additional information has become available to the person as to potential for carcinogenicity of the notified chemical;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a dye for textiles, 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 products 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(S)
Huntsman Advanced Materials Pty Ltd (ABN: 93 091627 879)
Gate 3, 765 Ballarat Rd
Deer Park VIC 3023

Chemiplas Australia Pty Ltd (ABN: 29 003 056 808)
Level 3, 112 Wellington Parade
East Melbourne VIC 3002

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: None

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: vapour pressure and dissociation constant

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
China, 2013
Korea, 2002
New Zealand, 2007

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Eriofast Blue 3R (Product containing the notified chemical at a concentration between 70% - 90%)

CAS NUMBER
323195-50-2

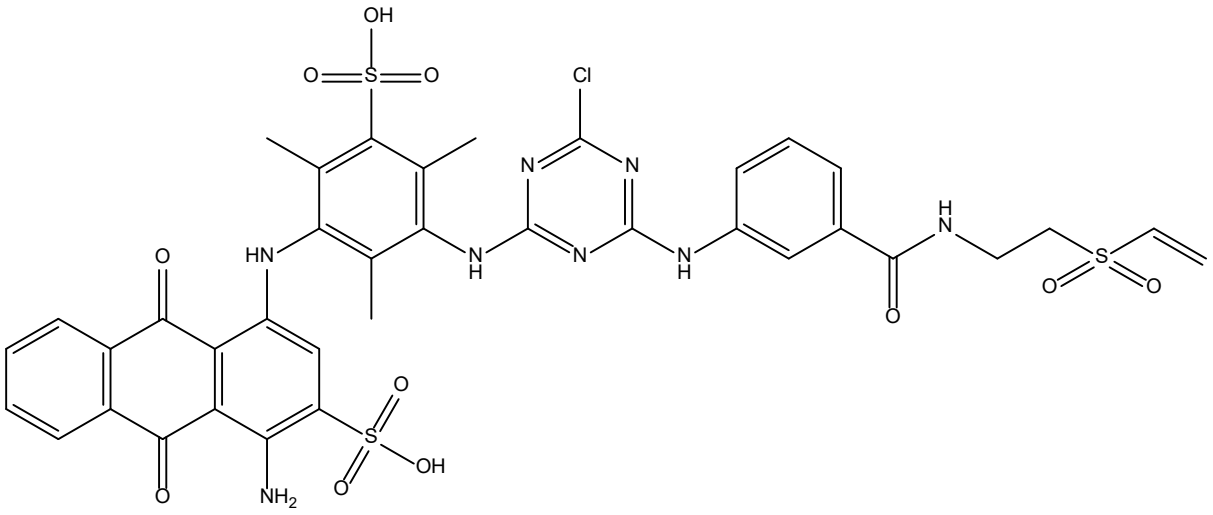
CHEMICAL NAME
2-Anthracenesulfonic acid, 1-amino-4-[[3-[[4-chloro-6-[[3-[[[2-(ethenylsulfonyl)ethyl]amino]carbonyl]phenyl]amino]-1,3,5-triazin-2-yl]amino]-2,4,6-trimethyl-5-sulfophenyl]amino]-9,10-dihydro-9,10-dioxo-, sodium salt (1:2).

OTHER NAME(S)

FAT 45401/A (or FAT 45'401/A)
Reactive Blue 272
Blue UL 1071

MOLECULAR FORMULA
$C_{37}H_{33}ClN_8O_{11}S_3.2\ Na$

STRUCTURAL FORMULA



. 2 Na

MOLECULAR WEIGHT
943.33 Da

ANALYTICAL DATA

METHOD	HPLC – UV/Visible
Remarks	Reference spectra were provided. The main reaction product (the notified chemical) was about 64.8% and was observed within the wavelength range of 220-800 nm.
TEST FACILITY	RCC Ltd

METHOD	^{1}H NMR
Remarks	Reference spectra were provided.
TEST FACILITY	RCC Ltd

METHOD	IR
Remarks	Reference spectra were provided.
TEST FACILITY	RCC Ltd

METHOD	UV/Visible
Remarks	Reference spectra were provided. The notified chemical was detected at pH 7.3, pH 1.1 and pH 12.9 with maximum absorption peaks observed at 204, 212 and 205 nm respectively.
TEST FACILITY	RCC Ltd

3. COMPOSITION

DEGREE OF PURITY
64.8%

IMPURITIES

Chemical Name	1-Amino-4-(3-amino-2,4,6-trimethyl-5-sulfophenylamino)-anthraquinone-2-sulfonic acid		
CAS No.	Unassigned	*Weight %*	0.6
Chemical Name	1-Amino-4-{3-[4-hydroxy-6-(3-(2-vinylsulfonylethylamino-carbonyl)-phenylamino-[1,3,5]triazine-2-ylamino]-2,4,6-trimethyl-5-sulfophenylamino}-anthraquinone-2-sulfonic acid		
CAS No.	Unassigned	*Weight %*	0.2

Chemical Name	3,5-Bis-(4-amino-2-sulfo-anthraquinone-ylamino)-2,4,6-trimethyl-benzene sulfonic acid		
CAS No.	Unassigned	*Weight %*	2.2
Chemical Name	2-Chloro-4,6-Bis-[5-(4-amino-2-sulfo-anthraquinone-ylamino)-1-sulfo-2,4,6-trimethyl-3-ylamino]-[1,3,5]triazine		
CAS No.	Unassigned	*Weight %*	5.5
Chemical Name	1-Amino-4-{3-[[6-(3-(2-vinylsulfonylethyl)-aminocarbonyl)-phenylamino]-4-[2-(2-(3-(4-chloro-6-(3-(4-amino-3-sulfo-anthraquinone-1-ylamino)-2,4,6-trimethyl-5-sulfophenylamino)-[1,3,5]triazine-2-ylamino)-phenyl-carbonylamino)-ethylsulfonyl)-ethoxy]-[1,3,5]triazine-2-ylamino]-2,4,6-trimethyl-5-sulfo-phenylamino}-anthraquinone-2-sulfonic acid		
CAS No.	Unassigned	*Weight %*	12.9

The notified chemical contains impurities in salt form; however, only acid form of the impurities is listed above.

