File No: STD/1385

June 2011

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Novacron Red LS-BN

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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 Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

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

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Huntsman Advanced Materials Pty Ltd (ABN: 93 091627879)
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
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, Methods of detection, Degree of purity, Hazardous impurities, Non-hazardous impurities, Import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Dissociation constant, Flash point, Acute inhalation toxicity, and In vivo genotoxicity study.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Novacron Red LS-BN (product containing the notified chemical at >75%)

MOLECULAR WEIGHT
>1000 Da (free acid form of main component).
All other known components have molecular weights > 500 Da, and in may cases > 1000 Da.

ANALYTICAL DATA
Reference UV/Vis, NMR, and HPLC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY Reaction mixture (>75% active in dyeing)

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Dark red powder or granule

Property	Value	Data Source/Justification
Melting Point	>400°C	Measured
Boiling Point	>400°C at 98.2 kPa	Measured
Density	1.728 x 10^3 kg/m^3 at 19.8°C	Measured
Vapour Pressure	8.96 x 10^{-44} kPa at 25°C	Measured
Surface Tension	66.1 mN/m at 20.2°C	Measured
Water Solubility	74.1 g/L	Measured
Hydrolysis as a Function of pH	$t_{½}$ = 2126 h at pH 4, 25°C $t_{½}$ = 100 h at pH 7, 25°C	Measured
Partition Coefficient (n-octanol/water)	log Pow = -4.32	Measured
Adsorption/Desorption	log K_{oc} < 1.25	Measured
Dissociation Constant	Not determined	The notified chemical is a salt and expected to be ionised under environmental conditions
Particle Size	Inhalable fraction (<100 μm): 100% Respirable fraction (<10 μm): 78% MMD* = 5.7 μm	Measured
Flash Point	Not determined	The notified chemical is a solid
Flammability (Solid)	Not determined to be flammable	Measured
Autoignition Temperature	300°C (auto-flammable)	Measured
Explosive Properties	Not explosive	Measured (study not provided)
Oxidizing Properties	Not an oxidant	Measured

* MMD = Mass Median Diameter

DISCUSSION OF PROPERTIES

For full details of tests on physical and chemical properties, refer to Appendix A. Note that particle size of imported material is larger than the tested material, as it in a non-dusting form.

Reactivity

The notified chemical is chemically stable and will not decompose under normal ambient conditions. It is also considered to be inert in terms of oxidizing properties. However, rapid hydrolysis will occur in alkaline solutions (>pH 9).

Dangerous Goods classification

Based on the submitted physical-chemical data in the above table, the notified chemical is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However, the data above do not address all Dangerous Goods endpoints. Therefore, consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

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

The product containing the notified chemical (>75% active in dyeing) will be imported into Australia in powder or granular form in robust anti-static polyethylene lined 25-kg fibreboard containers and transported from wharf to the contracted warehouse.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	5-20	5-20	5-20	5-20	5-20

PORT OF ENTRY
Melbourne or Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Chemiplas Australia Pty Ltd will supply the products containing the notified chemical directly to dye houses in Australia.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in polyethylene lined 25-kg fibreboard container and transported by road directly to the customer warehouses for storage.

USE
The notified chemical will be used in dye for cellulosic textiles for apparel, sheeting and other uses. It will be used in dye houses only.

OPERATION DESCRIPTION
The product containing the notified chemical (>75% active in dyeing) will be imported into Australia in anti-dusting form, either as a powder with a particle size range of 150-200 microns or as granules with a particle size of 300 microns. Although most of the notified chemical imported will be sold as received, a small amount (less than 100 kg per year) may be repacked into smaller containers as samples or for use in mill trials. Repackaging, if required, will take place at the importer's facility.

The notified chemical will be used in several dye houses nationally. At the dyehouses, the product containing the notified chemical will be weighed (approximately 2.5 kg on average per 500 L of water) in a dispensary equipped with local exhaust ventilation and poured through a hatch into the enclosed dyeing vat. In the enclosed dyeing vat, approximately 500 L of water is added to prepare the finished dye solution (containing 0.5% of the notified chemical). Small samples of the dye solution will be removed for quality control testing.

The dye solution containing the notified chemical will be transferred through an enclosed system to a tank, and then dispensed into an enclosed dyeing machine. The dyeing process is mainly automated with the cloth driven by mechanical rollers. The system is mainly enclosed to prevent splashes and spills. Manual handling of wet cloth will occur during transport to the wash off batch on a pin chain. However, the cloth will be wrapped up in plastic film.

Over 90% of the dye is bound covalently to the substrate (cloth) and excess dye is washed off. The textile is then dried by hydroextraction followed by heating. The moist cloth is always wrapped up in plastic film during various washing and drying processes. Cleaning and maintenance is performed by the machine operators. This involves flushing the holding and mixing tanks with water.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours per day)*	*Exposure Frequency (days per year)*
Transport drivers	5-10	0.5-12	30 - 60
Warehouse operators	4 – 8/site	0.5	100 – 150
Batch area operators (weighing, dissolving, transfer and QA sampling)	5 – 10/site	0.5	180 – 240
Dye machine operators (dyeing, fixing, cleaning and maintenance)	5 – 10/site	1	180 – 240

EXPOSURE DETAILS

Transport and Storage

Worker exposure to the notified chemical in neat form during importation, transportation and storage is not expected, except in the event of an accident where the packaging may be breached.

Preparation of dye solution and End Use Application

There is a possibility of dermal, ocular, and inhalation exposure to the notified chemical during weighing out and pouring of the notified chemical into the enclosed dyeing vat. However, exposure will be minimised by the anti-dusting formulation introduced, the use of purpose-designed dispensary, local exhaust ventilation and PPE such as elbow-length PVC gloves, safety glasses/faceshield and protective overalls when handling the notified chemical.

