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September 2007

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

C.I. Reactive Yellow 211

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.

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:

Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANTS
Huntsman Corporation Australia Pty Ltd (ABN 67 083 984 187) of 454-456 Somerville Road, West Footscray VIC 3012.

Chemiplas Australia Pty Ltd (ABN 29 003 056 808) of 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: Molecular Weight, Spectral Data, Purity, Hazardous Impurities, Non-Hazardous Impurities, Additives/Adjuvants, Import Volume, and 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
EU (2002), Switzerland (2002), USA (2002), China (2002), Korea (2002), and Philippines (2007).

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
1,5-Naphthalenedisulfonic acid, 3-[[2-(acetylamino)-4-aminophenyl]azo]-, disodium salt, reaction products with 2-[[4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]phenyl]sulfonyl] ethyl hydrogen sulfate, hydrolyzed

OTHER NAME(S)
3-[2-Acetylamino-4-[4-chloro-6-(4-vinylsulfonyl-phenylamino)-[1,3,5]triazine-2-ylamino]-phenylazo]-naphthalene-1,5-disulfonic acid disodium salt

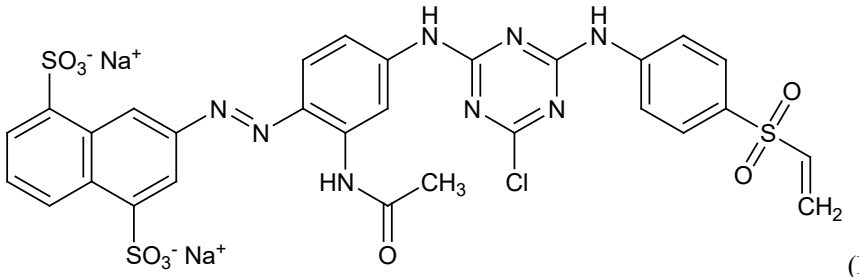
3-[[2-(Acetylamino)-4-aminophenyl]azo]-1,5-naphthalenedisulfonic acid disodium salt, reaction products with 2-[[4-[(4,6-dichloro-1,3,5-triazin-2-yl]amino]phenyl]sulfonyl]ethyl hydrogen sulfate, hydrolyzed

MARKETING NAME(S)
C.I. Reactive Yellow 211
Reactive Yellow 211
Novacron Yellow LS-RN
Cibacron Yellow LS-RN
Eriofast Yellow R
Polyamid Yellow DER 8824
FAT 45400

CAS NUMBER
319926-36-8

MOLECULAR FORMULA
Unspecified ($C_{29}H_{23}N_8O_9S_3ClNa_2$ for the main structure, shown below)

STRUCTURAL FORMULA



(Main structure shown)

MOLECULAR WEIGHT
<1,000 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, UV spectra, elemental analysis data and TLC data were provided.

3. COMPOSITION

DEGREE OF PURITY
>80%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa
Orange to dark orange powder, with no odour

Property	Value	Data Source/Justification
Melting Point	>400°C	Measured
Boiling Point	>400°C at 101.3 kPa ~726°C at 101.3 kPa	Measured Calculated
Density	1,340 kg/m^3 at 20.1°C	Measured
Vapour Pressure	3.43×10^{-26} kPa at 25°C	Estimated
Water Solubility	92.14 g/L at 20±0.5°C	Measured
Hydrolysis as a Function of pH	t½ >1 year at pH 4 and 7 t½ = 33 days (792 hrs) at pH 9	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = -2.7 at 20°C	Estimated
Surface Tension	69.1 mN/m at 20.6°C	Measured
Adsorption/Desorption	log K_{oc} = <1.32 at 20°C	Measured
Dissociation Constant	pKa range = -7.1 to 19.8	Calculated
Particle Size	Inhalable fraction (<100 µm): 100% Respirable fraction (<10 µm): 71.29% MMAD* = 6.2 µm	Measured
Flash Point	Not determined	Solid with a low vapour pressure.
Flammability	Not highly flammable	Measured
Auto-ignition Temperature	~296°C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Estimated

* MMAD = Mass Median Aerodynamic Diameter

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

The notifier has provided a statement describing their production of dust-free reactive dye formulations. Dependent on the dye, oil (undefined) may be added to achieve the dust-free formulation (to 0.1-1.0%). These dye formulations are routinely tested during production and the notifier claims that 99% of production runs are

dust-free as determined using a Lorenz apparatus. The particle sizes within these formulations is between 150 and 200 µm, or they are supplied in granules of ~300 µm.

Reactivity
The notified chemical is a reactive dye, containing both monochlorotriazine and vinylsulfone reactive groups. These groups are expected to react in use to form covalent links with cellulose-based fibres. Dyes such as the notified chemical are referred to as hot-dyeing dyes, as they are less reactive than other types of reactive dyes and require heat during use to induce reaction. Therefore, it is expected to be stable under normal handling conditions.

The notified chemical is non-oxidising and therefore not capable of causing fire or enhancing the risk of fire when in contact with combustible material. It is stable at room temperature and does not evolve any flammable gases in contact with water or humid air. Expected thermal decomposition products would be oxides of carbon, oxides of nitrogen and oxides of sulfur. Some hydrogen chloride is also possible.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported by sea as a 60-70% component of yellow dyestuff (eg Novacron Yellow LS-RN).