Residue of unsulphonated aromatic amines in the notified chemical was examined to be < 10 ppm using thin-layer chromatography with diazotation reaction for visual detection. Compared to the calibration solutions that showed positive dark colour, the notified chemical did not produce colour reaction.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Solid, blue powder

Property	Value	Data Source/Justification
Melting Point	> 400 °C	Measured
Boiling Point	> 400 °C at 101.3 kPa	Measured
Relative Density (D^{20}_4)	1.61 at 20 °C	Measured
Vapour Pressure	3.49×10^{-29} kPa at 25 °C	Calculated
Water Solubility	127.8 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	pH 7.0 $t_{1/2}$ > 1year at 25 °C pH 4.0 $t_{1/2}$ = 90 days 25 °C pH 9.0 $t_{1/2}$ = 52 days 25 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow = -2.3 at 20 °C	Measured
Surface Tension	48.8 mN/m at 20 °C ± 0.3 °C	Measured
Adsorption/Desorption	log K_{oc} < 1.32 at 25 °C	Estimated
Dissociation Constant	Strongest pKa(Acid): -1.2 ± 0.8 Strongest pKa(Base): 5.5 ± 1	Calculated using ACD ILab 2.0.
Particle Size	0.5 to 125 μm (MMD* < 21.18 μm) Inhalable fraction (< 100 μm): 98.06% Respirable fraction (< 10 μm): 30.81%	Measured
Flash Point	Not determined	The melting point is > 400 °C.
Flammability	Not highly flammable	Measured
Auto ignition Temperature	253 °C	Measured
Explosive Properties	Not determined	Theoretically assessed not to be classified as explosive material based on the expert statement provided by the notifier.
Oxidising Properties	Not determined	Theoretically assessed to be non-oxidising based on the expert statement provided by the notifier.

* MMD = Mass Median Diameter

DISCUSSION OF PROPERTIES

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

The notifier provided information about the dye dedusting process and the particle size distribution of the treated dyes. The dedusting processes involves the dye being treated with dedusting oils (0.5-2%) and/or are dedusted mechanically during the production process in order to minimise and protect workers from inhalation exposure. According to the notifier such treated dye will contain less than 20% inhalable and less than 1% respirable particles.

Reactivity
The notified chemical is expected to be stable under normal conditions of use. The notified chemical is a surface active substance.

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 in Australia. The notified chemical will be imported as a component of Eriofast Blue 3R at a concentration between 70% - 90%, in a form of non-dusting powder or granule. The imported finished product, Eriofast Blue 3R, will be used in the textile industry. No further reformulation and repackaging of the product containing the notified chemical will occur in Australia.

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

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

PORT OF ENTRY
Melbourne

IDENTITY OF RECIPIENTS
Chemiplas Australia Pty Ltd
Level 3, 112 Wellington Parade
East Melbourne VIC 3002

Huntsman Advanced Materials Pty Ltd
Gate 3, 765 Ballatat Road
Deer Park VIC 3023

TRANSPORTATION AND PACKAGING
The dye product, Eriofast Blue 3R, containing the notified chemical at a concentration up to 90% will be imported in non-dusting powder/granule form in anti-static polyethylene lined 20-kg fibreboard container and transported from wharf to the contracted warehouse for storage and further distribution.

USE
The imported Eriofast Blue 3R containing the notified chemical is a reactive dye for colouration of cotton and manufactured fibres including polyesters and polyamides. It will be used to dye textiles, which include domestic textile products used for apparel, sheeting and other uses. It will be used in industrial dye houses only. The concentration of the notified chemical in the final textile dye solution is < 1%.

OPERATION DESCRIPTION
At dye houses, Eriofast Blue 3R containing 70 - 90% of the notified chemical will be weighed in a dispensary equipped with local exhaust ventilation. The weighed dye containing the notified chemical will be dispensed through a hatch into the enclosed dyeing vat, where water will be added to prepare a dye solution. The notifier stated that the dye product will be imported in dried dedusted powder form which is expected to be treated with dedusting oils and/or to be mechanically dedusted during the production. When handling the dry powder of the dye, workers are expected to wear respiratory protection including dust masks or organic vapour cartridge respirators to minimise the possible inhalation of the dust. Appropriate use of additional PPE such as elbow-length PVC gloves, safety glasses/face shield and protective coveralls will reduce the potential for exposure

further. Once the dye is dissolved in water, the notified chemical will be present at < 1% in the final textile dye solution. Following fixation, the textile is washed free of unfixed dye in wash off baths, and dried by hydroextraction, followed by heating to 180 °C. Substrates which may be dyed include domestic textiles used for apparel, sheeting and other uses.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport drivers	0.5 - 12	30 -60
Warehouse operators	0.33	100-150
Batch area operators	0.33	~200
Dye machine operators	1	~200

EXPOSURE DETAILS

Transport and Storage
Transport and warehouse workers may experience dermal and ocular exposure only in the event of an accidental packaging breakage.

