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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Brown DER 8589 in NOVACRON® BLACK LS-N-01

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 the Environment and Water Resources.

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 334-336 Illawarra Road, Marrickville NSW 2204.

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FULL PUBLIC REPORT

Brown DER 8589 in NOVACRON® BLACK LS-N-01

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Huntsman Corporation Australia Pty Ltd (ABN: 67 083 984 187)
454-456 Somerville Road
West Footscray VIC 3012

AND

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)
No details are claimed exempt from publication.
Chemical Name; Other Names; CAS Number; Molecular Formula; Structural Formula; Molecular Weight; Spectral Data; Purity; Identity and % Weight of Toxic or Hazardous Impurities; Identity and % Weight of Non-Hazardous Impurities; Identity and % Weight of Additives/Adjuvants; Import Volume; Identity of Customer Sites

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Europe: Notified 2000; USA: Notified 2000; Switzerland: Notified 2001; Korea: Notified 2001; China: Notified 2004

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
NOVACRON® BLACK LS-N-01 (containing 20-30% notified chemical)

OTHER NAME(S)
BROWN DER 8589
FAT 40576
FAT 40576/A
FAT 40'576/A

MOLECULAR WEIGHT
>1000 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, LC, UV spectra were provided. Elemental analysis, water content and the content of hexane soluble unsulfonated primary aromatic amines were also determined.

3. COMPOSITION

DEGREE OF PURITY 80-95%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Dark brown/black powder

Property	Value	Data Source/Justification
Melting Point	> 400°C	Measured
Boiling Point	~860°C	Calculated
Density	1610kg/m^3 at 20.7°C	Measured
Vapour Pressure	2.24 x 10^{-33} kPa at 25°C	Calculated
Water Solubility	>200 g/L at 20°C	Measured (determined visually)
Hydrolysis as a Function of pH	$t_{½}$ > 1 year at pH 4, 25°C $t_{½}$ = 129 h at pH 7, 25°C $t_{½}$ = 3.7 h at pH 7, 50°C $t_{½}$ < 24 h at pH 9, 25°C	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} = -5.4 at 20°C	Estimated
Surface Tension	72.8 mN/m at 20.0±0.4°C	Measured
Adsorption/Desorption	K_{OC} ≥ 546 mL/g at 20°C.	Measured
Dissociation Constant	pKa << -7 to 3.9	Calculated
Particle Size	Inhalable fraction (<100 μm): ~7.31% <125 μm Respirable fraction (<10 μm): 0%	Measured
Flash Point	Not determined	
Flammability	Not highly flammable	Measured
Autoignition Temperature	~283°C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	The notified substance has a negative oxygen balance.

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

Reactivity
The notified chemical is considered to be non-oxidizing and is not capable of causing fire or enhancing the risk of fire when in contact with combustible material. No incompatible substances have been identified with the notified substance. The notified chemical is stable at room temperature and does not evolve any flammable gases in contact with water or humid air. The notified chemical is not considered to be an explosive as it is not thermally sensitive, not shock sensitive and not sensitive to friction. Conditions have not been identified which would contribute to the instability of the product; the product is considered to be stable under normal conditions of use. Typical decomposition products are oxides of carbon, oxides of nitrogen and oxides of sulfur. No other toxic gases/vapours have been identified.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported by sea as a 20 – 30% component of NOVACRON® BLACK LS-N-01 as granules or dedusted powder. NOVACRON® BLACK LS-N-01 is a mixture that contains azo dyes, including the azo dye notified as STD/1272, and one other.

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

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

PORT OF ENTRY
Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
Chemiplas Australia Pty Ltd

TRANSPORTATION AND PACKAGING
NOVACRON® BLACK LS-N-01 will be transported into Australia by ship in 25 kg fibreboard cartons, with inner PE liner. The product is transported by road from the dockside to the Chemiplas warehouse in Laverton North (Victoria), where it is stored until required for despatch to customers.

USE
The notified chemical is a reactive azo dye, used for the colouration of cotton and cotton-blend fibres (blended with synthetic fibres such as polyester, polyamide, elastane, or possibly blended with wool or silk). The notified chemical reacts with hydroxyl groups in cellulosic fibres during fixation to form covalent bonds with the fibre, such that high levels of wash and colourfastness are generated. The notified chemical will be used to dye domestic textiles that are intended for apparel, sheeting and other uses.

OPERATION DESCRIPTION
At customer dyehouses, the product containing the notified chemical (20-30% concentration) will be manually weighed into a dispensary and subsequently manually transferred to the blending vessel, both of which are equipped with local exhaust ventilation. Mixing and blending of dye components takes place in closed mixing vessels. Following blending, the notified chemical is present at <1% in the final textile dye solution. The textile dye solution will be applied to fabric at elevated temperatures.

The dyeing process is mainly automated once the dye is in solution (<1% notified chemical), with the cloth driven by mechanical rollers through the dyeing and washing steps in a mainly enclosed system. At the completion of the rinsing phase the wet fabric will be removed from the dyeing equipment by mechanical means, assisted by operators. Wet fabric will be carried on trolleys covered with plastic to prevent contamination and transported to either a centrifuge or mangle to remove excess water. The damp fabric will then be fed onto a pin frame for drying.

Cleaning and maintenance operations involve flushing the holding and mixing tanks with water.

For cotton fabrics, exhaustion is achieved by adding electrolyte (NaCl or Na_2SO_4) and fixation will be achieved by the addition of alkali (normally NaOH or $NaCO_3$, or both) at temperatures of 60-70ºC. The concentrations used of these additives will be dependent on the amount of dye used and the water to fabric ratio. Exhaustion rates of ~90-98% are expected, of which 80-95% will be bound to cellulosic fibres (fixed) and the remainder will react with water and alkali (allowing for variations between mills). The fabric will then be washed free of unfixed dye in a series of wash-off baths (five to eight) at temperatures ranging from 30ºC to 95ºC. At completion of this process, there is expected to be <1% of the hydrolysed dye on the fabric.

The dye containing the notified chemical has low affinity for polyester and elastane. For polyamide fabrics, the hydrolysed notified chemical dyes the fibre, with the majority expected to remain fixed. When used with wool or silk, some of the dye is fixed, and some hydrolysed dye (30% of the original amount) will be removed by rinsing and soaping processes.