Exposure will also be minimised by the use of an enclosed system to transfer prepared dye solution to the tank and also by the use of an enclosed dyeing machine. Exposure during manual handling of wet cloth during transportation to the wash off batch on a pin chain and during further washing and drying processes will be minimised by the use of plastic film to wrap up the wet cloth.

During cleaning and maintenance processes, dermal, ocular and inhalation (to aerosols) exposure could occur. Workers are expected to wear an organic vapour cartridge respirator, gloves, safety glasses and overalls to minimise exposure.

6.1.2. Public exposure

The product containing the notified chemical will only be available to industrial users. Therefore, the general public will not be exposed to the notified chemical as such. However, the general public may be exposed through the use of dyed textiles such as apparel, sheeting and other uses.

The notifier has stated that over 90% of the dye is bound covalently to the substrate (cloth). In this regard, the notifer has provided fixation/exhaustion curves and fastness results. The excess dye will be washed off, and the textile will be dried by hydroextraction followed by heating. During washing and drying, unfixed dye will be washed off. Although no leaching/bleeding study has been provided by the notifier, considering almost 100% fixation and low concentration 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 availability of 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 table below. Details of these studies can be found in 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
Rat, acute inhalation toxicity	not determined
Rabbit, skin irritation	not-irritating
In Vitro Test – Human Skin Model	not-corrosive
Rabbit, eye irritation	Irritating (persistent colouration of the eye)
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation up to 20% conc.
Rat, repeat dose oral toxicity – 28 days.	NOEL 200 mg/kg/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro - Chinees Hamster V79 Cells	non genotoxic

Toxicokinetics, metabolism and distribution.

No data were provided to assess toxicokinetics of the notified chemical. As the notified chemical is highly hydrophilic (log Pow < -4.32) with a high molecular weightt >1000 Da), dermal absorption of the notified chemical is expected to be limited. This is consistent with the lack of systemic effects in the acute dermal toxicity study.

Acute toxicity.
The notified chemical is of low acute oral and dermal toxicity, with oral and dermal LD50 of being >2000 mg/kg bw. Although no acute inhalation study has been conducted, exposure is expected to be limited by its low vapour pressure and the non-dusting form of the imported notified chemical.

Irritation and Sensitisation.
The notified chemical was not irritating to the skin of rabbit.

An *in vitro* study to assess the corrosive potential of the notified chemical by means of the Human Skin Model Test was also assessed. The study concluded that, under the conditions of the test reported, the notified chemical was non corrosive to skin.

In the eye irritation study provided, NICNAS noted that persistent slight red staining of conjunctivae and sclera was visible up to the end of the observation period (21 days) in two animals out of three tested. No other abnormal effect was noted in the eyes and no abnormal findings were also observed in the cornea or iris of any animal at any of the examination times. The persistency of slight red staining of conjunctivae and sclera in eyes is most likely due to the administration of red dye (test substance) per se rather than a reflection of any intrinsic property of the test substance.

It was not a skin sensitiser up to 20% concentration, based on the results of the LLNA in mice.

Repeated Dose Toxicity
In an oral toxicity study, rats were fed 50, 200 or 1000 mg/kg bw/day of the notified chemical for 28 days. There were no deaths and no test substance-related significant effects on clinical observations, mean food consumption, mean body weight gain, Functional Observational Battery tests, mean fore- & hindlimb grip strength values, and mean locomotor activity at any dose level. However, reddish colouration of the faeces was noted in all treated animals until day 4 of the recovery period. As this finding is commonly observed following oral administration of dyes, it is not considered to be an adverse finding.

There were also no test substance-related effects on laboratory finding. However, red colouration of individual urine samples was noted and was considered to be a result of excretion of the test substance by the kidneys. The urinary colouration was reversible after the recovery period.

Histological examination revealed changes in the kidneys of the males and females in the high-dose group, consisting of vacuolation of tubular epithelial cells with finely granular eosinophilic inclusions, and elevated degrees of severity and/or incidence of tubular basophilia. These changes persisted in the recovery animals. Tubular changes were paralleled by increased mean degree of severity and/or incidence of interstitial inflammatory infiltrates.

Necrosis of tubular cells (grade 1-2) was noted in rats of high dose group. This finding was not seen after the treatment period in control rats nor in rats of low dose and mid dose groups. The finding was nearly completely reverted in rats necropsied after the recovery period.

The No Observed Effect Level (NOAEL) was established as 200 mg/kg bw/day in this study, based on histological changes in the kidney in the rats of high dose group (1000 mg/kg bw/day).

Mutagenicity and Carcinogenicity
The notified chemical was found to be negative in a bacterial reverse mutation test, and also showed no evidence of clastogenicity in a Mammalian Chromosome Aberration Test, using Chinese Hamster V79 Cells. Therefore, based on the available information, the notified chemical is unlikely to be genotoxic.

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

NICNAS noted that the notified chemical is an azo dye and contains a functional group, which has a structural alert for carcinogenicity and mutagenicity. In general, azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. The azo linkage is the most labile portion of an azo dye molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecule into component amines. Some metabolism may also occur in the cells of the bladder wall, and during percutaneous absorption. Anaerobic intestinal bacteria are also capable of reductive

cleavage of the azo linkage.

The aromatic amines that arise from the azo reduction of azo dyes are thought to be activated through their *N*-oxidation by cytochrome P450 isozymes (SCCNFP, 2002). These *N*-hydroxylarylamines may be further glucuronated (activated) or acetylated (inactivated), which may influence their mutagenicity (Bartsch, 1981). Under acidic pH, they form reactive nitrenium ions that can alkylate bases in DNA, particularly the nucleophilic centres in guanine. This mechanism is thought to contribute to the carcinogenicity of many azo dyes.