Preparation of Dye Solution and End Use Application
There is a possibility of dermal, ocular and inhalation exposure to the notified chemical at concentrations up to 90% during weighing out and dissolving the dye. Dust formation of the notified chemical during the processes is possible; however, it will be reduced by the anti-dust nature of the dye products and use of local exhaust ventilation as proposed by the notifier. The notifier also states that the exposure of worker to the notified chemical will be minimised by the presence of appropriate engineering controls and the use of PPE such as dust mask, organic vapour cartridge respirator, gloves, coveralls and goggles when handling the dye containing the notified chemical. Exposure will also be minimised by the use of an automated enclosed system during the dyeing process to prevent splashes and spills where the cloth is driven by mechanical rollers.

When the dyed materials are transported to the wash off batch on a pin chain, dermal and ocular exposure to the notified chemical at < 1% concentration may occur during manual handling of dyed wet cloth. Inhalation exposure to the mist of the notified chemical (< 1% concentration) is also possible. However, the cloth will be wrapped in plastic film and thus exposure is expected to be minimal. During the subsequent washing and drying (hydroextraction) processes, dermal, ocular and inhalation exposure may occur to the diluted dye solution containing the notified chemical at < 1% concentration. Appropriate PPE will be used by workers during the processes to minimise the potential for exposure.

During cleaning and maintenance processes such as flushing the holding and mixing tanks with water, dermal, ocular and inhalation exposure (to mists and aerosols) to the notified chemical at < 1% concentration may occur. Workers are expected to wear an organic vapour cartridge respirator, gloves, safety glasses and coveralls to minimise exposure as proposed by the notifier.

6.1.2. Public Exposure

The dye product (Eriofast Blue 3R) containing the notified chemical will only be available to industrial users and will not be available to the general public. However, the general public may come into contact with the notified chemical through the use of dyed textiles such as apparel and sheeting.

According to the notifier, over 90% of the dye in the dye solution (containing < 1% of the notified chemical) will be strongly fixed to the fibre. Data on fixation/exhaustion curves of the notified chemical on fabric were provided by the notifier. The excess unfixed dye will be washed off, and the textile will be dried by hydroextraction followed by further heating. It is expected that there will be negligible unfixed residues of the

notified chemical available for further exposure after the process. Although no leaching/bleeding study was provided by the notifier, considering covalent chemical bond fixation and low concentration (< 1%) of the notified chemical in the dye solution, a significant amount of the notified chemical is not expected to be released from the dyed textile over time.

Therefore, considering the lack of commercial availability of dye containing the notified chemical to the public and the fact that a significant amount of the notified chemical is not expected to be released from the dyed textile over time, public exposure to the notified chemical is not considered to be significant.

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
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Severely irritating
Guinea pig, skin sensitisation	No evidence of sensitisation*
Rat, repeat dose oral toxicity – 28 days.	NOAEL 200 mg/kg bw/day
Mutagenicity – bacterial reverse mutation test	Non - mutagenic
Genotoxicity – *in vitro* chromosome aberration test	Non - genotoxic*

*Full study reports were not provided

Toxicokinetics, metabolism and distribution
The notified chemical is a blue powdery sodium salt, which serves as a reactive aromatic amine dye. The notified chemical is hydrophilic with a molecular weight > 500 Da and a partition coefficient of -2.3. Dermal absorption and accumulation of the notified chemical in fatty tissues is expected to be limited.

Acute toxicity
Acute oral and dermal toxicity studies showed that the lethal median dose (LD50) for the notified chemical was greater than 2,000 mg/kg bw/day. No mortality or any substance-related systemic effects or changes on organs were observed, indicating that the notified chemical exhibits low acute toxicity in rats.

Irritation and sensitisation
The notified chemical was non-irritating to the rabbit skin. Except for the blue staining of the treated skin, no erythema or oedema was observed during the entire study period.

The notified chemical was found to be severely irritating to the eyes of rabbits in an eye irritation study. Blue staining of the notified chemical affected the eyes of the test animals throughout the study. A primary irritation score of 4.00 was measured. Corneal opacity with maximum score of 4 persisted in all animals to the end of the study period. Swelling of conjunctivae with maximum scores of 4 was observed after 1 hour of treatment in all test animals and persisted up to 72 hours with scores of 1 in all animals.

The notifier provided a product record showing that the notified chemical was a non-sensitiser in a test in guinea pig. However, no details of the test were provided.

It is noted that the notified chemical contains halo-triazine and polarised alkene with sulphone group which are known structural alerts for skin sensitisation (QSAR Toolbox, v3.2).

Repeated dose toxicity
A 28 day oral toxicity study was conducted on the notified chemical in rats at the dose levels of 0, 50, 200 and 1,000 mg/kg bw/day. Statistically significant increases in creatinine and total bilirubin levels were noted in males and females treated with 1,000 mg/kg/day compared to controls. Statistically significant increases in total cholesterol, triglycerides and phospholipids were noted in males treated with 1,000 mg/kg/day when compared to controls. These changes were considered as treatment related and associated with lipid metabolism in liver and the histopathological changes noted in the kidney.

Increased absolute and relative kidney weights were noted in rats treated with 1,000 mg/kg bw/day. These effects persisted after the 2-week recovery period. Macroscopic findings showed bluish discolouration of kidneys, testes and mesenteric lymph nodes in all males treated with 1,000 mg/kg bw/day, which persisted after the 2 week recovery period with severity of lymphoid hyperplasia increasing slightly. Bluish discolouration of the kidneys was also noted in the females treated with 1,000 mg/kg bw/day and persisted after the 2 week recovery period.

Microscopic findings showing tubulonephrosis in kidneys were noted in all animals treated with 1,000 mg/kg bw/day, including the recovery group. Tubulonephrosis consisted of tubular epithelial vacuolation along with deposition of an exogenous, brownish pigment leading to tubular cell necrosis in a few animals and a higher incidence of tubular basophilia.