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/day)*	*Exposure Frequency (days/year)*
Transport drivers	1 – 5	20 – 30 min/day	50 – 100
Warehouse operators	3 – 6	20 min/day	100 – 150
Batch area operators	4 – 8	20 min/day	180 – 240
Dye machine operators	4 – 8	60 min/day	180 – 240

EXPOSURE DETAILS
Transport and warehouse workers are not expected to be directly exposed to the notified chemical, except in the event of a spill or leak.

Dye machine operators are likely to be exposed to the imported product (containing 20-30% notified chemical) by inhalation, ingestion, skin contact or eye contact while manually weighing out the dye and adding it to mixing vessels to form the dye solution. The imported product is supplied as granules or dedusted powder, reducing the potential for inhalation exposure. Dye machine operators will wear personal protective equipment (PPE) that includes gloves, coveralls, goggles and a dust mask/breathing apparatus. Local and general exhaust ventilation will be present where the solid product containing the notified chemical is handled. EASE modelling of the weighing process was performed to estimate dermal/inhalation exposure of workers to the notified chemical. The following assumptions were used for these estimates: direct handling, non-dispersive use (only used by workers with knowledge of the processes and use of controls), and intermittent contact level (assumed to be 2-10 events per day). The predicted dermal exposure to the notified chemical is 0.03-0.3 mg/cm^2/day. This is equivalent to 0.7-7 mg/kg bw/day, based on assumptions outlined by the European Commission (EC, 2003).

The dyeing process is mainly automated once the dye is in solution (<1% notified chemical), with the cloth driven by mechanical rollers through the dyeing and washing steps. The system is mainly enclosed to prevent splashes and spills. Some manual handling of wet cloth will occur during some steps of the process. Therefore, dermal and possibly ocular exposure to the dye solution is possible, though likely to be low (also confirmed by EASE modelling).

Cleaning and maintenance of the machines will be performed by the machine operators. During this process, inhalation, dermal and ocular exposures are possible, but a significant proportion of the residue in the machines is expected to be hydrolysed dye. Workers involved with cleaning of machines will wear an organic vapour cartridge respirator, gloves, safety goggles and overalls.

6.1.2. Public exposure
The imported product containing the notified chemical will be available only to industrial end-users. Fabrics that are dyed with the notified chemical may be used for apparel and sheeting (and other uses), with which members of the public would be expected to make frequent dermal exposure. However, the notified chemical belongs to a class of dyes that reacts with fabrics, becoming covalently bound during the exhaustion and fixation steps of the dyeing process (Smith, 1993). Less than 1% of the free, hydrolysed dye is expected to remain in dyed fabrics, and this unfixed material is likely to be removed upon washing of fabrics by consumers. Therefore, public exposure to the notified chemical is not likely 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	Low toxicity oral LD50 >2000 mg/kg bw
Rat, acute dermal toxicity	Low toxicity LD50 >2000 mg/kg bw
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Severely irritating
Guinea pig, skin sensitisation – adjuvant test	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOEL = 50 mg/kg bw/day NOAEL = 200 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	Non mutagenic

Genotoxicity – in vitro mammalian chromosome aberration test	Genotoxic
Genotoxicity – in vivo mammalian erythrocyte micronucleus test	Non-genotoxic

Toxicokinetics, metabolism and distribution
Based on its molecular weight (>500) and $logP_{ow}$ (<0), the notified chemical is not expected to be readily absorbed transdermally or from the gastrointestinal tract. Nonetheless, the effects observed in the mouse micronucleus and 28-day repeat oral dose toxicity studies suggest that it will at least be partly absorbed from the gastrointestinal tract. In addition, impurities and azo reduction species produced by intestinal bacteria may be more readily absorbed than the notified chemical (Chung, 1983).

Once absorbed, the notified chemical is likely to be metabolised to some extent, with one mechanism being reduction of the azo linkage to form aromatic amines (see below).

Ultimately, the metabolites of azo dyes are excreted in the urine and bile (Brown & DeVito 1993, referenced in Øllgaard *et al* 1998 and Fuji 2007). Sulfonation of azo dyes appears to decrease their toxicity by enhancing their urinary excretion, and that of their metabolites. Orange coloured urine observed in the mouse micronucleus study is likely to be indicative of the urinary excretion of metabolites of the notified chemical.

Acute and repeat dose (sub-acute) toxicity
The notified chemical was found to be of low acute toxicity by the oral and dermal route (LD50 > 2000 mg/kg bw).

In the 28-day repeat dose oral toxicity study, abnormal laboratory findings and effects in the liver, spleen and kidneys were observed in animals treated with 1000 mg/kg bw/day. When treated with 200 mg/kg/day, marginal reticulocytosis and significantly increased urine pH were seen in females. Based on these results, the No Observed Effect Level (NOEL) was established as 50 mg/kg bw/day and the No Observed Adverse Effect Level (NOAEL) as 200 mg/kg bw/day. Such effects do not warrant classification in accordance with the Approved Criteria (NOHSC, 2004).

The notified chemical is a solid that contains particles in the inhalable range. Given its high water solubility, accidental inhalation of the particles would likely result in its dissolution and ultimately ingestion.

Irritation and sensitisation
The notified chemical was found to be non-irritating to the skin, based on a rabbit skin irritation study. The notified chemical is considered to be severely irritating to the eyes, based on the persistence of mild irritation effects until the end of the study period (24 days) in two animals (slight reddening of the nictitating membrane in one animal, and slight reddening of the sclera in another animal).

Reactive dyes have been reported as the causative agents in sensitisation of the public to textiles (Estlander, 1988; Manzini et al, 1996). However, the notified chemical was not found to be sensitising to the skin of guinea pigs in a maximisation study. Also, relatively few sulfonated azo dyes have been demonstrated to be skin sensitisers (Øllgaard *et al* 1998). Therefore, the notified chemical is unlikely to cause skin sensitisation in exposed humans.

Reactive dyes that are formulated as respirable particles have also caused respiratory sensitisation in workers handling them for a period of years, for example during weighing procedures (Alanko *et al,* 1978; Docker *et al,* 1987; Topping *et al*, 1989). As such, the notified chemical may induce respiratory sensitisation, however, further testing is required to confirm this possibility.