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The analysis of the notified chemical found that it contains very low levels of primary unsulfonated aromatic amines as an impurity. As such, the impurities are unlikely to contribute to carcinogenicity of the notified chemical.

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

Health hazard classification
Based on the data provided, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase:

R41: Risk of serious eye damage

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. Considering the possibility of persistency of the dye in the eyes if exposed, eye protection should be used to prevent any exposure to the notified chemical.

There is a risk for potential occupational exposure (dermal, ocular and inhalation) to the notified chemical during various processes involving the notified chemical such as importation, transport, storage, weighing out and pouring of the notified chemical into the enclosed dyeing vat. During importation, transport and storage, the risk of occupational exposure is minimal and considered acceptable as workers will only be exposed to the notified chemical in the case of an accident involving damage to the packaging and to wrapping.

The notified chemical is a dark red powder, with the majority of particles in the respirable range (<10 μm - 78%). However, the commercial form of the notified chemical that will be imported into Australia will contain anti-dusting agent and will be imported either as a powder with a particle size range of 150-200 microns or as granules with a particle size of 300 microns.

Therefore, the risk of inhalation exposure during weighing out and pouring of the notified chemical into the enclosed dyeing vat is expected to be low. The exposure during weighing out and pouring of the notified chemical into the enclosed dyeing vat will also be minimised by the use of purpose-designed dispensary, local exhaust ventilation, and PPE such as elbow-length PVC gloves, safety glasses/faceshield and protective overalls during these procedures.

The risk of occupational exposure during preparation of dye solution and end use application of dye solution containing 0.5% notified chemical is also expected to be low and considered acceptable, as enclosed system will be used to transfer prepared dye solution to the tank and dyeing machine is also an enclosed and mainly automated system with the cloth driven by mechanical rollers. If the dyeing machine has to be opened in the case of malfunction, exposure will be minimised with the use of eye protection, gloves and overalls.

Furthermore, as a plastic film will be used to wrap up wet cloth, the risk of occupational exposure during manual handling of wet cloth, during transportation to the wash off batch on a pin chain and during further washing and drying processes, is also expected to be low and considered acceptable. Also, due to the covalent linkage of the dye to the substrate, it is not expected that there will be any exposure to the free chemical following the washing steps.

During cleaning and maintenance, as workers will wear a respirator with an organic vapour cartridge, gloves, safety goggles and overalls and they will only be exposed to a low concentration of the notified chemical (0.5%), the risk of occupational exposure is expected to be low and considered acceptable.

Considering the dye formulation with an anti-dusting agent, and use of engineering controls and PPE, the risk to workers from using the product containing the notified chemical is not considered to be unacceptable.

6.3.2. Public health

The product containing the notified chemical will only be available to industrial users. Therefore, the general public will not be exposed to the notified chemical. However, the general public may be exposed to the notified chemical through the use of dyed textiles such as apparel, and sheeting.

The notified chemical will be present at a low concentration (0.5%) in the dye. The notifier states that almost 100% of the notified chemical will be bound covalently to the substrate (cloth) (as shown in fixation/exhaustion curves and fastness study provided). It is also noted that dyed textile material will be dried by hydroextraction followed by heating and it is expected that unfixed dye will be washed off during washing and heating cycles. Therefore, a significant amount of the notified chemical is not expected to be released from the dyed textile over time.

Therefore, considering all of the above and the hazard profile of the notified chemical, the risk of public exposure to the notified chemical is not considered to be significant and considered acceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

No manufacture or reformulation of the notified chemical will take place locally. Therefore, there will be no environmental exposure associated with this process in Australia. Release to the environment may occur in the event of an accident during transport or storage. Repacking of the dye (< 100 kg/annum) may take place however the release from this process is expected to be negligible.

RELEASE OF CHEMICAL FROM USE

Nearly all of the dye containing the notified chemical will be removed from the import container liners by shaking and < 1 kg of the chemical per annum will remain as residue. Waste due to spills is expected by the notifier to be < 10 kg per annum. Dissolution of the dye takes place in an enclosed vat and the dye is pumped through a closed system to the dyeing machine. Therefore, release of the chemical is not expected at this stage.

The dye will be used to colour cellulosic textiles by the exhaust dyeing method. Once the dye has diffused into the fibre matrix it reacts with active sites on the substrate producing strong covalent bonds. The fabric is then dried and steamed to fix the dye to cellulose and then the unreacted dye is washed off. Fixation data provided by the notifier indicates that the fixation rate of the notified chemical to be > 90%. The notified chemical adsorbed to the fabric with the dye will not be released to the environment. The rinsate generated via fabric rinsing should contain up to 10% of the notified chemical imported.

The dye washed off the fabric will be discharged to the dyehouse effluent system, where cationic flocculation will be used to remove the anionic dyestuff. The treated effluent containing traces of the notified chemical will be disposed of to the sewer and sludge containing the notified chemical will be disposed of to landfill. The dye will be used in a small number of dyehouses and is not expected to be used in country dyehouses.

RELEASE OF CHEMICAL FROM DISPOSAL

Any solid waste generated at the dyehouse including the residue in empty import containers will be disposed of as chemical waste. Articles containing dye are expected to be eventually disposed of to landfill.