In the epididymides, tubular epithelial vacuolation was observed in males treated at 1,000 mg/kg bw/day. After the 2-week recovery period, this finding increased in severity. In some males, this finding was accompanied by interstitial oedema.

Based on these observations, a No Observed Adverse Effect Level (NOAEL) was established at 200 mg/kg bw/day for the notified chemical.

Mutagenicity/Genotoxicity
The notified chemical showed no evidence of mutagenicity in a bacterial reverse mutation assay.

A summary of results from an *in vitro* mammalian chromosome aberration study was provided by the notifier. Cells exposed to the notified chemical at 625 µg/mL for 4 hours in the presence of metabolic activation showed significant increase in aberration frequency. However, this result was not reproduced in another experiment. No details of the study were provided. There was no sufficient evidence of clastogenicity for the notified chemical.

The notified chemical contains amino anthraquinone which is known to have the potential for DNA intercalation causing mutations (QSAR Toolbox, v3.2).

Carcinogenicity
No data were provided to assess the carcinogenicity of the notified chemical.

The notified chemical contains aromatic amines and amino anthraquinones. A small number of the aromatic amines are classified as being carcinogenic or potentially carcinogenic to humans (SCCNFP, 2002). Based on the structural formula provided for the notified chemical, it may be metabolised *in vivo* to release aromatic amines. However, release of carcinogenic amines listed in the SCCNFP report is not expected. The presence of sulfonate groups in the notified chemical is likely to slow systemic uptake and enhance excretion of the chemical and its potential metabolites; however, the extent of these mitigating effects is unclear. There are also studies on anthraquinone dyes exhibiting carcinogenicity in workers (IARC Monograph, 2012). In the absence of a carcinogenicity study on the notified chemical, the potential for carcinogenicity cannot be completely ruled out.

It is also noted that, based on the analysis report provided by the notifier, residues of primary unsulfonated aromatic amines in the notified chemical that may be of carcinogenic concern were determined to be < 10 ppm.

Toxicity for reproduction
No data were provided to assess the potential for reproductive and/or developmental toxicity of the notified chemical.

Health 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 following table.

Hazard classification	***Hazard statement***
Eye irritation (Category 1)	H318 – Causes serious eye damage

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(s):

R41: Risk of serious damage to eyes

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The primary risk to workers from exposure to the notified chemical is eye irritation. In addition, its potential for carcinogenicity cannot be completely ruled out. Workers may be possibly exposed (via dermal, ocular and inhalation) to the notified chemical during various processes involving the notified chemical such as importation, transport, storage and textile processing.

Transport and storage

During importation, transport and storage, occupational exposure is minimal as workers would only be exposed to the notified chemical in the case of an accident involving damage to the packaging. Use of PPE would further reduce the potential for exposure.

Textile process

The notified chemical is a solid blue powder, with the percentages of respirable and inhalable particles at 30.81% and 98.06% respectively. Eriofast Blue 3R containing the notified chemical at 70 - 90% that will be imported into Australia will contain anti-dusting agent and the notifier states that such treated dye will normally contain less than 20% inhalable and less than 1% respirable particles. The health risk caused by inhalation exposure during weighing out and pouring the dye containing the notified chemical into the enclosed dyeing vat is expected to be low, since apart from the anti-dust treatment, engineering controls including local exhaust ventilation and enclosed dyeing vat and PPE such as gloves, safety goggles, organic vapour cartridge and protective coveralls are expected to be used during these procedures.

The health risk associated with occupational exposure during preparation of dye solution and end use application of dye solution containing < 1% notified chemical is also expected to be low, as enclosed and mainly automated systems will be used to transfer prepared dye solution and to process the textiles with the cloth driven by mechanical rollers. Workers are expected to wear eye protection, gloves and coveralls. When manual handling of wet cloth occurs, plastic films will be used to wrap up the wet cloth to minimise the potential for exposure.

The risk associated with occupational exposure during transfer of the wet cloth to the wash off batch on a pin chain and during further washing and drying processes is also expected to be low. Due to the covalent linkage of the dye to the substrate, it is not expected that, following the wash and fixation steps, there will be significant residual of free chemical available for further exposure.

Maintenance

During cleaning and maintenance, as workers will wear PPE such as a respirator with an organic vapour cartridge, gloves, safety goggles and coveralls, the risk associated with occupational exposure is expected to be low.

Overall, provided that the recommended controls are being adhered to, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health

The dye product (Eriofast Blue 3R) containing the notified chemical will only be available to industrial users. Therefore, the general public is not expected to come into contact with the notified chemical at significantly high concentrations. However, the general public will come into contact with dyed textiles such as apparel and sheeting. The concentration of the notified chemical in the final dye solution for textiles is < 1%.

The notifier states that over 90% of the notified chemical in the dyed textile will be bound covalently to the substrate (cloth). The dyed textile will be washed, dried by hydroextraction followed by further heating. It is expected that unfixed dye will be eliminated during the washing and heating cycles. Significant amount of the notified chemical is not expected to be released from the treated textiles over time.

Therefore, based on the available information, 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

Since the notified chemical will not be manufactured or repacked locally, there will be no environmental exposure associated with these processes in Australia.