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

TRANSPORTATION AND PACKAGING
Novacron Yellow LS-RN (60-70% notified chemical) will be transported to Australia by ship in 25 kg robust fibreboard cartons, with inner PE liner (UN approved for road/rail transport), held within a shipping container. These will be transported from the dockside to Chemiplas's contracted warehouse in Laverton North (Victoria), where it will be unloaded from the shipping container and stored until required for despatch to customers. No repackaging or reformulation will occur.

USE
The notified chemical is a reactive azo dye, used for the colouration of polyamide, cotton and cotton-blend fibres. 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 dye-houses in Victoria and New South Wales, the sales product containing the notified chemical will be weighed in a dispensary equipped with local exhaust ventilation. The weighed powder will be added to the blending vessel, also under local exhaust ventilation. Mixing and blending of dye components takes place in closed mixing vessels. The notified chemical will be present at <1% in the final textile dye solution, which will be applied to fabric at elevated temperatures.

- 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). The concentrations used of these additives will be dependent on the amount of dye used and the water to fabric ratio. Exhaustion rates of 85-95% are expected, of which 70-82% will be bound to cellulosic fibres and of which 18-30% will react with water and alkali (the vinyl sulfone and chlorotriazine groups hydrolyse to form hydroxyl species). Following fixation, the bath will be discharged to drain. The fabric will then be washed free of unfixed dye in a series of wash-off baths (six to eight) at temperatures ranging from 50ºC to 95ºC. This washing will remove the hydrolysed, unfixed dye, leaving <0.3% of the hydrolysed dye on the fabric.
- For polyamide, exhaustion and fixation will be achieved through the use of elevated temperatures alone (≥100ºC). Polyamide fabrics will be washed under strong alkaline conditions to remove all unfixed dye, but low levels of unfixed dye are expected to require removal from these fibres.

At the completion of the rinsing phase the wet fabric will be removed from the dyeing equipment by

mechanical means, assisted by operators wearing protective clothing and gloves. 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.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (min/day)*	*Exposure Frequency (days/year)*
Transport drivers	1-5	20-30	50-100
Warehouse operators	4-8	20	100-150
Batch area operators	5-10	20	180-240
Dye machine operators	5-10	60	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 60-70% notified chemical) by inhalation, ingestion, skin contact or eye contact while weighing out the dye and adding it to mixing vessels to form the dye solution. The imported product is supplied as a non-dusting formulation in which all of the particles are greater than 150 μm in diameter, reducing the potential for inhalation exposure. Dye machine operators will wear personal protective equipment (PPE) that includes gloves, coveralls, goggles and a dust mask. Local and general exhaust ventilation will be available where the solid product containing the notified chemical is handled.

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.

Cleaning and maintenance of the machines will be performed by the machine operators. This will involve flushing the holding and mixing tanks with water. 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 0.3% 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 and Result	*Assessment Conclusion*
Rat, acute oral	Low toxicity, LD_{50} >2,000 mg/kg bw
Rat, acute dermal	Low toxicity, LD_{50} >2,000 mg/kg bw
Rat, acute inhalation	Not performed
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Severely irritating
Guinea pig, skin sensitisation – adjuvant test	No evidence of sensitisation.
Rat, oral repeat dose toxicity – 28 days	NOEL not established (50-1,000 mg/kg bw/day)
Genotoxicity - bacterial reverse mutation	Non-mutagenic
Genotoxicity – *in vitro* chromosome aberration test	Genotoxic
Genotoxicity – *in vivo* micronucleus assay	Non-genotoxic

Toxicokinetics, metabolism and distribution

Based on its molecular weight and $logP_{ow}$, 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).

The inhalation of respirable particles of the notified chemical is possible, given the particle size of the powder (MMAD = 6.2 µm). The notified chemical would be likely to dissolve and be retained in mucus, and be ultimately ingested. Significant absorption from the lung is not probable, but given the reactivity of the notified chemical, some reaction with and/or toxicity towards cells of the lung epithelium is possible.

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).

In the 28-day repeat oral dose toxicity study, orange staining of the cage bedding was observed, indicating that the notified chemical may be excreted into urine at high doses. No other indications on the toxicokinetic behaviour of the test substance could be derived from the results of the available studies.

Acute and repeat dose (sub-acute) toxicity

In the test for acute oral and dermal toxicity, the notified chemical was applied to rats at a single dose level of 2000 mg/kg bw. Neither mortality nor any substance-related systemic effects or changes on body weights and organs were seen. In the mouse micronucleus study, a single oral dose of 2000 mg/kg bw showed reduced spontaneous activity, abnormal abdominal position and eyelid closure were noted as systemic toxic effects. However, it is possible that these symptoms resulted from gastrointestinal distress and not intoxication *per se*.

In the 28-day repeat dose oral toxicity study at 1,000 mg/kg bw/day, a treatment-related reduction of mean locomotor activity was noted during the first measurement interval, which is similar to the effects observed in the mouse micronucleus study.

Also in the repeat dose study, a reversible decrease in testis and epididymis weight was observed in all treated males (50-1,000 mg/kg bw/day). The effect was slight – a reduction of ~10% in average relative or absolute weight – but was statistically significant. No histopathological effects were noted. Decreases in testis weight may be an indication of male reproductive toxicity (Foster, 1997). Given that the effect was observed for both testis and for epididymis, an effect on spermatogenesis is possible, though none was observed in any histological studies performed (no effect reported). No data on spermatid count, or prostate or seminal vesicle function were available within the study design to determine more detailed mechanisms of toxicity.