7.1.2 Environmental fate

Studies submitted by the notifier indicated the notified chemical is not readily biodegradable or inherently biodegradable, however the notified chemical may hydrolyse under environmental conditions. The potential for bioaccumulation of the notified chemical is low due to its very high water solubility, large molecular weight and low log P_{OW}. It is expected that most of the dye not fixed to textiles (< 10% of the imported notified chemical) will be flocculated and collected and disposed according to State/Territory regulations. Due to its high water solubility, notified chemical residues released to STPs are not likely to be removed from influent by adsorption to sludge. However, notified chemical released to surface waters is expected to disperse and degrade. The majority of imported notified chemical will be covalently bound to textiles (> 90%) or contained in flocculated sludge and will ultimately end up in landfill. In landfill the notified chemical may be mobile and is expected to degrade slowly by biotic and abiotic pathways forming water and oxides of carbon, nitrogen, sulfur and inorganic salts.

For the details of the environmental fate studies, refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The dye will be used in a small number of dyehouses. The environmental hazard has been determined for a typical dyehouse located in a metropolitan area. The Predicted Environmental Concentration (PEC) is estimated below assuming a single point release and an estimated maximum daily use of 100 kg (annual import volume/work days = 20,000 kg / 200 days).

Calculation Factor	Value
Typical use of notified chemical	100 kg
Amount of notified chemical in wastewater (fixation rate 90%)	10 kg
Typical daily volume of dye house wash-water effluent	2.90 ML
Concentration in dyehouse effluent	3.45 mg/L
Dilution factor in sewage treatment plant	1:100
Concentration in effluent from sewage treatment plant	34.5 µg/L
Predicted environmental concentrations (PECs) in receiving waters	
PEC River (Dilution Factor 1:1)	34.5 µg/L
PEC Ocean (Dilution Factor 1:10)	3.45 µg/L

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
Daphnia Toxicity (48 h)	EC50 > 100 mg/L	Not harmful
Algal Toxicity (72 h)	E_rC50 > 120 mg/L	Not harmful
Inhibition of Bacterial Respiration	IC50 > 1000 mg/L	No toxic effect

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is considered to be not harmful to fish, aquatic invertebrates and algae, and therefore is not classified for acute or long-term hazard.

7.2.1 Predicted No-Effect Concentration

The lower limit of the median effect concentrations from ecotoxicological studies on the notified chemical was used to calculate the PNEC. An assessment factor of 100 was used as acute toxicity endpoints are available for the effects of the notified chemical on aquatic species from three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish), EC50 (Daphnia)	> 100	mg/L
Assessment Factor	100	
PNEC:	> 1000	µg/L

7.3. Environmental risk assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	34.5	1000	0.0345
Q - Ocean	3.45	1000	3.45×10^{-3}

The Risk Quotients (Q = PEC/PNEC) for the worst case discharge scenario have been calculated to be < 1 for the river and ocean compartments. Furthermore, flocculation is expected to efficiently remove the notified chemical from the dyehouse waste stream. If released to surface waters, the notified chemical is expected to disperse and degrade. It is not expected to bioaccumulate or have significant effect on aquatic biota. Therefore, the notified chemical is not expected to pose an unacceptable risk to the aquatic environment based on its reported use pattern.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the data provided, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)], with the following risk phrase:

R41: Risk of serious eye damage

The classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Eye irritation	Category 1	Causes serious eye damage

Human health risk assessment

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

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable 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 unacceptable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - R41: Risk of serious eye damage

- Use the following risk phrase for products/mixtures containing the notified chemical:

- Conc ≥ 10%: Xi; R41
- 5% ≤ conc < 10%: Xi; R36

Material Safety Data Sheet

- The MSDS provided by the notifier should be amended as follows:
  - Include R41hazard classification and safety phrase with respect to eyes

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced and as diluted for use:
  - Local exhaust ventilation during weighing out and pouring of the notified chemical into the enclosed dyeing vat.
  - Enclosed and automated processes during dye preparation and end use dye application.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced and as diluted for use:
  - Avoid contact with eyes.
  - Do not inhale dust/vapour.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced and as diluted for use:
  - Chemical resistant gloves
  - Goggles
  - Coveralls

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

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

1) Under Section 64(1) of the Act; if
   - the notified chemical is introduced in other than a non–dusting form and/or in which greater than 25% of particles have a diameter less than 10μm;

or

(2) Under Section 64(2) of the Act; if
   - the function or use of the chemical has changed from dye for cellulosic textiles, or is likely to change significantly;
   - the amount of chemical being introduced has increased from 20 tonnes/annum, 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.

Material Safety Data Sheet
The MSDS of a product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

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 of up to 400°C under the conditions of the test. Decomposition started at about 280°C.
Test Facility	RCC Ltd (2008a)

Boiling Point >400°C at 98.2 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 of up to 400°C under the conditions of the test. Decomposition started at about 280°C.
Test Facility	RCC Ltd (2008a)

Density 1.728×10^3 kg/m^3 at 19.8°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 (2008b)

Vapour Pressure 8.96×10^{-44} kPa at 25°C

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Determined using Modified Watson Correlation method. The boiling point used in this calculation was 1041.19°C, which was estimated using Meissner's method.
Test Facility	RCC Ltd (2008c)

Water Solubility 74.1 g/L

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Simplified Flask Method with HPLC Analysis. The test substance was ground in a mortar and 3 samples of 4 g were each added to about 20 mL water and stirred for 24 hours. After filtration (0.2 µm Nylon) the solution was diluted by a factor of 1:5000 with water. The test substance was quantified by HPLC and water solubility calculated.
Test Facility	RCC Ltd (2008d)

Hydrolysis as a Function of pH

Method	OECD TG 111 Hydrolysis as a Function of pH. EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ (hours)*
4	25	2126
4	50	323
4	70	60
4	80	48
7	25	100
7	50	7
7	60	2
7	70	1
9	50	unstable

Remarks As the test substance was found to be unstable at pH 4, 7 and 9, further testing was

performed in order to calculate the rate constant. Two peaks were observed. Results for the main peak are reported (the second peak had a half life less than the main peak at pH 4 ($t_{½}$ = 485 h) and no hydrolysis observed at pH 7 and 9).