RELEASE OF CHEMICAL FROM USE

The dye containing the notified chemical will be supplied to customers in a non-dusting powder or granules for direct use in dye houses in Victoria and New South Wales. The notified chemical will be used in a batch dyeing process in closed automated systems in the textile industry. The notified chemical will be fixed permanently (chemical covalent bound) to the fabric material with a high degree of fixation (99%). Consequently, a release value of 1% to waste water is expected. Following fixation, the textile will be washed free of unfixed dye in wash off baths, and dried by hydro-extraction, followed by heating to 180°C. Process waters will be pre-treated before discharge to the waste water treatment plant. Purification steps such as membrane filtration, flocculation with appropriate agents or treatment with ozone are expected to further lower the concentration of dye in the resulting waste water. A reduction in the dye concentration in waste water of at least 50% will be achieved with these additional treatments prior to the treatment in an industrial waste water treatment plant. Empty packaging is expected to contain approximately 0.1% of the imported product.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of notified chemical will share the fate of articles in which it is incorporated. These articles are expected to be disposed of to landfill at the end of their useful life. Empty packaging is expected to contain approximately 0.1% of the imported product (0.08% of notified chemical). Therefore, the annual maximum of 0.8 kg (1,000 kg × 0.08%) is likely to be disposed of to landfill. The treated effluent containing the notified chemical will be disposed of to the sewer.

7.1.2. Environmental Fate

A hydrolysis study on the notified chemical indicated that it is hydrolytically stable in water at pH 7.0 and not stable at pH 4.0 and 9.0 (pH 4.0 $t_{1/2}$ = 90 days and pH 9.0 $t_{1/2}$ = 52 days). The notified chemical is not readily biodegradable (6% in 28 days) nor inherently biodegradable. Therefore the notified chemical has the potential to be persistent in the environment. The potential for bioaccumulation of the notified chemical is low due to its very high water solubility, large molecular weight and charge and low log P_{OW}. Notified chemical released to sewer is not likely to be removed from the water column during sewage treatment plant (STP) processes as it has a low soil absorption coefficient (Koc) and is not expected to degrade rapidly. The notified chemical released to STPs is therefore expected to reach surface waters.

The majority of the notified chemical incorporated into dyed textiles is expected to share the fate of the articles in which it will be incorporated and is likely to ultimately be sent to landfill. The notified chemical fixed into dyed goods is not expected to be mobile nor bioavailable. In landfill or water, the notified chemical is expected to eventually degrade abiotically and biotically to form water, oxides of carbon, nitrogen, sulfur and metal salts.

For details of the fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A Predicted Environmental Concentration (PEC) has been determined based on the notifier's information for the operational procedures at the dyehouse. It was assumed that a maximum of 10 kg of the notified chemical (i.e. 10 kg of the imported dye/day × 80%) would be used at the dyehouse per day with a total of 10% released to sewer based on a conservative 90% fixation rate. The notified chemical concentration entering the STP was calculated as the mass of unfixed notified chemical released to sewer per day divided by the volume of rinsate per day (given by the notifier as 40,000 L). Additional dilution of the wastewater was indicated by the notifier to occur within the total dyehouse effluent (dilution factor ≥ 5), release to country sewer (dilution factor 3), and at receiving waters (dilution factor 10). It has been estimated by the notifier that 50% of the notified chemical will be removed during secondary STP processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Maximum daily use of notified chemical	8	kg/day
Chemical in dyehouse wastewater released to STP per day	0.8	kg/day
Rinsate usage in dyehouse	40,000	L/day
Amount in the rinsate	20	mg/L
Dilution in to total Mill effluent (at least 5 times)	4	mg/L
Dilution to sewer (3:1)	1.33	mg/L
Removal of notified chemical within STP	50%	
Dilution Factor – River	1	
Dilution Factor – Ocean	10	
PEC – River:	66.67	µg/L
PEC – Ocean:	6.67	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 66.67 µg/L may potentially result in a soil concentration of approximately 0.44 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 2.22 mg/kg and 4.44 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity (48 h)	EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity (72 h)	E_rC50 > 100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	IC50 > 1,000 mg/L	Not inhibitory to bacterial respiration

Based on the endpoints for toxicity of the notified chemical to aquatic organisms, 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. Based on its measured acute toxicity, biodegradability and expected low bioaccumulation potential, the notified chemical is not formally classified under the GHS for the chronic hazard.

As the notified chemical is a dye which results in coloured media, the modified algal test has demonstrated that the observed growth inhibition effect on algae was caused in part due to the indirect effect of light absorption in the coloured test solutions. However, the experimental data does not suggest that the algal growth is inhibited solely as a result of a reduction in light intensity.

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 common endpoint for all the test species (fish, daphnia, and algae). Since acute ecotoxicity endpoints for aquatic species from three trophic levels are available, an assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Fish).	> 100	mg/L
Assessment Factor	100	
PNEC:	> 1,000	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	66.67	> 1,000	< 0.067

Q - Ocean:	6.67	> 1,000	< 0.007

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 readily biodegradable in the environment. However, it is not likely to be significantly bioavailable based on its expected low potential to bioaccumulate. On the basis of the assessed use pattern, it is unlikely to result in ecotoxicologically significant concentrations in aquatic environment. Therefore, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point > 400 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks The test substance did not melt at temperature up to 400 °C under the conditions of the test. After the test, the sample was still powder and the colour changed to black. The notified chemical lost up to 21% of its mass.
Test Facility RCC Ltd (2001a)

Boiling Point > 400 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks The test substance did not boil at temperature up to 400 °C under the conditions of the test. No endothermic peaks were detected in the temperature range of 25 – 400 °C. At the end of the experiment, the test substance was lost up to 21% of its mass and changed into black powder.
Test Facility RCC Ltd (2001b)

Relative Density 1.61 at 20 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Determined using the gas comparison pycnometer method.
Test Facility RCC Ltd (2001c)

Vapour Pressure 3.49×10^{-29} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Calculated using Modified Watson Correlation
Test Facility RCC Ltd (2001d)