The possible acute or chronic effects of inhalation of respirable particles of the notified chemical are not known.

Irritation and sensitisation

The notified chemical showed no irritant effects to skin or eyes, but did cause irreversible staining to the conjunctivae of all animals treated in the eye irritation study.

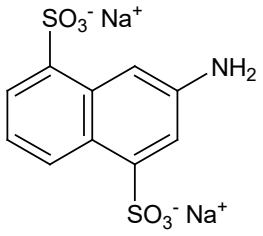
Reactive dyes have been reported as the causative agents in sensitisation of the public to textiles (Estlander, 1988; Manzini et al, 1996). However, the notified chemical was not found to be sensitising to the skin of guinea pigs in a maximisation study.

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 K *et al,* 1978; Docker *et al,* 1987; Topping *et al*, 1989). Human case studies of respiratory sensitisation to reactive dyes that are similar in structure to the notified chemical have been reported. It is not known if the notified chemical can induce respiratory sensitisation, but it has the potential based on the literature for similar chemicals.

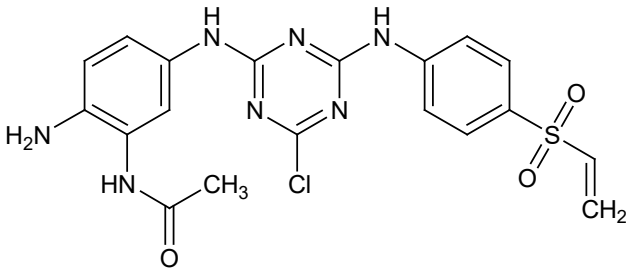
Mutagenicity/carcinogenicity

The notified chemical was not mutagenic to bacteria *in vitro*, but was clastogenic to cultured mammalian cells, with and without S9 activation. It was not found to be clastogenic in an *in vivo* mouse bone marrow micronucleus assay, but on the basis of the lack of effect on the PCE/NCE ratio, some doubt exists as to whether the notified chemical had reached the target tissue to induce the formation of micronuclei. Given the positive *in vitro* chromosome aberration test data, 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). 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 may also be metabolised or broken down by azo reduction into two main arylamine species:



1



2

1. 3-amino-1,5-naphthalenedisulfonic acid (CAS 131-27-1, 52085-24-2 and 14170-43-5 [Na salt]): This species has been found to be non-mutagenic in a range of Ames studies (JETOC, 1996). No further information regarding the possible mutagenicity of this species is available in the literature.

   This species contains 2-naphthylamine as a component of its structure. 2-Naphthylamine is a known human carcinogen, and is classified internationally as a Group 1 carcinogen (IARC, 1987; RoC, 2005). It is also listed on the "banned list" of the annexes of the EU SCCNFP/0495/01 (SCCNFP, 2002). The US EPA, in their category of concern for β-naphthylamine, express concern about azo dyes that may release a β-naphthylamine (sulfonated on the distal ring to the amine group) upon azo reduction:

   > *"Based on analogy to β-naphthylamine per se, members of the class are considered potential carcinogens. A number of mono- and disulfonated β-naphthylamines are positive in the Ames assay, and some are active in the mouse lung adenoma assay. The presence of one or two sulfonate groups or the sulfatoethylsulfone group is likely to slow systemic uptake and enhance excretion, however, the extent of these mitigating effects is unknown… Concern is restricted to sulfonated ß-naphthylamines where not more than two sulfonate or sulfatoethylsulfone group(s) are on the ring distal to the ß-amino group. The Agency has sufficient data to indicate that ß-naphthylamines where a sulfonate group is on the proximal ring are unlikely to be carcinogenic."* (US EPA, 2002)

   As this azo reduction species contains a sulfonate group that is on the proximal ring to the amine group, it is unlikely to be carcinogenic according to the US EPA criteria.

2. *N*-[2-amino-5-[(4-chloro-6-[[4-(ethenylsulfonyl)phenyl]amino]-1,3,5-triazin-2-yl)amino]phenyl]acetamide: No information regarding the mutagenicity of this exact species is available in the literature. However, it contains two potential functional groups that might indicate concern for mutagenicity: a vinyl sulfone and an aromatic amine. For the vinyl sulfone group, the known data for the notified chemical appears to mirror the concern for chemicals containing these groups, expressed by the US EPA as follows:

   > *"For those who inhale or ingest a vinyl sulfone, there is an oncogenicity concern and mutagenicity concerns based on the potent mutagenicity of vinyl sulfone (VS) and methylvinyl sulfone (MVS). VS and MVS are mutagenic in the L5178Y TK+/- mouse lymphoma gene mutation assay in vitro. Evaluation of small colonies indicates that genotoxicity is due to a clastogenic mechanism. This is confirmed by evaluating the lymphoma cells for chromosome aberrations and micronuclei. MVS also induces effects upon the spindle apparatus in Chinese hamster lung cells in vitro, indicating an*

aneugenic (aneuploidy-inducing) activity. A direct-acting Michael addition-type reaction may be the mechanism of action. Although MVS and divinyl sulfone (DVS) are both reported as negative in vivo in the mouse micronucleus assay (inconclusive, only males tested), and in the dominant lethal assay, the Agency has determined that these negatives are not sufficient to remove concern for vinyl sulfone-containing new chemicals as potential mutagens and carcinogens... Nearly all new chemicals in the category have been water-soluble, fiber-reactive dyes with molecular weights <1,000."