Mutagenicity/carcinogenicity
The notified chemical was not mutagenic to bacteria *in vitro*, under the conditions of the Ames test used. The Ames test performed was a standard test, according to OECD Test Guideline 471. However, the test guideline strongly recommends the use of alternative procedures for the detection of mutagenicity of azo dyes, as it is recognised that the standard procedure may not be sufficiently sensitive for these chemicals. Modified tests, such as that of Prival and Mitchell, utilise a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out (Prival and Mitchell, 1982). This modified test is thought to yield a greater detection of mutagenic azo dyes. As such, mutagenicity of the notified chemical cannot be ruled out on

the basis of the studies performed.

The notified chemical was clastogenic to cultured mammalian cells, in the presence of metabolic activation. It was not found to be clastogenic in an *in vivo* mouse bone marrow micronucleus assay. As there was no effect on the PCE/NCE ratio in this assay, some doubt exists as to whether the notified chemical reached the bone marrow. However, it should be noted that there were some significant haematological changes, particularly in female animals of the 28 day repeat dose study. The available results are not sufficiently conclusive to allay all concern for mutagenicity and/or carcinogenicity in exposed humans.

Azo dyes as a class are a concern for their potential induction of mutagenicity and carcinogenicity (Combes and Haveland-Smith, 1982). In general, the degree of correlation between mutagenicity study results and the carcinogenicity of azo dyes as a class is poor, likely due to their complex metabolism *in vivo* (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998). Mutagenicity from azo dyes may result from the intact chemical or from amines formed by reductive metabolism or degradation. Reductive cleavage or degradation into component aromatic amines is a major mechanism leading to the genotoxicity of azo dyes (SCCNFP, 2002). The aromatic amines that arise from the azo reduction and cleavage of azo dyes are thought to be activated as mutagens through their *N*-oxidation by cytochrome P450 isozymes. This mechanism is thought to contribute to the carcinogenicity of many azo dyes, and as a result, azo dyes should be assessed for toxicity and classified similarly to their component amines (DFG, 1988, quoted in Golka *et al*, 2004). The notified chemical is not expected to be reductively cleaved to release any of the restricted aromatic amines specified in either the Appendix to EC Directive 76/769/EEC (EC 2004) or the annexes of EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into a number of arylamine species, some of which may be mutagenic/carcinogenic.

The notified chemical also fits into the US EPA category of concern for vinyl sulfones (US EPA, 2002), due to the presence of vinyl sulfone groups and vinyl sulfone precursors. Such groups are considered to be of concern for their potential oncogenicity and mutagenicity. During its use in dyeing textiles, the reactive vinyl sulfone groups of any of the notified chemical that does not react with the fabric will be hydrolysed to form hydroxyl species (Smith, 1993). These hydrolysis products are expected to be of lower concern for mutagenicity than the notified chemical, due to the loss of the reactive functional groups.

Overall, these results do not rule out the notified chemical as a possible mutagen or carcinogen. However, the weight of evidence does not meet the criteria for classification. Carcinogenicity would need to be determined by further testing.

Based on the irreversible effects observed in the eye irritation study, the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

R41 - Risk of serious damage to eyes

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Transport and warehouse workers would only be exposed to the notified chemical in the event of a spill or leak. The risk arising from occupational exposure to the notified chemical can be divided into exposure to the powdered solid, the dye solution, and to the dyed cloth.

Powdered solid notified chemical

Batch area operators may be exposed to solid product containing 20-30% notified chemical while weighing out the dye and during addition of the dye powder to solution. Dermal/inhalation/ocular exposure of workers is estimated to be 0.7-7 mg/kg bw/day. A dermal NOEL/NOAEL was not determined, however, a NOEL of 50 mg/kg bw/day and NOAEL of 200 mg/kg bw/day was established in a 28 day oral study in the rat. Use of the NOEL results in a margin of exposure (MOE) of 71, whilst use of the NOAEL results in a MOE of 286. The MOE suggests that the risk is not acceptable if workers are exposed to the notified chemical repeatedly. The health risk of the notified chemical is likely to be significant upon inhalation, given its potential for mutagenicity and/or respiratory sensitisation (in the absence of sufficient negative test data). In addition, the health risk arising from ocular exposure is also significant, given the persistent irritant effects observed in the eye irritation study.

It should be noted that the current practice is for workers to manually weigh the powdered notified chemical

and add it to dye solution under conditions where engineering and other measures are in place to limit occupational exposure to the notified chemical, including the use of dedusted powder formulations, local exhaust ventilation, and appropriate PPE such as dust masks, goggles, gloves and overalls. However, it is recommended that these procedures be modified such that automated processes to exclude manual handling of the notified chemical be used. Under such conditions it is expected that the risk to workers from exposure to the notified chemical would be considered acceptable.

Dye solution
Exposure of dye machine operators to the notified chemical in solution is unlikely during the majority of the dyeing process as the machinery is largely enclosed and mostly automated. These workers may experience predominantly dermal and ocular exposure to dye solution (<1%) – both during the manual handling stages of the dyeing process (notified chemical and hydrolysed dye in solution) and during the cleaning of the dye equipment (expected to be mostly hydrolysed dye). The health risk from ocular exposure is expected to be significant, but dermal exposure is expected to present a lower risk (as described above). The wet fabric will be wrapped in plastic during handling, and workers will wear gloves, overalls and goggles to prevent incidental exposure. These measures are expected to significantly reduce worker exposure to the notified chemical in dye solutions. During dyeing processes, dye machine workers will require eye and skin protection to avoid exposure to splashes and accidental exposure.

Dyed cloth
After fixation of the dye to the textile and washing off of unfixed dye, the remainder of the notified chemical will be covalently linked to the fabric and thus expected to be unavailable to cause significant exposure.

6.3.2. Public health
The product containing the notified chemical will be available only to industrial end users. The dyed cloth may be used for apparel, sheeting and other uses. The notified chemical is covalently linked to the cloth after the dyeing process. Colourfastness test results indicate a high degree of fastness of the notified chemical to dyed textiles. Therefore, there will be significant exposure to the dyed product, but exposure to the notified chemical is not likely to be significant.