Test Facility Harlan Laboratories (2009a)

Partition Coefficient (n-octanol/water) log Pow = -4.32

Method In-house method

Remarks Based on the results of the preliminary test, neither the flask method nor the HPLC-method were applicable for the determination of the log P_{OW}. Therefore, the value was estimated from the ratio of solubilities of the test substance in water (74.098 g/L) and n-octanol (3.577 mg/L), respectively.

Test Facility RCC Ltd (2008d)

Adsorption/Desorption log K_{oc} < 1.25

Method OECD TG 121: Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)

Remarks Stock solution was prepared by dissolving the test substance in a solvent mixture of water and methanol (45:55; v/v). An aliquot of stock solution was diluted with solvent mixture to obtain a test solution with concentration of 117.75 µg/mL. The pH was determined to be 6.9. The test solution was injected 3 times in a HPLC system and its retention time was compared with several reference compounds. The test substance had a shorter retention time than the reference item with the lowest log K_{oc} and hence is an upper limit is reported.

Test Facility Harlan Laboratories (2009b)

Particle Size

Method EC, Directorate General JRC, Joint research Centre. 'Particle Size Distribution, Fibre Length and Diameter Distribution' Draft Guidance Documents, EUR 20268 EN (2002), Part 5.2 "Laser scattering / diffraction".

Range (µm)	*Mass (%)*
<1.1	5
<1.7	10
<5.7	50
<14	90

Remarks The particle size was found to range from approximately 0.3 to 300 µm. The mass median diameter (MMD) was determined to be 5.7 µm.

Test Facility RCC Ltd (2008e)

Flammability (Solid) Not determined to be flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
EC Directive 92/69/EEC A.12 Flammability in contact with water
EC Directive 92/69/EEC A.13 Pyrophoric properties.

Remarks The test substance could not be ignited with flame during the preliminary test (contact time of about 2 minutes). Therefore, no main test was performed. The test substance also did not ignite spontaneously when on contact with water or humid air and shows no risk with respect to pyrophoric properties.

Test Facility RCC Ltd (2008f)

Autoignition Temperature 300°C (auto-flammable)

Method	EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks	Applying a linear increase in temperature of about 0.5ºC/min, the test substance showed an exothermic reaction starting at about 295ºC. At the end of the measurement, the test item was carbonised and coloured black. The test substance is auto-flammable under the conditions of the test.
Test Facility	RCC Ltd (2008g)

Surface Tension 66.1 mN/m at 20.2ºC

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	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 not a surface active substance.
Test Facility	RCC Ltd (2008h)

Oxidizing Properties Not an oxidant

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	The test substance showed no burning at all or only surface burning.
Test Facility	RCC Ltd (2008i)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD
- Species/Strain: Rat/HanRcc:WIST
- Vehicle: Water
- Remarks - Method: Animals were treated by oral gavage

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 (female)	2000	0
2	3 (female)	2000	0

- LD50: >2000 mg/kg bw
- Signs of Toxicity: A slightly ruffled fur was observed in 2 animals at 3 hours after treatment and persisted up to 5 hours after treatment. Soft faeces were observed in all animals 5 hours after treatment. Discoloured purple faeces or purple/black faeces were observed in all animals 5 hours after treatment & persisted up to test day 2.
- Effects in Organs: No macroscopic findings were recorded at necropsy. All group 2 animals were found to have an enlarged spleen at necropsy.

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

TEST FACILITY: RCC Ltd (2008j)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD
- Species/Strain: Rat/ HanRcc:WIST
- Vehicle: Water
- Type of dressing: Semi-occlusive
- Remarks - Method:

RESULTS

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

- LD50: >2000 mg/kg bw
- Signs of Toxicity - Local: No clinical signs were observed during the course of the study.
  A slight red staining was observed in all males & females on test day 2 and persisted up to test days 3 and 5.
  A very slight erythema was observed in 2 males from test day 6 up to test days 10 & 15 (the end of observation period). Scaling was recorded in one male on test day 4 & 5 and in two males on test day 5. Additionally, one male was observed with scabs on test days 10 & 15.
- Signs of Toxicity - Systemic: None
- Effects in Organs: None

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

TEST FACILITY Harlan Laboratories Ltd (2009b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Vehicle Water
Observation Period 10 days
Type of Dressing Semi-occlusive
Remarks - Method The duration of application was four hours. The scoring of skin reactions was performed at 1, 24, 48 and 72 hours as well as 7 & 10 days after removal of the dressing.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	0	0	0	0
Oedema	0	0	0	0	0	0

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

Remarks - Results Marked red staining of the skin produced by the test substance (red dye) was observed in all animals at 1 hour after treatment, which persisted slightly up to the 72-hour reading or up to 7 days after treatment. Due to this staining, no assessment of erythema formation could be made at 1 hour after the treatment.
From 24 hours after the treatment onwards, the intensity of the staining was reduced and a full assessment of skin reactions was possible. Neither erythema nor any other skin reactions were observed at any of the observation times.

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

TEST FACILITY Harlan Laboratories Ltd (2009c)

B.4. Corrosion – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 431: In Vitro Skin Corrosion: Human Skin Model Test (Original Guideline adopted April 13, 2004)

Treatment:
Duplicate EpiDerm tissues were treated with the test item, positive, and negative controls for 2 different treatment intervals: 3 minutes and 1 hour.
After pre-incubation of EpiDerm tissues was completed (1 hour for each treatment medium), medium was replaced by 0.9 mL fresh assay medium in all four 6-well plates. 50 µl of deionised water (negative control) was added into the first insert atop the EpiDerm tissue. The procedure was repeated with the second tissue. It was proceeded with test item and the positive control in the same manner until all tissues of the same treatment interval were dosed. The 6-well plates were placed into the incubator (37±1.5ºC, 0.5% CO_2).
After the end of the treatment interval, the first insert was removed

immediately from the 6-well plate. Using a wash bottle, the tissue was gently rinsed with PBS to remove any residual test material. Excess PBS was removed by gently shaking the insert and blotting the bottom with blotting paper. The insert was placed in the prepared holding plate. It was proceeded with test item and the positive control in the same manner until all EpiDerm tissues were dosed.