Water Solubility 127.8 g/L at 20 °C

Method OECD TG 105 Water Solubility.
Remarks Flask Method. The individual results from each sample did not differ by more than 15%. Therefore, the study is considered to be valid. The water solubility was not corrected for the purity of the notified chemical.
Test Facility RCC Ltd (2001e)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	$t_{½}$
7	25	> 1 year
4	25	90 days
9	25	52 days

Remarks The notified chemical was found to be stable at pH 7.0 and 50 °C. Therefore, no further testing was performed at this pH-value. The notified chemical was not stable at pH 4.0 and pH 9.0. Further testing was performed at different temperatures in order to calculate the rate constant (k_{25}) and the half-life time of the hydrolysis at pH 4.0 and pH 9.0 at 25°C.
Test Facility RCC Ltd (2001f)

Partition Coefficient (n-octanol/water) log Pow = -2.3 at 20 °C

Method OECD TG 107/117 Partition Coefficient (n-octanol/water).
Remarks HPLC Method and Flask Method. In the preliminary test, a very good solubility in water and a very poor solubility in n-octanol were found indicating a partition coefficient below -2. Hence, a main test according to OECD Guidelines 107/117 (either HPLC or flask shaking method) could not be applied. Therefore, the partition coefficient of the notified chemical was estimated using the solubility data in n-octanol (as obtained in the preliminary test) and in water. The water solubility of the notified chemical was stated to be 127.8 g/L. The result was used for the calculation of the partition coefficient. The tabulated values represent rounded results, which were obtained by calculation using the exact raw data.
Test Facility RCC Ltd (2001g)

Surface Tension 48.8 mN/m at 20 °C ± 0.3 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Concentration: Surface tension of the notified chemical was determined at a concentration of about 0.1% in water. Based on the criteria outlined in the EEC Guidelines, the notified chemical is a surface active substance.
Test Facility RCC Ltd (2001h)

Adsorption/Desorption log K_{oc} < 1.32 at 25 °C

Method OECD TG 121 Adsorption Coefficient - High Performance Liquid Chromatography (HPLC) Method.
Remarks The test item solution was injected three times and the combined reference solution was injected six times. The log Koc was calculated using a regression curve (log k' vs. log Koc) and was found to be < 1.32 which is equal to a Koc value of < 21. This value indicates that the notified chemical is mobile and will not be absorbed by organic carbon in soil.
Test Facility RCC Ltd (2001i)

Dissociation Constant Strongest pKa (Acid): -1.2 ± 0.8
Strongest pKa (Base): 5.5 ± 1

Method Calculated using ACD ILab 2.0.
Remarks The notified chemical is a salt and has acid and base groups that are expected to dissociate.
Test Facility RCC Ltd (2001j)

Particle Size 0.5 to 125 µm (MMD < 21.18 µm)

Method European Commission, Directorate General XII- JRC, Science Research and Development-Joint Research Centre. "Particle Size Distribution, Fibre Length and Diameter Distribution" Guidance Document, ECB/TM/February 1996.

Range (µm)	*Cumulative mass percentage (%)*
< 0.5	0.25
< 1	2.44
< 5	19.63
< 10	30.81
< 100	98.06
< 175	99.91

Remarks The identity of the test substance was missing based on the study report. However, in the *Material and Methods* section of the report, the identity of the test substance was reported as FAT 45'401/A. The test substance was dispersed in 2-propanol at room temperature and ultrasonificated for 15 minutes before use. The particle size was measured by laser diffraction. The particle size was found to range from approximately 0.5 to 125 µm. The mass median diameter (MMD) was determined to be < 21.18 µm.
Test Facility RCC Ltd (2001k)

Flammability Not highly flammable

Method	EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks	The test substance could not be ignited with flame during the preliminary test (contact time of about 2 minutes). In contact with the ignition source, the test substance gleamed and a black residue was formed.
Test Facility	RCC Ltd (2001l)

Autoignition Temperature 253 °C

Method	EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks	Applying a linear increase in temperature of about 0.5 °C/min, the test substance showed an exothermic reaction starting at about 158 °C which produced a maximum temperature of 620 °C in the sample cube. At the end of the measurement, the test item was carbonized and coloured grey to black. The test substance is auto-flammable under the conditions of the test.
Test Facility	RCC Ltd (2001m)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical (approximately 60% in purity)

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain	Rat/HanCrl: WIST Han (Glx:BRL) BR
Vehicle	Bi-distilled water
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Slightly blue faeces were observed in females and males for up to day 2 and day 3 respectively. Slight diarrhoea was noted in both females and males for up to day 1 and day 2 respectively. The study authors reported these signs as passive non-toxic effects of the notified chemical.
	The body weights were within the range of the strain and age.
Effects in Organs	No macroscopic findings were observed at necroscopy.
Remarks - Results	

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

TEST FACILITY: RCC Ltd (2001n)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical (approximately 60% in purity)

METHOD: OECD TG 402 Acute Dermal Toxicity.
EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).