(US EPA, 2002)

In addition to concern from this group, many unsulfonated aromatic amines are also generally of concern for mutagenicity (Bartsch, 1981). Given these concerns, this species has the potential to be mutagenic, based on its structural formula.

In addition to these concerns, azo dyes in general are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al*, 1998). The identity of these species is known for the notified chemical, but their mutagenic potential is unknown. The identified impurities are in general likely to display similar or lower genotoxic properties to the notified chemical, but in some cases might display greater genotoxicity.

During its use in dyeing textiles, the reactive chlorotriazine and 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.

The available data is not sufficient to rule out the notified chemical as a possible mutagen or carcinogen. However, based on the available evidence, the notified chemical cannot be classified as genotoxic.

Classification

Based on the irreversible conjunctival staining seen in then 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

If inhalation exposure was to occur, the health risk of the notified chemical is likely to be significant, given its potential for mutagenicity and/or respiratory sensitisation (in the absence of sufficient negative test data). It is not possible to quantitate the toxic risk of repeated exposure to the notified chemical, as a NOEL was not established. Batch area operators may be exposed to solid product containing 60-70% notified chemical while weighing out the dye and during addition of the dye powder to solution. Inhalation/ingestion exposure during these procedures is expected to be low, as workers will operate under conditions where engineering and other measures are in place to limit occupational exposure to respirable particulates (such as local exhaust ventilation and powder-free formulations). In addition, workers will wear suitable dust masks or particulate filter respirators. Considering the expected low exposure, the risk to workers handling the notified chemical is expected to be minimal given that the proposed controls are expected to be used.

The health risk arising from ocular exposure is significant, given the irreversible conjunctival staining observed in the eye irritation study. The exposure of batch area workers may occur during these steps, but should be limited by the wearing of goggles and by the other controls in place. These workers' health risk from dermal exposure to the powdered notified chemical is expected to be minimal, given its low acute dermal toxicity and lack of skin sensitising ability. In addition to the above controls, dermal exposure will be limited by the use of PPE such as gloves and overalls.

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 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 FROM USE
Release of the notified chemical to the environment during transport or storage is not expected, however this may occur in the unlikely event of an accident.

Almost all of the dye containing the notified chemical will be removed from the import containers by shaking into mixing vessels. Potential sources of environmental exposure at this stage include spillage, which is estimated at <5 kg/year (0.1% import volume), and container residues, estimated at <1 kg/year (0.02% import volume). Any spillages will be contained and either swept up together with dust binding material or sucked up using an industrial vacuum cleaner, and then placed in sealed and labelled containers. The solid waste and contaminated containers will be disposed of by landfill or incineration though landfill is more likely. The Material Safety Data Sheet recommends that any residues remaining after containment be flushed away with water.

Dissolution of the dye takes place in an enclosed vat, after which the dye is pumped through a closed system to the dyeing machine. Given that this process occurs in a closed system, there are limited opportunities for release to the environment.

The dye will be used to colour cotton and cotton blend textiles, and polyamide fabrics by exhaust dyeing. Due to variation between the three dye-houses, exhaustion rates of between 85-95% and fixation of 70-82% may be expected. The remaining 18-30% will react with hydroxyl groups of water, thus rendered inert and retained in the rinsate. This hydrolysed dye will be discharged to the dye-house 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 dye-houses, including solids obtained from dye flocculation (up to 30% import volume), from container residues (0.02% import volume), and from spillages (0.1% import volume) are said to either go to landfill or be incinerated; however, landfill is the more likely option. Incineration is the preferred method of disposal due to the ready water solubility and potential high mobility of the notified chemical, however landfill is more likely due to local regulatory requirements. The treated effluent containing minor traces of the notified chemical will be disposed of to the sewer.

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 the fabric and be either disposed of to landfill or incinerated.

7.1.2 Environmental fate

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

Solid waste generated throughout the dyeing process from spillage, container residue and dye rinsate flocculation is expected to contain up to 30% of the total import volume for the notified chemical. The waste will either be incinerated, in which case the notified chemical will produce water, oxides of carbon, nitrogen and sulphur and some hydrogen chloride.

Due to the high water solubility and low $\log K_{oc}$ value for the notified chemical, any waste that is disposed of to landfill is not expected to adsorb to soil; instead, it is expected to move readily through the soil column. Similarly, any notified chemical as a component of unfixed dye that is not captured through flocculation at the end of the dyeing process is not likely to adsorb to sludge, and will be released directly to the aquatic environment. The notified chemical is not readily biodegradable, but it has low potential for bioaccumulation due to its low $\log K_{ow}$ value.