If any residual, unfixed dye remains on the dyed fabric (after industrial fixation and washing), it is likely to be a hydrolysed species of lower concern for mutagenicity (see above). Residual dye is likely to be removed from fabrics during domestic washing.

Should any azo reduction occur on the fabric (for example through the action of bacterial skin flora or photolysis), this would not be expected to liberate arylamine species that are of concern for mutagenicity.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
No manufacture or reprocessing of the notified chemical will take place. Therefore, there will be no environmental exposure associated with this process in Australia.

Release to the environment may occur in the unlikely event of an accident during transport or storage.

RELEASE OF CHEMICAL FROM USE
Nearly all of the dye containing the notified chemical will be removed from the import container liners by shaking. Less than 0.5% of the chemical per annum will remain as residue. Given the potential for recovery and reuse and the high unit cost of the dyestuff the waste due to spills is expected to be less than 0.5% 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 notified chemical is not expected at this stage.

The dye will be used to colour cotton and cotton blend textiles by exhaust dyeing. Fixation is performed at 60-70°C with the fixation rate expected to be ~85%. The notified chemical adsorbed to the fabric will not be released to the environment. The rinsate, generated via fabric rinsing, contains ~15% of the import volume of the notified chemical. This will represent a major route of environmental exposure. The rinsate will be

discharged to the dyehouse effluent system, where flocculation will be used to remove the dyestuff. The treated effluent containing minor traces of the notified chemical will be disposed of to the sewer, where the sludge/solids will be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL
Any solid wastes generated in the dyehouses, including container residues, will either go to landfill or be incinerated. Incineration of the notified chemical will produce water, oxides of carbon, nitrogen and sulphur. Incineration is the preferred method of disposal due to the ready water solubility of the notified chemical.

Once bound to the fabric the notified chemical is expected to remain fixed throughout the useful life of the fabric. Hence it will share the fate of fabric and be either disposed of in landfill or incinerated.

7.1.2 Environmental fate

Three tests relating to biodegradability were presented. The results indicate that the notified chemical is poorly biodegradable. For the details of the environmental fate studies please 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 dyehouses located in two general locations, one metropolitan based and the other country based. The Predicted Environmental Concentration (PEC) is estimated below:

Calculation Factor	Country Dyehouse (Low volume STP discharge)	City Dyehouse (High volume STP discharge)
Typical use of product expected per day	50.000 kg	50.000 kg
Amount of notified chemical	11.100 kg	11.100 kg
Concentration in wastewater (fixation rate 85%)	1.665 kg	1.665 kg
Typical daily volume of dye wash-water effluent	0.400 ML	0.400 ML
Concentration in dye wash water	4.1625 mg/L	4.1625 mg/L
Typical daily volume of dye house wash-water effluent	2.900 ML	2.900 ML
Concentration in dyehouse effluent	574.138 µg/L	574.138 µg/L
Dilution factor in sewage treatment plant	1:10	1:100
Concentration in effluent from sewage treatment plant	57.414 µg/L	5.741 µg/L
Predicted environmental concentrations (PECs) in receiving waters		
PEC Ocean (Dilution Factor 1:10)	5.741 µg/L	0.574 µg/L
PEC River (Dilution Factor 1:1)	57.414 µg/L	5.741 µg/L

These calculations assume that no dye is removed in treatment of the different waste effluents and represent the worst case scenario for dyehouses.

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	LC50 >100 mg/L	Not harmful
Daphnia Toxicity	E_iC50 >100 mg/L	Not harmful
Algal Toxicity	E_rC50 53* mg/L	Effect due to reduction in light only

Inhibition of Bacterial Respiration	E_iC50 >1000 mg/L	No toxic effect

*The algal toxicity test demonstrated that growth reduction was attributable to the reduction in light intensity from the coloured test substance, rather than due to direct toxicity effects. Thus, a real toxic effect of the notified chemical on the growth of algae was excluded up to the highest test concentration of 110 mg/L.

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
E_iC50 (Alga)	53	mg/L
Assessment Factor	100	
PNEC:	530	µg/L

7.3. Environmental risk assessment

Using the PEC and PNEC values derived above, the following Risk Quotients have been calculated.

Risk Assessment (Country Dyehouse)	PEC µg/L	PNEC µg/L	Q
Q - River:	57.414	530	0.108
Q - Ocean:	5.741	530	0.011

Risk Assessment (City Dyehouse)	PEC µg/L	PNEC µg/L	Q
Q - River:	5.741	530	0.011
Q - Ocean:	0.574	530	0.001

These calculations show that the exposure to fish, daphnia, algae and waste water treatment bacteria is at levels unlikely to cause any significant effect. At higher release rates, there is still unlikely to be any significant effect on these species. Once in the aquatic environment, the notified chemical is expected to swiftly dilute to undetectable concentrations, and undergo biotic and abiotic degradation and an adequate safety factor exists for use in country locations.

With a fixation rate of ~ 85%, ~15% of the imported volume of the notified chemical will enter the sewer in the rinsate from fabric rinsing following dyeing. The high water solubility and low Kow value indicate that the notified substance is not likely to adsorb to sludge. However, effluent flocculation is expected to effectively precipitate the notified chemical. The solids containing the notified chemical would be disposed of through incineration or as chemical waste along with other solid waste (due to spills, leaks and container residues) from the dyehouse. Incineration is the preferred option because of the high water solubility and potential mobility of the material. Incineration of the sludge or waste containing the notified chemical will produce oxides of carbon, oxides of nitrogen, oxides of sulphur, and other main elements in the ash.

If the dye containing the notified chemical is disposed of to landfill the residues may be mobile. Although the notified chemical cannot be considered as readily biodegradable it would degrade very slowly via biotic and abiotic processes. Disposal to landfill, if any, will be as chemical waste, therefore, the risk of leaching to the water table is significantly reduced. The fate of the dye and the notified chemical bound to the fabrics would be the same as that of the fabrics. The fabrics may be disposed of to landfill, where the notified chemical would remain inert.

The potential for bioaccumulation is low due to the very high water solubility, large molecular weight and the low lipid solubility and log Kow of the notified chemical.