MTT Assay:
For MTT (2*H*-tetrazolium, 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-, bromide (1:1)), two 24-well plates were prepared before end of the tissue pr-warming period. 300 µl of the MTT solution was added to each well and the plates were kept in an incubator (37±1.5ºC, 0.5% CO_2), until use.
After the treatment procedure was completed for all tissues of each time point, cell culture inserts were transferred from the holding plates to the MTT-plates. Further procedures were performed to complete the MTT assay. Finally, optical density was measured at 570 nm without reference filter. Mean values were calculated from the 3 wells per tissue.

Remarks - Method

After treatment with the negative control, the absorbance values were well above the required acceptability criterion of mean OD ≥ 0.8 for both treatment intervals thus showing the quality of the tissues. Treatment with the positive control induced a decrease in the relative absorbance as compared to the negative control to 21.9% for the 3 minutes treatment interval and 8,2% for the 1 hour treatment intervals, thus ensuring the validity of the test system.

Acceptance criteria:
The absolute optical density (OD) 570 nm of the negative control tissues in the MTT-test is an indicator of tissue viability obtained in the testing laboratory after shipping and storing procedure and under specific conditions of the assay. Tissue viability is meeting the acceptance criterion if the mean OD of the two tissues is OD ≥ 0.8.
An assay is meeting the acceptance criterion if mean relative tissue viability of the Positive Control is ≤ 30%.

RESULTS

Remarks - Results

The notified chemical did not stain the MTT solution blue colour after 1 hour incubation and therefore, it was concluded that the notified chemical did not reduce MTT.

Dose group (treatment interval)	Mean Absorbance @ 570 nm of Tissue 1 and Tissue 2	Relative absorbance (% of negative control)
Negative Control (3 min)	1.758	100.0
Positive Control (3 min)	0.385	21.9
Test substance (3 min)	1.522	86.6
Negative Control (1 hr)	1.533	100.0
Positive Control (1hr)	0.125	8.2
Test substance (1 hr)	0.147	74.8

After treatment with the test substance, the relative absorbance values were decreased to 86.6% after 3 minutes treatment and 74.8% after 1 hour treatment. Both values were well above the threshold for corrosivity (50% for 3 minutes treatment and 15% for 1 hour treatment). Therefore, the test

substance was not considered corrosive.

CONCLUSION The notified chemical was considered to be non-corrosive under the conditions of the test.

TEST FACILITY RCC Ltd (2008k)

B.5. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Observation Period 21 Days
Remarks - Method Observation period was extended to 21 days due to red staining of the eye tissues.

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	1	72 hrs	0
Conjunctiva: chemosis	0	0	0	0	0	0
Conjunctiva: discharge	0	0	0	0	0	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results No clinical signs were observed during the course of study & there was no mortality.
The test substance (red dye) produced red staining of the eye. Marked staining was observed in the treated eyes of all animals one hour after treatment. From 24 hours after treatment, slight red staining was noted in the treated eyes, persisting up to 14 days in one animal, and up to the end of the observation period (21 days) in the other two animals.
From 24 hours to 72 hours after exposure to the test substance, slight reddening of the conjunctivae was noted in all animals, Thereafter, no abnormal findings were observed in the treated eye of any animal until 21 days after treatment.

CONCLUSION NICNAS noted that persistent slight red staining of conjunctivae & sclera was visible up to the end of the observation period (21 days) in the two animals. No other abnormal effect was noted in the eyes. No abnormal findings were also observed in the cornea or iris of any animal at any of the examination. It is likely that the persistency of slight red staining of conjunctivae & sclera is most likely due to the administration of red dye (test substance) per se and rather than a reflection of any intrinsic property of the test substance.
NICNAS also noted that eye irritation scores were below the classification for eye irritation, according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004). However, as the notified chemical caused persistent eye colouration that lasted in 2 animals up to 21 days, the notified chemical was considered to cause serious eye damage, according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004). Therefore, the notified chemical is classified with the risk phrase 'Risk of serious eye damage (R41)'.

TEST FACILITY Harlan Laboratories Ltd (2009d)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain Rat/Wistar
Route of Administration Oral – gavage
Exposure Information Total exposure days: 28 days;
Dose regimen: 7 days per week;
Post-exposure observation period: 14 days recovery
Vehicle Water
Remarks - Method No significant deviation from test protocol.
Recovery animals were sacrificed after 14-day treatment-free recovery period.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5/sex	0	0
II (low dose)	5/sex	50	0
III (mid dose)	5/sex	200	0
IV (high dose)	5/sex	1000	0
V (control recovery)	5/sex	0	0
VI (high dose recovery)	5/sex	1000	0

Mortality and Time to Death
All animals survived until schedule necropsy.

Clinical Observations
No significant clinical observations were noted in animals administered the test substance. The mean food consumption and mean body weight developments were unaffected by the administration of the test substance. However, reddish colouration of the faeces was noted in all treated animals, until day 4 of the recovery period. As this finding is commonly observed following oral administration of dyes, it is not considered to be an adverse finding.