The notified chemical as a component of fixed dye on textiles (minimum of 70% import volume) will form covalent bonds with reactive groups on cotton and polyamide fabrics and is therefore expected to share the same fate as the end use product, i.e. landfill.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC was calculated based on effluent releases from a low volume STP discharge country dye-house, and a high volume STP city dye-house, which undergoes 10-fold greater dilution than effluent from the former. Given that the dyeing process is unlikely to operate 365 days per year, the PEC has been calculated based on the typical usage of the notified chemical for a day during which the dyeing process occurs. A worst-case scenario has been calculated in which no waste dye is removed from the sewer, including by flocculation.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
	Country dye-house	City dye-house
Typical daily use of product	4 kg/day	4 kg/day
Amount of notified chemical	2.6 kg/day	2.6 kg/day
Concentration in wastewater (fixation rate = 70%)	0.78 kg	0.78 kg
Typical daily volume of dye washwater effluent	400,000 L	400,000 L
Concentration in dye washwater	1950 µg/L	1950 µg/L
Typical daily volume of dye-house washwater effluent	2,900,000 L	2,900,000 L
Concentration in dye-house effluent	269 µg/L	269 µg/L
Dilution factor in sewage treatment plant	1:10	1:100
Concentration in effluent from sewage treatment plant	26.9 µg/L	2.69 µg/L
Dilution Factor - River	1.0	1.0
Dilution Factor - Ocean	10.0	10.0
PEC - River	26.9 µg/L	2.69 µg/L
PEC - Ocean	2.69 µg/L	0.269 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	LC50 > 100 mg/L	Non-toxic
Daphnia Toxicity	EC50 > 100 mg/L	Non-toxic
Algal Toxicity	ErC50 > 100 mg/L	Non-toxic
Inhibition of Bacterial Respiration	EC50 > 1,000 mg/L	Non-toxic

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
LC50 (Fish).	100 mg/L
Assessment Factor	100
Mitigation Factor	1.0
PNEC	1,000 µg/L

The notified chemical was non-toxic to three trophic levels with all endpoints being above 100 mg/L. A safety factor of 100 was used.

7.3. Environmental risk assessment

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q - River	26.9	1,000	0.027
Q - Ocean	2.69	1,000	0.003

From the country dye-house PEC/PNEC ratio, a value of 0.027 is calculated for the risk quotient for the aquatic environment. The notified chemical is therefore not expected to pose an unacceptable risk to the aquatic environment. This calculation has been based on a worst-case scenario and flocculation (where it occurs) is expected to remove most of the notified chemical from effluent. The notified chemical released to the aquatic environment is expected to remain persistent in solution due to its low biodegradability potential. It is not expected to become associated with sediments.

While the molecular weight is less than 1,000 Da, the notified chemical is not expected to bioaccumulate due to its very high water solubility and low $logP_{ow}$ value. In addition the notified chemical is non-toxic to three trophic levels of aquatic organisms.

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, 2004). The classification and labelling details are:

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

and

As a comparison only, 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***
Health Hazard	1	Causes serious eye damage
Environmental Hazard	-	Classification not possible for environment

Human health risk assessment
Under the conditions of the occupational settings described, the risk to workers is considered to be acceptable. Eye and skin protection will be required during all dyeing operations. Respiratory protection will also be required during handling of powdered forms of the notified chemical.

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
On the basis of the PEC/PNEC ratio, 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 to <10% Xi: R36 Irritating to eyes.*
    - *≥10% Xi: R41 Risk of serious damage to eyes.*

Material Safety Data Sheet

- The MSDS provided by the notifier should be amended as follows:
  - *The occupational hygiene section should recommend handling procedures that avoid the formation of dusts.*
  - *The method of disposal should be amended to recommend disposal to landfill only.*

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 where feasible*

- 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, 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 containment and either sweeping up together with dust binding material or sucking up using an industrial vacuum cleaner, and then placing in sealed and labelled containers.

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a reactive textile dye, or is likely to change significantly;
  - the amount of chemical being introduced has increased from five 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.

No additional secondary notification conditions are stipulated.

Materials Safety Data Sheet
The MSDS of 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	Determined using a differential scanning calorimeter (DSC).
Test Facility	RCC (2001b)

Boiling Point >400°C at 101.3 kPa (measured)
~726°C at 101.3 kPa (calculated)

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	The notified chemical did not boil under the conditions of the test, determined using a differential scanning calorimeter. The calculated value was determined using Meissner's method.
Test Facility	RCC (2001c), RCC (2001e)

Density 1,340 kg/m^3 at 20.1°C

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

Vapour Pressure 3.43×10^{-26} kPa at 25°C (estimated)

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Estimation was performed using the calculated boiling point and the Modified Watson Correlation.
Test Facility	RCC (2001e)

Water Solubility 92.14 g/L at 20±0.5°C

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Based on a preliminary test determining the water solubility to be about 114.8 g/L, the Flask Method was chosen for the main test. Approximately 6 g of the test substance was shaken in a flask with 25 mL water at ~30°C for 24, 48 and 72 hours respectively. After equilibration for 24 hours, the supernatant was centrifuged, filtered and diluted 1:2500 with acetonitrile. Quantitation was performed using HPLC as the analytical method.
Test Facility	RCC (2001f)

n-Octanol Solubility 0.17 g/L at 20±0.5°C

Method	Modified flask method.
Remarks	The test substance was mixed with n-octanol and stirred at room temperature for about 24 hours. After centrifuging and filtration, the supernatant was diluted with acetonitrile. Quantitation was performed using HPLC as the analytical method.
Test Facility	RCC (2001h)

Hydrolysis as a Function of pH t½ >1 year at pH 4 and 7
t½ = 33 days (792 hrs) at pH 9

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 <hours or days>*

4	25°C	>8760
7	25°C	>8760
9	25°C	792
9	29°C	435
9	39°C	93
9	50°C	21

Remarks Preliminary and main test solutions were prepared by dissolving the test substance in pH buffered solutions to 130-150 µg/mL, and then treating in an ultrasonic bath for 5 minutes. Aliquots of solutions at each temperature/pH combination were taken at 0, 2.4 and 120 hours, and analysed using HPLC as the analytical method. The test substance was found to be stable at pH 4 and 7 and unstable at pH 9 in the pre-test at 50°C. Therefore, further testing was performed at pH 9 at 29°C and 39°C. From these results the half-life at 25°C was estimated.