Therefore, based on the proposed use pattern and volume, the notified chemical is not expected to pose an unacceptable risk to the aquatic environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification and labelling details are:

- 5 ≤ conc < 10%: Xi: R36 Irritating to eyes.
- ≥10%: Xi: R41 Risk of serious damage to eyes.

Human health risk assessment
The risk to workers from handling of the notified chemical is only considered to be acceptable if further measures are taken to automate the processes for weighing and transferring of the notified chemical so as to exclude manual handling. Respiratory protection and eye protection are required during handling of the powdered notified chemical. Skin and eye protection are required during dyeing operations.

When used in the proposed manner the risk to the public is considered to be acceptable, as the notified chemical will be covalently bound to dyed fabrics.

Environmental risk assessment
The notified chemical is not considered to pose a risk to the environment based on its reported use pattern.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified chemical:
  - R41 Risk of serious damage to eyes.

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - 5 ≤ conc < 10%: Xi: R36 Irritating to eyes.
  - ≥10%: Xi: R41 Risk of serious damage to eyes.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - Local exhaust ventilation where there is potential exposure to the solid product
  - Isolation controls during weighing and transfer operations of the solid product

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid dust formation
  - Avoid inhalation of dust
  - Avoid exposure to eyes and skin
  - Clean spills immediately, taking care to avoid dust formation

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical in dye solutions:
  - Avoid exposure to eyes and skin
  - Clean spills immediately

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Dust mask or respirators capable of removing all product particles
  - Gloves, overalls and goggles or face-shield

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical in dye solutions:
  - Gloves, coveralls and goggles or face-shield

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 by incineration or to sealed 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
- additional data becomes available on the genotoxicity or 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 reactive textile dye, or is likely to change significantly;
- the amount of chemical being introduced has increased from 5 tonnes, or is likely to increase, significantly;
- if 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 MSDS of products 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 > 400ºC

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Capillary method
Test Facility Ciba (2000d)

Boiling Point ~ 860ºC

Method Calculated using Meissner's method (Lyman 1990).
Test Facility RCC (2000b)

Density 1610 kg/m^3 at 20.7 ± 0.1ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Gas comparison pycnometer
Remarks Performed on the notified chemical with nominal purity of ~59%.
Test Facility RCC (2000c)

Vapour Pressure ~2.24 x 10^{-33} kPa at 25ºC

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Calculated using the Modified Watson Correlation (Lyman 1990).
Test Facility RCC (2000b)

Water Solubility >200 g/L at 20˚C

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Water solubility was determined visually. As the result exceeds the limit of 150 g/L, the flask method was not carried out.
Test Facility Ciba (2000e)

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)*	*t½*
4	25	>365 d
7	25	129 h
7	50	3.7 h
9	25	<24 h

Remarks Analysis was performed using HPLC.
Test Facility Ciba (2000f)

Partition Coefficient (n-octanol/water) log Pow = -5.4 at 20ºC

Method OECD 107 Partition Coefficient (n-octanol/water): Shake Flask Method
OECD TG 117 Partition Coefficient (n-octanol/water) , HPLC Method.
EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks Neither the flask method nor the HPLC-method were applicable for the determination of the partition coefficient. Therefore, the value was estimated from the solubility of test substance in water and n-octanol, being 476.09 and 2.07 g/L, respectively.

Test Facility RCC (2000d)

Surface Tension 72.8 mN/m at 20.0±0.4˚C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.

Remarks Concentration: 0.1%. The determination was accomplished by means of a tensiometer, using the ring method. The notified chemical should not be regarded as a surface active substance.

Test Facility RCC (2000e)

Adsorption/Desorption – screening test $K_{OC} \geq 546$ mL/g at 20°C.

Method OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method with HPLC analysis.
EC Directive 2001/59/EC C.18 Adsorption - Desorption Using a Batch Equilibrium Method with HPLC analysis.

Soil	*Soil Type USDA*	*Organic Carbon Content g/100 g dry soil*	*K' mL/g*	*K'_{OC} mL/g*	*K'_{OM} mL/g*
Speyer	Loamy Sand	2.19	24.9	1139	660
Sisseln	Sandy Clay Loam	1.71	9.3	546	317
Les Barges	Silt Loam	3.64	24.2	665	386

Remarks The screening test revealed a strong adsorption of test substance on all three soils tested. At a concentration of 5.41 mg/L (sum of four components) and soil samples of 5 g, the amount of test substance adsorbed was 83.3% for soil Speyer 65.1% for soil Sisseln and 82.9% for soil Les Barges. Correspondingly, from the quantity absorbed, 10.1%, 22.8% and 11.3% could be desorbed for the three soils, respectively.

The notified chemical can therefore, be regarded as having low mobility in all three soils.

Test Facility RCC (2000f)

Dissociation Constant pKa = << -7 to 3.9

Method OECD TG 112 Dissociation Constants in Water (calculated).

Remarks The notified chemical does not dissociate or protonate in the environmentally relevant pH range. The behaviour of the notified chemical in aqueous solutions is dominated by the strongly acidic groups. The molecule is negatively charged and is present in anionic form over the whole environmentally relevant pH range.

Test Facility RCC Ltd (2000g)

Particle Size

Method European Commission, Document ECB/TM/February 1996: "Particle Size Distribution Fibre Length and Diameter Distributions", Guidance Document

Range (μm)	*Mass (%)*		
	Sample 1	*Sample 2*	*Average*
< 75	2.94	0.10	1.52

< 125	11.79	2.82	7.31
< 250	44.67	13.07	28.87
< 500	95.9	77.38	86.64
< 1000	99.79	96.66	98.23
< 2000	100	100	100

Remarks Performed on notified chemical with nominal purity of ~59%.
Two samples of approximately 50 g each were sieved through mesh sizes of 75, 125, 250, 500, 1000, 2000 μm by constant shaking for 20 minutes. The quantities that passed through the sieves were weighed and the percentage of the total mass calculated.
Significant variability between the two batches was observed. This was considered to be due to tendency of the test substance to form agglomerates, even after sieving.
Test Facility RCC (2000h)

Flammability Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks Performed on notified chemical with nominal purity of ~59%.
In contact with the ignition source, the test substance glowed red, evolving no smoke but a strong smell. The glowing continued without contact of the ignition source. The velocity of the propagation was about 70 mm during 30 minutes. Therefore, the burning time of the test item over a distance of 200 mm was determined to be longer than 4 minutes, therefore, it was not necessary to perform the main test. A grey to black carbonised residue remained. The notified chemical is not flammable under the conditions of the test.
Test Facility RCC (2000i)