No significant finding was also evident during the Functional Observational Battery tests at week 4. Similarly, no test substance related differences in the mean fore- & hindlimb grip strength values or the mean locomotor activity were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No significant laboratory finding was noted. However, colouration of individual urine samples was noted and was considered to be a result of excretion of the test substance by the kidneys. The urinary colouration was reversible after the recovery period.

Effects in Organs
At 50 mg/kg bw/day, reduction in mean relative epididymide weights was noted after four weeks of treatment. At 50 and 200 mg/kg bw/day, reduced mean absolute epididymide weights were noted after four weeks of treatment. However, as no effect on epididymide weights were noted at 1000 mg/kg bw/day, this effect was unlikely to be test substance related and is therefore, not considered to be an adverse effect. Also, at 1000 mg/kg bw/day, no differences to the mean absolute and relative organ weights were seen after the treatment period, when compared with the control animals of either sex.

Histological examination revealed changes in the kidneys of the males and females in the high-dose group, consisting of vacuolation of tubular epithelial cells with finely granular eosinophilic inclusions. Elevated degrees of severity and/or incidence of tubular basophilia were also noted in this group. These changes persisted in the recovery animals. Tubular changes were paralleled by increased mean degree of severity and/or incidence of interstitial inflammatory infiltrates. An increase in hyaline droplets also occurred in high-

dose males. These kidney changes were thought to be treatment-related.

Necrosis of tubular cells (grade 1-2) was noted in rats of high dose group. This finding was not seen after the treatment period in control rats nor in rats of low dose and mid dose groups. The finding was nearly completely reverted in rats necropsied after the recovery period. Tubular changes were deemed to be of an adverse nature.

Remarks – Results
Toxicologically relevant changes occurred in the kidneys in both sexes of the high-dose group.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 200 mg/kg bw/day in this study, based on histological changes in the kidney in the high dose group (1000 mg/kg bw/day).

TEST FACILITY Harlan Laboratories Ltd (2009g)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Test 1: plate incorporation
Test 2: pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2 uvrA

Metabolic Activation System S9 fraction Phenobarbital/β-naphthoflavone induced rat liver

Concentration Range in Main Test
a) With metabolic activation: 33-5000 µg/plate.
b) Without metabolic activation: 33-5000 µg/plate.

Vehicle Deionised Water

Remarks - Method No significant protocol deviations.
In the pre-experiment, the concentration range of the test item was 3-5000 µg/plate. TA100 & WP2 uvrA experiments were repeated in Test 1 due to contamination. The pre-experiment is reported as Test 1. Since no toxic effects were observed at up to 5000 µg/plate, this concentration was chosen as maximal concentration. In Test 2, concentration used were 33, 100, 333, 1000, 2500 & 5000 µg/plate.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	Not performed	Not performed	Negative
Test 2	Not performed	>5000	>5000	Negative
Present				
Test 1	>5000	Not performed	Not performed	Negative
Test 2	Not performed	>5000	>5000	Negative

Remarks - Results No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the notified chemical at any dose level, neither in the presence nor absence of metabolic activation.
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 (2008l)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (>75%)

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	Chinese Hamster
Cell Type/Cell Line	V79
Metabolic Activation System	S9 fraction Phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Deionised Water
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (hrs)*	*Harvest Time (hrs)*
Absent			
Test 1	19.5, 39.1, 78.1, 156.3*, 312.5*, 625*, 1250, 2500, 5000	4	18
Test 2	3.9, 7.8, 15.6, 31.3*, 62.5*, 125*, 250, 500, 1000	18	18
Present			
Test 1	19.5, 39.1, 78.1*, 156.3*, 312.5*, 625, 1250, 2500, 5000	4	18
Test 2	75, 100, 200, 300, 350*, 400*, 450*	4	18

*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				
Test 1	Not performed	>625.0	>625.0	Negative
Test 2	Not performed	>500.0	>500.0	Negative
Present				
Test 1	Not performed	>312.5	>312.5	Negative
Test 2	Not performed	>450.0	>450.0	Negative

Remarks - Results Under the experimental conditions reported, the test item did not induce chromosome aberrations as determined by the chromosome aberration test in V79 cells (Chinese hamster cell line) *in vitro*.

Ethylmethane sulfonate (EMS) and cyclophosphamide (CPA) were used as positive controls and showed distinct increases in cells with chromosomal aberrations.

CONCLUSION The notified chemical was not clastogenic to Chinese Hamster V79 Cells treated in vitro under the conditions of the test.

TEST FACILITY Harlan Laboratories Ltd (2009g)

B.9. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	Ethanol:Water (30:70)
Remarks - Method	No significant protocol deviations. The highest test item concentration, which can be technically used was a 20% solution in ethanol/deionised water (30+70). The test item

concentrations in the main study were chosen based on the preliminary test.
Four animals were used per group.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	461.4	--
5	604.3	1.31
10	759.7	1.65
20	705.9	1.53
Positive Control (α-Hexylcinnamaldehyde)		
0 (vehicle control)	665.9	--
5%	1188.2	1.78
10%	1225.8	1.84
25%	3244.9	4.87

Remarks - Results

The animals did not show any clinical signs during the course of the study and no cases of mortality were observed.
The test item did not result in 3-fold or greater increase in the stimulation index with any of the test item concentration.
Positive control was conducted with α-Hexylcinnamaldehyde previously in February 2008. The positive control induced a stimulation index of >3, thereby confirming the suitability of the test system.

CONCLUSION

There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical up to 20% concentration, under the conditions of the test.