The half-life time of the test substance at 25°C was determined to be:

- pH 4 and 7: > one year
- pH 9: 33 days (792 hours)

Test Facility RCC (2001g)

Partition Coefficient (*n*-octanol/water) log P_{ow} = -2.7 at 20°C

Method Ratio of solubilities in *n*-octanol and water

Remarks Neither the HPLC Method, nor the Flask Method could be used to determine the partition coefficient for the notified chemical directly. Therefore, the partition coefficient was estimated based on the solubility of the notified chemical in both *n*-octanol and water.

Test Facility RCC (2001h)

Surface Tension 69.1 mN/m at 20.6°C

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

Remarks Determined using a tensiomenter, the ring method and a notified chemical concentration of 1 mg/mL. The notified chemical was considered to be not surface-active.

Test Facility RCC (2001o)

Adsorption/Desorption log K_{oc} = <1.32 at 20°C

Method OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method.

Remarks The test substance was dissolved in a solution of methanol and water, and was injected into a HPLC column containing polar and non-polar sites, along with 6 reference solutions with known logK_{oc} values. Based on the regression curve (capacity factor vs logK_{oc}), the logK_{oc} of the test substance was determined to be <1.32, indicating a very low adsorption potential.

Test Facility RCC (2001i)

Dissociation Constant pKa range = -7.1 to 19.8

Method OECD TG 112 Dissociation Constants in Water.

Remarks The structural formula of the notified chemical was used to estimate its pKa values:

Acidic/basic group	Estimated pKa	Estimation Method
Arenesulfonic acid 1*	-7.0	Hammett
Arenesulfonic acid 2*	-7.1	Hammett
Azobenzene**	<0	General estimation
Amide	19.8	Taft
Secondary Amine	0.4	Taft
Triazine (×3)	~-5.6	Hammett

* Arenesulfonic acids are strong acids and are completely dissociated in aqueous solution.

** An exact value for the dissociation constant of protonated azo groups (strong acids) could not be estimated, but these groups are not expected to be protonated in aqueous solution.

The strongly acidic sulfonate groups are expected to dominate in aqueous solution; thus, it is expected to be in anionic form over the environmental pH range.

Test Facility RCC (2001j)

Particle Size

Inhalable fraction (<100 μm): 100%
Respirable fraction (<10 μm): 71.29%
Mass Median Aerodynamic Diameter (MMAD) = 6.2 μm

Method EC Directorate General XII-JRC: Particle Size Distribution, Fibre Length and Diameter Distribution.

Range (μm)	*Mass (%)*
<0.5	2.27%
<1.0	6.85%
<2.0	16.56%
<3.0	25.59%
<4.0	33.76%
<5.0	41.35%
<10.0	71.29%
<20.0	95.63%
<30.0	99.90%

Remarks The particle size distribution was determined using the laser diffraction technique.
Test Facility RCC Ltd (2001k)

Flammability

Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The notified chemical could not be ignited with a flame after a contact time of 2 minutes in a preliminary test. In contact with the ignition source, the surface of the notified chemical became black. Therefore, no main test was performed.
Test Facility RCC (2001m)

Auto-ignition Temperature

~296°C

Method 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks Upon increasing the temperature of the sample, an exothermic reaction started at around 250°C (as measured by thermocouple). A maximum temperature of 573°C was measured in the sample during the reaction.
Test Facility RCC (2001n)

Explosive Properties

Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks The notified chemical was not thermally sensitive, shock sensitive or sensitive to friction, and was thus considered to be not explosive.
Test Facility Institute of Safety & Security (2001)

Oxidizing Properties

Not oxidising

Method EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks Based on structural analysis of the notified chemical and the calculated oxygen balance.
Test Facility RCC (2001p)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

Test Substance	Notified chemical
Method	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 92/69/EEC B.1 tris – Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/HanCrl: WIST Han(Glx:BRL)BR
Vehicle	PEG 300
Remarks – Method	No significant protocol deviations.

Results

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

LD50	>2,000 mg/kg bw
Signs of Toxicity	No deaths occurred during the study. No clinical signs were noted during the course of the study.
Effects in Organs	No macroscopic findings were observed at necropsy.
Remarks – Results	None.
Conclusion	The notified chemical is of low toxicity via the oral route.
Test Facility	RCC (2001q)

B.2. Acute toxicity – dermal

Test Substance	Notified chemical
Method	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/HanCrl: WIST Han(Glx:BRL)BR
Vehicle	PEG 300
Type of dressing	Semi-occlusive.
Remarks – Method	No significant protocol deviations. The applied test substance solution was 0.33 g/mL (6 ml/kg bw).