Autoignition Temperature ~283 °C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks Performed on notified chemical with nominal purity of ~59%.
The maximum temperature of the sample during the exothermic reaction was 565 °C. After measurement, the test substance was carbonised and black in colour.
Test Facility RCC (2000j)

Explosive Properties Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks Performed on notified chemical with nominal purity of ~59%.
The notified chemical is not considered to be an explosive as it is not thermally sensitive, not shock sensitive and not sensitive to friction.
Test Facility Institute of Safety & Security (2000)

Oxidizing Properties Not oxidising

Method Expert statement
Remarks The oxygen balance of the notified chemical is negative, meaning that there is a surplus of carbon atoms. Therefore, it is expected that the notified chemical is incapable of causing fire or enhance the risk of fire when in contact with combustible material.
Test Facility RCC (2000k)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical (~59% purity)

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
- Species/Strain: Rat/HanIbm: WIST (SPF)
- Vehicle: Bi-distilled water
- Remarks - Method: No significant protocol deviations

RESULTS

Number and Sex of Animals	*Dose mg/kg bw*	*Mortality*
3 F	2000	0
3 M	2000	0

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: Slight ataxia and tachypnea were observed in one female animal from 1 to 5 hours after the administration, and tachypnea persisted until test day 4. Slight emaciation (2 females) and rales (1 female) were additionally observed on test days 3 and 4. No clinical signs were observed in treated males.

  Some animals showed slight body weight loss at either one week or two weeks following treatment, but had recovered by the end of observation period.
- Effects in Organs: No macroscopic findings were observed at necropsy.

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

TEST FACILITY: RCC Ltd (2000l)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical (~59% purity)

METHOD: OECD TG 402 Acute Dermal Toxicity.
- Species/Strain: Rat/HanIbm: WIST (SPF)
- Vehicle: Bi-distilled water
- Type of dressing: Semi-occlusive.
- Remarks - Method: No significant protocol deviations

RESULTS

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

- LD50: >2000 mg/kg bw
- Signs of Toxicity – Local/Systemic: None
- Effects in Organs: None
- Remarks - Results: Red staining on the skin produced by the test item was observed in all animals on days 2 and 3.

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

TEST FACILITY RCC Ltd (2000m)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 (1 M, 2 F)
Vehicle Bi-distilled water
Observation Period 72 h
Type of Dressing Semi-occlusive.
Remarks - Method No significant protocol deviations

RESULTS

Remarks - Results Light red staining by the test item of the treated skin was observed in all animals after removal of the dressing and until the 48-hour observation in one female or during the whole study period in the other animals. The staining did not have an influence on the skin reaction reading.
No irritation effects were observed throughout the duration of the study.

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

TEST FACILITY RCC Ltd (2000n)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain Rabbit/New Zealand White
Number of Animals 3 (1 M, 2 F)
Observation Period 24 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[a]	0.3	0[b]	1	24 days	1
Conjunctiva: chemosis	0	0	0.3	1	48 hr	0
Conjunctiva: discharge	0	0	0	0	-	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
[a]Average calculated based on scores at 48 and 72 hours only, due to test material staining.
[b]Average calculated based on scores at 72 hours only, due to test material staining.

Remarks - Results The conjunctivae were not assessable in all animals at 1 hour, and the nictitating membrane up to 10 days, due to staining by the test material. In all animals, the nictitating membrane was slightly reddened at day 14, diminishing in two animals to be cleared by day 17 in one of them, and

day 21 in the other. In the remaining animal, this effect was still present at the end of the study (day 24). The conjunctivae, when visible, were slightly reddened in one animal from 24 hours to 14 days, and in another animal at 24 hours. A slight swelling of the conjunctivae and/or nictitating membrane was noted in all animals at the one hour reading, persisting in one animal until the 24 hour reading.

The sclera was not visible in all animals at the one hour reading due to the test item staining. When visible, the sclera was slightly reddened and disappeared by day 7 in one animal, day 24 in another, and was present at the end of the study in the remaining animal (different animal than the one in which redness of the conjunctivae persisted).

Red-grey staining of the treated eyes by the test item was observed in all animals from 1-hour to 14 days after treatment, before diminishing to clear by day 17 or 21. Grey to red-grey remnants of the test item in the eye or conjunctival sac were observed in two animals at the 1-hour reading.

CONCLUSION The notified chemical is severely irritating to the eye based on irreversible staining seen in the sclera and conjunctivae.

TEST FACILITY RCC Ltd (2000o)

B.5. Skin sensitisation

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 406 Skin Sensitisation – adjuvant test
Species/Strain Guinea pig/Ibm: GOHI; SPF-quality
PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal: 30%
topical: 25%
MAIN STUDY
Number of Animals Test Group: 10 M Control Group: 5 M
INDUCTION PHASE Induction Concentration:
intradermal: 30%
topical: 50%
Signs of Irritation It was not possible to determine whether erythema were present due to black staining of the skin by the test item. No oedema were observed.
CHALLENGE PHASE
1st challenge topical: 25%
Remarks - Method No significant protocol deviations

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY RCC Ltd (2000p)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain Rat/HanIbm: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

Dose mg/kg bw/day	*Number and Sex of Animals*	*Mortality*
0	5/sex	0 %
50	5/sex	0 %
200	5/sex	0 %
1000	5/sex	0 %
0 (recovery)	5/sex	0 %
1000 (recovery)	5/sex	0 %

Mortality and Time to Death

All animals survived until scheduled necropsy.

Clinical Observations

No test item-related toxic signs were noted during daily or weekly observations, or during functional observational battery. The mean fore- and hindlimb grip strength of test item-related animals compared favourably with those of the controls, and no test-item related effects upon locomotor activity were observed.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

Increased creatinine levels, decreased phosphorus and chloride levels, and decreased total bilirubin levels were found in females treated with 1000 mg/kg/day when compared with the controls. These differences were considered to be test item-related effects, possibly associated with renal changes and anaemia. However, these changes were reversible during the recovery period. All other differences to the control values were either without a clear dose-response relationship or within the 95% tolerance limits of the historical control data, and therefore considered to be incidental.