Species/Strain	Rat/HanCrl:Wist Han (Glx; BRL)BR
Vehicle	Bi-distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	Slight blue skin and residue of the notified chemical were noted in all animals from day 2 to day 6. It was present up to day 7 and 8 in two males. These effects were considered to be non-toxic effect of the notified chemical. Crusts on the back were noted on day 7 and 8 in one male which was considered incidental. The body weights were within the range of the strain and age.
Signs of Toxicity - Systemic	There were no unscheduled deaths or systemic responses observed during

	the study period.
Effects in Organs	No macroscopic findings were observed at necropsy.
Remarks - Results	

CONCLUSION	The notified chemical of low toxicity via the dermal route.
TEST FACILITY	RCC Ltd (2001o)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical (approximately 60% in purity)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White, SPF
Number of Animals	3 (2F + 1M)
Vehicle	None (the test substance was moistened with bi-distilled water before application)
Observation Period	14 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.
Remarks - Results	No erythema and oedema was observed during the entire study period. However, there was blue staining observed in all test animals after 1 hour of treatment which prevented the initial observations for erythema. A light blue staining was observed in all animals up to the 10 day reading. No clinical signs of systemic toxicity and no mortality were observed in the study. The body weights were within the range of the strain and age.
CONCLUSION	The notified chemical non-irritating to the skin.
TEST FACILITY	RCC Ltd (2001p)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical (approximately 60% in purity)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White, SPF
Number of Animals	3 (2F + 1M)
Observation Period	21 days
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness[#]	1	-	1	1	< 10 days	0
Conjunctiva: chemosis	1.33	1.67	1.67	3	< 7 days	0
Corneal opacity	1.33	0	3	4	> 21 days	4
Iridial inflammation	0	0	0	0	Nil	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
# Redness of conjunctivae was not assessable at 24 hours for animals 1 and 3, and at all three time points for animal 2, due to blue staining of the test substance.

Remarks - Results	No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

Marked blue staining was observed in two animals up to 24 hours and up to 72 hours in one animal, which prevented the assessment. Light blue stain was observed in all animals till the end of the study period (21 days). A primary irritation score of 4.00 was measured. Corneal opacity with a score of 1 was observed in one female animal at 24 hours and in male animal starting at 72 hours with a score of 4. It is noted that corneal opacity scores of 4 appeared to all test animals at day 7 and persisted in all animals till the end of the study period. The study authors considered this is due to the blue staining caused by the notified chemical. Swelling of conjunctivae with scores up to 4 was observed after 1 hour of treatment in all animals and persisted up to 72 hours with scores of 1.

A slight to moderate watery discharge was observed in all animals 1 hour after treatment. Severity of the discharge increased in the male at 24 hours to a moderate mucus discharge which cleared after 7 days of treatment. In case of the females, a slight mucus discharge was observed at 24 hours of treatment.

CONCLUSION The notified chemical is severely irritating to the eye.

TEST FACILITY RCC Ltd (2001q)

B.5. Repeat dose toxicity

TEST SUBSTANCE Notified chemical (approximately 60% in purity)

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, HanBrl:WIST (SPF)
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	Bi-distilled water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5F + 5M	0	0/10
low dose	5F + 5M	50	0/10
mid dose	5F + 5M	200	0/10
high dose	5F + 5M	1,000	0/10
control recovery	5F + 5M	0	0/10
high dose recovery	5F + 5M	1,000	0/10

Mortality and Time to Death
There were no unscheduled deaths during the study.

Clinical Observations
Soft faeces were noted in males and females treated with 1,000 mg/kg bw/day due to the large amount of the notified chemical administered and were not considered to be related to systemic toxicity by the study authors. Dark blue-coloured faeces were noted in males and females and the severity was dependant on the dose concentrations. Persistence of this effect was noted in the high dose recovery group for several days during the recovery period. Blue discolouration of the bedding were also noted in males and females treated with 1,000 mg/kg bw/day. These effects were also not considered by the study authors to be indications of systemic toxicity.

No significant treatment-related differences in mean daily food consumption, mean bodyweight or mean bodyweight gain were noted during the treatment and recovery periods.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No treatment related differences in the haematology and urinalysis parameters were noted during the treatment or recovery periods. Statistically significant increases in creatinine and total bilirubin levels were noted in males and females treated with 1,000 mg/kg/day compared to control groups. Statistically significant increases in total cholesterol, triglycerides and phospholipids were noted in males treated with 1,000 mg/kg/day. These changes were considered as treatment related and associated with lipid metabolism in liver and the histopathological changes noted in the kidney.

Effects in Organs

Increased absolute and relative kidney weights were noted in rats treated with 1,000 mg/kg bw/day. These effects persisted after the 2-week recovery period. Macroscopic findings showed bluish discolouration of kidneys, testes and mesenteric lymph nodes in all males treated with 1,000 mg/kg bw/day, which persisted after the 2 week recovery period with severity of lymphoid hyperplasia increasing slightly. Bluish discolouration of the kidneys was noted in the females treated with 1,000 mg/kg bw/day and persisted after the 2 weeks recovery period. Bluish discolouration or discoloured foci in the lungs of two females was also noted at 1,000 mg/kg/day after the two week recovery.

Microscopic findings showing tubulonephrosis in kidneys were noted in all animals treated with 1,000 mg/kg bw/day including the recovery group. Tubulonephrosis consisted of tubular epithelial vacuolation along with the deposition of an exogenous, brownish pigment leading to tubular cell necrosis in a few animals and a higher incidence of tubular basophilia.
In the epididymides, tubular epithelial vacuolation was observed in males treated at 1,000 mg/kg bw/day. After the 2-week recovery period, this finding increased in severity. In some males, this finding was accompanied by interstitial oedema.

Remarks – Results

Toxicologically significant effects were observed in both sexes with the highest dose of 1,000 mg/kg/day.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 200 mg/kg bw/day in this study based on adverse effects observed on kidneys of both sexes, epididymides and mesenteric lymph nodes of males treated at 1,000 mg/kg bw/day.

TEST FACILITY	RCC Ltd (2001r)

B.6. Genotoxicity – bacteria reverse mutation test

TEST SUBSTANCE	Notified chemical (approximately 60% in purity)
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 (Test 1) Pre incubation procedure (Test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction Phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 33-5,000 µg/plate b) Without metabolic activation: 33-5,000 µg/plate
Vehicle	Deionised water
Remarks - Method	No significant protocol deviations. In the pre-experiment, the concentration range of the test item was 3-5,000 µg/plate. Since no toxic effects were observed at up to 5,000 µg/plate, this concentration was chosen as maximal concentration.