Results

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

LD50	>2,000 mg/kg bw
Signs of Toxicity - Local	Test item remnants were noted on day 2 (all animals) and persisted in one female until test day 11. No local signs of toxicity were observed during the study period.
Signs of Toxicity - Systemic	No systemic signs of toxicity were observed during the study period.
Effects in Organs	No macroscopic findings were observed at necropsy.
Remarks – Results	None.
Conclusion	The notified chemical is of low toxicity via the dermal route.
Test Facility	RCC (2001r)

B.3. Irritation – skin

Test Substance	Notified chemical
Method	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Test substance was moistened with distilled water.
Observation Period	72 hours
Type of Dressing	Semi-occlusive
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			
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	Light orange staining by the test article of the treated skin area was observed in all animals from 1 to 24 hours after treatment, and this was considered to be due to the nature of the test substance rather than irritation. No mortality or clinical signs were observed.
Conclusion	The notified chemical is non-irritating to skin.
Test Facility	RCC (2001s)

B.4. Irritation – eye

Test Substance	Notified chemical
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 male, 2 females)
Observation Period	21 days
Remarks – Method	No significant protocol deviations.

Results

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.67	0.33	0.33	1	>48 hours	0
Conjunctiva: chemosis	0.33	0	0	2	>24 hours	0
Conjunctiva: discharge	-	-	-	-	-	-
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.

Remarks – Results	No scoring of conjunctival discharge was reported, although a slight, watery discharge was observed in all animals 1 hour after treatment. Light orange staining of the eyes was observed in all animals throughout the study period, which was still present at study termination on day 21. All other results indicate a non-irritant nature.

Conclusion	The notified chemical is severely irritating to the eye, based on its ability to cause permanent discolouration.
Test Facility	RCC (2001t)

B.5. Skin sensitisation

Test Substance	Notified chemical
Method	OECD TG 406 Skin Sensitisation – Maximisation test. EC Directive 96/54/EC B.6 Skin Sensitisation - Maximisation test.
Species/Strain	Guinea pig/Ibm: GOHI (Himalayan spotted)
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 3% (in distilled water) topical: 10% (in distilled water)
MAIN STUDY	
Number of Animals	Test Group: 10 males Control Group: 5 males
Induction phase	Induction Concentration: intradermal: 10% (in distilled water or FCA/saline) topical: 40% (in distilled water)
Signs of Irritation	Moderate/confluent erythema was observed 24 hours after epidermal induction, which had receded to discrete/patchy erythema by 48 hours. No oedema was observed.
Challenge phase	
Topical challenge	topical application: 10% (in distilled water)
Remarks – Method	The maximum non-irritating concentrations were selected as the highest concentrations that could technically be administered (and because no irritation was observed in the preliminary studies at these concentrations).

Results

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after challenge*	
		24 h	*48 h*
Test Group	10	0	0
Control Group	10	0	0

Remarks – Results	The test substance stained the skin slightly red-brown, but this did not interfere with the scoring of erythema or oedema. No deaths or signs of systemic toxicity occurred during the study. None of the control and test animals showed skin reactions after the challenge treatment.
Conclusion	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
Test Facility	RCC (2001u)

B.6. 28-day repeat dose oral toxicity

Test Substance	Notified chemical
Method	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/HanBrl:WIST (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

Vehicle	PEG 300
Remarks – Method	No significant protocol deviations.

Results

Dose (mg/kg bw/day)	*Number and Sex of Animals*	*Mortality*	
	0	5M, 5F	0
	0 (recovery)	5M, 5F	0
	50	5M, 5F	0
	200	5M, 5F	0
	1,000	5M, 5F	0
	1,000 (recovery)	5M, 5F	0

Mortality and Time to Death

All animals survived until scheduled necropsy.

Clinical Observations

No clinical signs of toxicity were noted at any dose level during daily cage observations or weekly behavioural observations (weeks 1-3). During pre-test and treatment, minor ocular findings were noted in the right eye of one male treated with 200 mg/kg bw/day. This finding was considered to be incidental.

Lower locomotor activity in males and females treated with 1,000 mg/kg bw/day was observed, and this was considered to be treatment-related. The locomotor activity of rats treated with 50 or 200 mg/kg bw/day was similar to the controls.

Orange discolouration of cage bedding was noted in animals dosed with 1,000 mg/kg bw/day, beginning from treatment day 20 onwards, and on day 1 of recovery (in high-dose recovery animals).

No effects on food consumption, body weight or body weight gain were noted at any dose level.

Laboratory Findings

Clinical chemistry

At 1,000 mg/kg bw/day, significantly higher chloride levels and significantly higher sodium levels were noted in males and females respectively. These values remained within the 95% tolerance limits of the historical control values and were considered to be incidental.

All other differences noted after 4 weeks treatment in the rats treated with 50 and 200 mg/kg bw/day were, in the absence of dose-response relationship, considered to be incidental.

Haematology

After 4 weeks of treatment, the mean thromboplastin time of the males treated with 200 mg/kg bw/day was prolonged when compared with the control males. In the absence of a dose-response relationship, this finding was considered to be incidental.

In females treated with 1,000 mg/kg bw/day, slightly elevated reticulocyte counts were noted after 4 weeks treatment when compared with the controls. The difference remained within the 95% tolerance limits of the historical control values and was therefore considered to be unrelated to the test item.

Urinalysis

Males treated with 1,000 mg/kg bw/day had significantly higher urinary osmolality when compared with the controls. In the absence of similar findings in females, this finding was considered to be incidental.