Haematology

Reticulocytosis, characterised by higher absolute and relative reticulocyte counts, as well as a significant shift in the ratio of high florescent reticulocytes from low fluorescent reticulocytes was noted in both sexes treated with 1000 mg/kg/day and, to a lesser extent (not statistically significant) in the females treated with 200 mg/kg/day. Increased methaemoglobin levels in both sexes and decreased haemoglobin levels in females treated with 1000 mg/kg/day were found when compared with the controls. These were considered to be test item-related changes indicative of compensated anaemia. These changes were reversible during recovery.

Urinalysis

Significant and dose-related increase of the urine pH was found in both sexes treated with 1000 mg/kg/day as well as with 200 mg/kg/day in females when compared with the controls. This difference was considered to be test item-related but it was reversible. The urinalysis parameters of the remaining animals were unaffected when compared with the controls.

Effects in Organs

Statistically significant higher absolute liver weights (but not relative liver weights) were found in females treated with 1000 mg/kg/day, but there was no associated dose-response relationship. All other absolute and relative organ weights compared favourably with those of the controls after four weeks treatment. The minor differences noted in post-recovery absolute and relative organ weights were considered not to be of toxicological significance.

Reddish discoloration of the kidneys was noted after recovery in the males previously treated with 1000 mg/kg/day. This finding was considered to be possible residual effect caused by the test item. No other macroscopic changes were attributed to treatment with the test item.

Morphologically, a minimal increase in the mean severity of microvesicular fatty vacuolation of the hepatocytes was noted in the livers of animals treated with 1000 mg/kg/day. There was an increase in the mean severity of extramedullary erythropoiesis in the spleens of animals treated with 1000 mg/kg/day. The changes

in the kidneys consisted of an increase in the mean severity of hyaline droplets in the tubular epithelium of males treated with 1000 mg/kg/day and an increased incidence and severity of lipofuscin deposition in females treated with 1000 mg/kg/day. With the exception of the lipofuscin deposition observed in the kidney of the females treated with 1000 mg/kg/day, all findings reverted after the two-week recovery period.

Remarks – Results

A number of effects including abnormal laboratory findings and target organs (liver, spleen, and kidneys) were found in animals treated with 1000 mg/kg/day. At 200 mg/kg/day, only marginal reticulocytosis and significant increased urine pH were seen in females. No treatment-related effects were found at 50 mg/kg/day.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 50 mg/kg bw/day and the No Observed Adverse Effect Level (NOAEL) as 200 mg/kg bw/day based on the results of this study.

TEST FACILITY RCC Ltd (2000q)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure/Pre incubation procedure

Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System S9 mix derived from Phenobarbital induced Wistar rat liver.

Concentration Range in Main Test a) With metabolic activation: 33, 100, 333, 1000, 2500, 5000 µg/plate
b) Without metabolic activation: 33, 100, 333, 1000, 2500, 5000 µg/plate

Vehicle Deionised water

Remarks - Method The positive controls used in the TA98, TA1537 and WP2 uvrA strains (without metabolic activation) were not those specifically recommended by the test guideline. In addition, 2-Aminoanthracene was used as the only positive control for assays with metabolic activation. The test guideline advises against this.

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	>5000	≥2500	Negative
Test 2**		>5000	≥2500	Negative
Present				
Test 1*	>5000	>5000	≥2500	Negative
Test 2**		>5000	≥2500	Negative

*Plate incorporation test
**Pre-incubation test

Remarks - Results Precipitation was observed at test concentrations of ≥2500 µg/plate. No cytotoxicity or increases in the number of revertant colonies was observed at any dose level in the presence or absence of metabolic activation.

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

TEST FACILITY RCC Ltd (2000r)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain Chinese hamster
Cell Type/Cell Line V79 cells
Metabolic Activation System S9 mix derived from Phenobarbital induced Wistar rat liver.
Vehicle Deionised water
Remarks - Method No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	75*, 150*, 300*, 600, 900, 1200	4 hr	18 hr
Test 2	25, 50, 100*, 150, 200*, 250*	18 hr	18 hr
Test 3	100, 150*, 200*, 250	28 hr	28 hr
Present			
Test 1	12.5, 25, 50*, 100*, 150*, 200	4 hr	18 hr
Test 2	12.5, 25, 50*, 100*, 150, 200*	4 hr	28 hr

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	~300	>1200	Negative
Test 2	>250	>250	Negative
Test 3	100-150	>250	Negative
Present			
Test 1	100-150	>200	Positive
Test 2	150-200	>200	Positive

Remarks - Results In the presence of S9 mix the aberration rates (Test 1: 8.0% and 7.5%, respectively; Test 2: 19.5%) were biologically relevant and statistically significant increases were observed after treatment with 100 and 150 µg/mL (Test 1) and with 200 µg/mL (Test 2) compared to the solvent control (Test 1: 1%; Test 2: 1.5%).

CONCLUSION The notified chemical was clastogenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY RCC Ltd (2000s)

B.8. Genotoxicity – in vivo

TEST SUBSTANCE Notified chemical (~59% purity)

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain Mouse/NMRI
Route of Administration Oral – gavage.
Vehicle Deionised water
Remarks – Method No significant protocol deviations.

Dose mg/kg bw	*Number and Sex of Animals*	*Sacrifice Time hours*
0	6/sex	24

500	6/sex	24
1000	6/sex	24
2000	6/sex	24
2000	6/sex	48
40 (positive control, CP)	6/sex	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity	>2000
Genotoxic Effects	>2000
Remarks - Results	The mean number of normochromatic erythrocytes (NCE) was not increased after treatment with the test material as compared to the mean value of NCEs of the vehicle control, indicating that the test material has no cytotoxic properties in the bone marrow. The mean values of micronuclei observed after treatment with the test material were below or near to the value of the vehicle control group.

CONCLUSION The notified chemical was not clastogenic under the conditions of this in vivo Mammalian Erythrocyte Micronucleus Test.