RESULTS

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

Remarks - Results

No toxic effects of any of the five tester strains was observed following treatment with the notified chemical at any dose level, either in the presence or absence of metabolic activation. No substantial increase in revertant colony number was noted in the study. The study authors concluded that there was no tendency of higher mutation rates with increasing concentrations of the notified chemical in the range below the generally acknowledged border of biological significance.

The positive controls (sodium azide, 2-aminoanthracene, 4-nitro-o-phenylene-diamine, methyl methane sulfonate) showed a distinct increase in induced revertant colonies, confirming the efficacy of the test system.

CONCLUSION

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

TEST FACILITY

RCC Ltd (2001s)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 A Ready Biodegradability: DOC Die-Away Test.
Inoculum	Activated Sludge
Exposure Period	28 Days
Auxiliary Solvent	None Reported
Analytical Monitoring	Dissolved organic carbon (DOC)
Remarks - Method	The test was conducted in accordance with the test guideline above with no significant deviation from the protocol reported.

RESULTS

Test substance		*D (+) - Glucose*	
Day	*% Degradation*	*Day*	*% Degradation*
1	7	1	56
10	5	7	98
14	3	10	96
21	4	21	96
28	6	28	100

Remarks - Results	All validity criteria were met. The biodegradation of the notified chemical was determined as 6% after 28 days of incubation. The test item did not reach the pass level of 70% for ready biodegradability in the DOC Die-Away Test either within the 10-day window or after 28 days of incubation.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Solvias AG (2001a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static.
Species	Zebra fish (*Brachydanio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Liquid Chromatography
Remarks – Method	Tested in accordance with the test guideline without significant deviation from the protocol. Good Laboratory Practices (GLP) was followed.

RESULTS

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

LC50	> 100 mg/L at 96 hours.
LOEC	> 100 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. In the control and at the test

concentration of 100 mg/L no mortality or other signs of intoxication were determined during the test period of 96 hours. Therefore, the 96-hour NOEC (highest concentration tested without toxic effects after the exposure period of 96 hours), and the 96-hour LC0 of the notified chemical to zebra fish were determined to be at least 100 mg/L. The NOEC and the LC0 might even be higher than this concentration, but concentrations in excess of 100 mg/L have not been tested, according to the guidelines. The 96-hour LOEC (lowest concentration with toxic effects), the 96-hour LC50 and the 96-hour LC100 were clearly higher than 100 mg/L. These values could not be quantified due to the absence of toxicity of the notified chemical at tested concentration,

CONCLUSION The notified chemical is not harmful to fish.

TEST FACILITY RCC Ltd (2001t)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None Reported
Water Hardness	214 mg $CaCO_3$/L
Analytical Monitoring	Liquid Chromatography
Remarks - Method	The test was carried out according to the test guideline above without significant deviation from the protocol. Good Laboratory Practices (GLP) was followed.

RESULTS

Concentration mg/L	*Number of D. magna*	*Number Immobilised*	
Nominal		*24 h*	*48 h*
4.3	10	0	0
9.4	10	0	0
21	10	0	0
45	10	0	0
100	10	0	0

LC50 > 100 mg/L at 48 hours

Remarks - Results All validity criteria for the test were satisfied. No immobilisation of Daphnia was observed in the control and at any of test item concentrations.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates.

TEST FACILITY Solvias AG (2001b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 32, 100 mg/L and a control.
Auxiliary Solvent	None Reported
Water Hardness	24 mg $CaCO_3$/L

Analytical Monitoring	High-performance liquid chromatography (HPLC) and UV/Vis-detection
Remarks - Method	The test was carried out according to the test guideline above without significant deviation from the protocol. Good Laboratory Practices (GLP) were followed. As the test substance is a dye which results in coloured media, the test method was modified to differentiate between a reduced growth of algae due to real toxic effects of the test substance and the algal cells or due to an indirect effect, a reduced algal growth by light absorption in coloured test solutions. Two experiment parts were used: Part A used the usual algal toxicity test protocol. Erlenmeyer flasks containing test substance and algae were covered with glass dishes containing untreated test water. Algal growth inhibition in these vessels would be due to any toxic effects in addition to reduced light intensity. Part B used the same procedure but replaced the contents of the glass dishes with the coloured test substance. The Erlenmeyer flasks contained algae but no test substance. Thus Part B results show the algal growth inhibition due to light absorption only.

RESULTS

	Biomass		*Growth*	
	E_bC50 (95% CI) mg/L at 72 h	*NOE_bC (mg/L)*	*E_rC50 (95% CI) mg/L at 72 h*	*NOE_rC (mg/L)*
Part A-Test solutions (coloured)	25 (8.7-222)	Not reported	> 100 (n.d)	Not reported
Part B-No test substance	54 (31-135)		>100 (n.d)	

Remarks - Results	This modified algal test has demonstrated that the observed growth inhibition effect on *Scenedesmus subspicatus* was caused in part due to the indirect effect of light absorption in the coloured test solutions. A real toxic effect of the test item on the growth of *Scenedesmus subspicatus* can be excluded up to the highest test concentration of 100 mg/L.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	RCC Ltd (2001u)

C.2.4. 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: 3.2, 10, and 32 mg/L of mg/L Actual: 25.6, 64, 160, 400 and 1000 mg/L
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	
IC50	> 1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The EC50 was out of the tested concentration range (> 1000 mg/L).
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	Solvias AG (April 2001c)

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