TEST FACILITY

RCC Ltd (2008m)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Aerobic activated sludge from domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption measured as pressure drop by electrode type manometer
Remarks - Method	No significant deviations from the test guidelines were reported

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation**	*Day*	*% Degradation**
5	0	5	80.5
10	2	10	90.5
14	0	14	93.5
20	4	20	95.0
28	4	28	97.0

*Based on mean of 2 replicates

Remarks - Results	The results tabulated above are based on COD. There was no net oxygen consumption or carbon dioxide evolution in the abiotic control. The test substance had no apparent toxic or inhibitory effect on the inoculum, based on the results from the toxicity control. All validity criteria for the test were satisfied. The test substance was found to be not biodegradable under the test conditions.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	RCC Ltd (2008n)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302C Inherent Biodegradability: Modified MITI Test (II)
Inoculum	Aerobic activated sludge from domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption measured as pressure drop by electrode type manometer
Remarks – Method	The following modifications were made: Activated sludge from only one source was used, the holding period for the activated sludge was maximum of 7 days, the test was conducted at 22°C and only BOD was measured. No other deviations to protocol were reported. The test item was test at 80 mg/L. Sodium benzoate was used at the reference substance at 100 mg/L concentration. Inoculum control, procedural control and toxicity control were also conducted.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	-	1	-
3	0	3	65
7	0	7	78
10	0	10	86
14	0	14	91
21	0	21	92
28	0	28	93

Remarks – Results: The toxicity control attained 68% degradation after 14 days confirming that the test substance was not inhibitory to activated sludge bacteria. The reference substance showed the expected degradation profile, confirming the suitability of the inoculums and the validity of the test conditions. The deviations to protocol are not expected to significantly alter the outcome of the test.

CONCLUSION: The notified chemical is not inherently biodegradable

TEST FACILITY: RCC Ltd (2008o)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Semi- static
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – Semi- static

Species: Zebra fish (*Brachydanio rerio*)
Exposure Period: 96 hours
Auxiliary Solvent: None
Water Hardness: 125 mg $CaCO_3$/L
Analytical Monitoring: HPLC
Remarks – Method: Following a range-finding test a limit test was conducted in accordance with the guidelines above and with GLP compliance. No deviations to protocol were reported.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0
100	98-104	7	0	0	0	0	0

LC50: > 100 mg/L at 96 hours (based on nominal concentration)
NOEC: > 100 mg/L at 96 hours (based on nominal concentration)
Remarks – Results: The analytically determined mean test item concentration in the test medium at the start and the end of the test medium renewal periods of 24 hours was 98 and 104% of the nominal value, respectively. Under the test conditions, the test substance was stable during the test period of 96 h. Therefore, all reported results are related to the nominal concentration. All validity criteria for the test were satisfied.

CONCLUSION: The notified chemical is not harmful to fish

TEST FACILITY: Harlan Laboratories (2009h)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Static EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Liquid chromatography
Remarks - Method	Following a range-finding test a definitive test was conducted in accordance with the guidelines above and with GLP compliance. No deviations to protocol were reported.

RESULTS

Nominal Concentration mg/L	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	20	0	0
4.5	20	0	0
10	20	0	0
22	20	0	0
46	20	0	0
100	20	0	0

LC50	> 100 mg/L at 48 hours (based on nominal concentration)
NOEC	≥ 100 mg/L at 48 hours (based on nominal concentration)
Remarks - Results	The analytically determined test substance concentration in the test medium in the highest nominal concentration was 115% of nominal value. Under the test conditions, the test substance was stable during the test period of 48 h. Therefore, all reported results are related to the nominal concentration. All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates.
TEST FACILITY	RCC Ltd (2008p)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 1, 3.2, 10, 32, 100 mg/L Actual: 0.52 (half of LOQ), 2.1, 7.8, 29, 120 mg/L (52%, 66%, 78%, 89%, 120% of nominal)
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Liquid chromatography
Remarks - Method	As the test substance is a dye resulting in coloured test media, the light intensity was reduced. The protocol was modified by running two parallel experiments. Experiment A used the standard algal toxicity test protocol. Experiment B used light source which was filtered through a solution of the test substance prior to exposure of the algae in a separate container in order to control for the effects of the changes in the light.

RESULTS

Experiment	*Biomass*		*Growth*	
	E_bC50 (95% CI) mg/L at 72 h	*NOEC mg/L*	*E_rC50 (95% CI) mg/L at 72 h*	*NOEC mg/L*
A	23 (9.7-68)	0.52	>120 (CI n.d.)	7.8
B	63 (44-99)	11 (EC10)*	>120 (CI n.d.)	31 (EC10)*

CI: confidence interval, n.d.: could not be determined
*NOECs not reported for Experiment B, EC10 value reported.

Remarks - Results

At the end of the test, the analytically determined test item concentrations in the analysed test media varied in the range from 52 to 117% of the nominal value. Therefore, the reported biological results are based on the geometric means of the concentrations measured at the start and end of the test.

The difference in the growth rates between Experiments A and B exceeded the criteria of 10% for the two highest concentration tested (29 and 120 mg/L). Thus, a toxic effect of the test item could not be excluded and the reduced growth rate could be entirely explained by reduced light intensity. All test validity criteria were satisfied.

CONCLUSION

The notified chemical is not harmful to algae (based on E_rC50)

TEST FACILITY

RCC Ltd (2008q)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum: Activated sludge from domestic wastewater treatment plant
Exposure Period: 3 hours
Concentration Range: Nominal: 1000 mg/L
Actual: Not reported
Remarks – Method: No significant protocol deviations were reported

RESULTS

IC50: > 1000 mg/L
NOEC: 1000 mg/L
Remarks – Results: The IC50 for the reference substance (3,5 dichlorophenol) was calculated to be 18 mg/L which was within the guideline range, confirming the suitability of activated sludge used.

CONCLUSION

The notified chemical is not inhibitory to microbial respiration

TEST FACILITY

RCC Ltd (2008r)

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