TEST FACILITY RCC Ltd (2000t)

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. EC Directive 92/69/EEC C.4-A Biodegradation: Determination of the " Ready" Biodegradability: Dissolved Organic Carbon (DOC) Die-Away Test
Inoculum	Activated sludge from a wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	HPLC
Remarks - Method	No significant protocol deviations.

RESULTS

Test substance		*D(+)-Glucose*	
Day	*% degradation*	*Day*	*% degradation*
3	3	3	93
7	4	7	96
10	2	10	96
14	4	14	98
21	4	21	99
28	3	28	98

Remarks - Results	All test validity criteria were satisfied. The test substance was found to be not biodegradable under the test conditions.
CONCLUSION	The notified chemical cannot be classed as ready biodegradable.
TEST FACILITY	Solvias AG (2000a)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD 302B Inherent Biodegradability: Zahn-Wellens/EMPA Test EC Directive 88/302/EEC C.9 Biodegradation: Zahn – Wellens Test
Inoculum	Aerobic activated sludge from a wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	HPLC
Remarks – Method	No significant protocol deviations

RESULTS

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
5	1	5	6
9	1	9	86
12	3	12	96
16	4	16	98
21	3	21	
23	4	23	
28	2	28	

Remarks – Results: The test substance was found to be not biodegradable under the test conditions.

CONCLUSION: The notified chemical cannot be classed as ready biodegradable.

TEST FACILITY: Solvias AG (2000b)

C.1.3. Biochemical/chemical oxygen demand (BOD/COD)

TEST SUBSTANCE: Notified chemical

METHOD: EC Directive 92/69/EEC C.5 Degradation: Biochemical Oxygen Demand
EC Directive 92/69/EEC C.6 Degradation: Chemical Oxygen Demand

Inoculum: Seeding water taken from the aeration tank of a domestic sewage treatment plant

Exposure Period: 5 days

Auxiliary Solvent: None

Analytical Monitoring: HPLC

Remarks – Method: No significant protocol deviations. Reference item: Potassium hydrogenphthalate.

RESULTS

BOD (5 days)	*COD*	*BOD/COD*
0 mg O_2/g	919 mg O_2/g	0

Remarks – Results: All test validity criteria were satisfied.

CONCLUSION: This test supports the findings in the biodegradability tests that the notified chemical is poorly biodegradable.

TEST FACILITY: Solvias AG (2000c)
Solvias AG (2000d)

C.1.4. Potential for Bioaccumulation

The notified substance has low potential to bioaccumulate. This is based on the water solubility of the test substance (>200 g/L) and the partition coefficient (log P_{OW} = -5.4).

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test – 96-hour static test.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish- 96-hour static test.

Species: Zebra fish (*Brachydanio rerio*)

Exposure Period: 96 h

Auxiliary Solvent: None

Water Hardness: 142 mg $CaCO_3$/L

Analytical Monitoring	HPLC
Remarks – Method	No significant protocol deviations

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	105.3	7	0	0	0	0	0

LC50	>100 mg/L at 96 hours
NOEC	100 mg/L at 96 hours
Remarks – Results	Measured concentrations ranged from 105.0 to 105.5% of the nominal concentration during the exposure period and hence the results are based on the nominal concentration. No sublethal effects were observed in the control or the treatment concentration during the test period. All test validity criteria were satisfied.

CONCLUSION The notified chemical is not harmful to zebra fish (*Brachydanio rerio*)

TEST FACILITY Solvias AG (2000e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – 48-Hour.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – 48-Hour Immobilisation test

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	231 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	No significant protocol deviations.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	20	0	0
4.3	4.45	20	0	0
9.4	9.95	20	0	0
21	21.40	20	0	0
45	48.95	20	0	0
100	107.10	20	0	1

E_iC50	>100 mg/L at 48 hours
NOEC	45 mg/L at 48 hours
Remarks - Results	During the test the measured test concentrations in the analysed samples were in the range of 101.9 – 108.7% of the nominal value at the start and 101.9 – 108.9% at the end of exposure. Hence the results were based on nominal concentrations only.

CONCLUSION The notified chemical is not harmful to *Daphnia magna*.

TEST FACILITY Solvias AG (2000f)

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* CHODAT
Exposure Period	72 hours
Concentration Range Nominal	1.1, 3.5, 11, 35, 110 mg/L
Concentration Range Actual	0.94, 2.96, 9.30, 29.8, 98.9 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	The test method was modified to quantify the algicidal effect of the test item (Experiment A) and the growth inhibition effect caused by reduced light intensities in the coloured test solutions (Experiment B). The test was performed in buffered test medium to keep the pH of the test media constant during the test period. No significant protocol deviations.

RESULTS

Experiment	*Biomass*		*Growth*	
	E_bC_{50} *mg/L at 72 h*	NOE_bC *mg/L*	E_rC_{50} *mg/L at 72 h*	NOE_rC *mg/L*
A	28 (95% CI: 16 - 53)	3.5	53 (95% CI: 37 - 86)	3.5
B	16 (95% CI: 12 - 21)	<3.5	40 (95% CI: 30 - 55)	<3.5

Remarks - Results	The quantification of the test item concentrations was based on the main compound. In the freshly prepared test media, 96-102% of the nominal values were found. In aged samples (72 hours), the values decreased slightly to 71-78% of the nominal values. The decrease was considered to be due to hydrolysis as the test substance has been shown to be unstable at pH 7 and 9. The mean measured concentrations ranged from 84-90% of nominal. Therefore, the reported biological results are based on the nominal concentration. This modified algal test has demonstrated that the observed growth inhibition effect of the test item on *Scenedesmus subspicatus* was caused only by an indirect effect, the light filter effect in the coloured test solutions. Thus a toxic effect of the test item on the algal cells can be excluded up to the highest test concentration of 110 mg/L.
CONCLUSION	The notified substance had no toxic effect on the algae up to 110 mg/L.
TEST FACILITY	RCC Ltd (2000u)

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	Aerobic activated sludge from wastewater treatment plant
Exposure Period	3 hours
Concentration Range	25.6 – 1000 mg/L

Nominal	
Remarks – Method	No significant protocol deviations

RESULTS

IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	All test validity criteria were satisfied.

CONCLUSION	The notified substance had no toxic effect on the bacteria up to 1000 mg/L.
TEST FACILITY	Solvais AG (2000g)

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