Effects in Organs

Organ Weights

After the treatment period, treated males (all dose groups) had slightly lower testes and epididymides weights when compared with the controls (relative and absolute). These differences were not supported by macroscopic or microscopic changes and were considered by the investigators to be the result of incidentally large control values rather than an indication of toxicity. After recovery, the testis weights of treated rats had recovered such that they compared favourably with control rats.

Macroscopic Findings

All findings (renal pelvis dilation, thickened thymus, size differences, ovarian cysts, incomplete lung deflation) noted after treatment and recovery were considered to be incidental. All were considered to be within the range of normal biological variation for rats of the strain and age used.

Microscopic Findings

There were no test item-related histopathological findings in rats sacrificed after treatment or after recovery. All of the microscopic findings encountered were considered to have arisen spontaneously.

Remarks – Results

The investigators concluded that treatment-related findings were generally restricted to the reduced locomotor activity in males and females treated with 1,000 mg/kg bw/day, and thus set the No Observed Effect Level (NOEL) as 200 mg/kg bw/day on this basis.

However, the effects on relative and absolute testis and epididymis weight observed in all treated males (50-1,000 mg/kg bw/day), in the absence of further data on testis parameters (e.g. spermatid count), cannot be considered incidental. The claimed lack of dose-dependency observed for this effect may in fact be due to a maximal effect being observed at the lowest dose tested. The argument presented for abnormally large control organ weight values was not consistent with: (1) the body weight data for control animals (the body weights of treated males was similar to or exceeded that of control animals), (2) with the organ weight data for other organs (not higher for control animals), or (3) the organ weight data relative to brain weight (a significant reduction in testis and epididymis relative weights were observed).

Conclusion

The No Observed Effect Level (NOEL) for the notified chemical could not be established from the results of this study.

Test Facility RCC (2001v)

B.7. Genotoxicity – bacteria

Test Substance Notified chemical

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

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

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

Concentration Range in Main Test a) With metabolic activation: 33-5,000 µg/plate.
b) Without metabolic activation: 33-5,000 µg/plate.

Vehicle De-ionised water

Remarks – Method No significant protocol deviations.

Results

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

Remarks – Results The notified chemical, despite its nature as an azo dye, was not examined using a modified test that includes a reductive pre-incubation step (eg Prival and Mitchell, 1982), as is recommended by the OECD TG 471. Therefore, the results of this study are indicative of the mutagenicity of only the notified chemical itself, not of any potential reductive cleavage products.

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

of the test.

Test Facility RCC Ltd (2002b)

B.8. Genotoxicity – *in vitro*

Test Substance Notified chemical

Method OECD TG 473 *In vitro* Mammalian Chromosomal 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 cells
Metabolic Activation System Phenobarbital/β-naphthoflavone-induced rat liver S9 mix
Vehicle Deionised water
Remarks – Method No significant protocol deviations. Due to the inhomogeneous results obtained from scoring only 100 metaphase plates, 200 metaphase plates were scored.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	15.6*, 31.3*, 62.5*, 125.0, 187.5, 250.0	4 hours	18 hours
Test 2	31.3*, 62.5*, 93.8*, 125.0, 187.5*, 250.0	4 hours	28 hours
Absent			
Test 1	18.8, 37.5*, 56.3*, 75.0*, 112.5*, 150.0	4 hours	18 hours
Test 2A	7.5, 15.0*, 22.5, 30.0*, 45.0, 60.0*	18 hours	18 hours
Test 2B**	15.0, 22.5, 30.0, 45.0 15.0, 30.0, 45.0. 60.0, 90.0*	28 hours	28 hours

* Cultures selected for metaphase analysis.
** Test 2B was repeated due to a lack of expected toxicity.

Results

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	≥223.4	>250.0	≥62.5	Positive
Test 2		>93.8	≥62.5	Positive
Absent				
Test 1	≥111.7	>75.0	>150.0	Negative
Test 2A	≥55.9*	>30.0	>60.0	Negative
Test 2B		>60.0	>90.0	Negative

* Exposure to 55.9 µg/mL notified chemical continuously for 24 hours (preliminary test).

Remarks – Results In Test 2, 16.0% of cells treated with 187.5 µg/mL in the presence of S9 mix showed exchanges, adding weight to the biological relevance of the positive result.

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

Test Facility RCC Ltd (2002c)

B.9. Genotoxicity – *in vivo*

Test Substance Notified chemical

Method	OECD TG 474 Mammalian Erythrocyte Micronucleus Test. EC Directive 2000/32/EC Annex 4C
Species/Strain	Mouse/NRMI
Route of Administration	Oral – gavage
Vehicle	Deionised water
Remarks – Method	No significant protocol deviations. Five males and five females were evaluated from each test group.

Dose (mg/kg bw)	*Number and Sex of Animals*	*Sacrifice Time (hours)*
0	6M, 6F	24
500	6M, 6F	24
1,000	6M, 6F	24
2,000	6M, 6F	24
2,000	6M, 6F	48
40 (cyclophosphamide)	6M, 6F	24

Results

Doses Producing Toxicity	Animals treated with 2,000 mg/kg bw showed reduction of spontaneous activity, abnormal abdominal position and eyelid closure as signs of systemic toxicity.
Genotoxic Effects	No significant increase in the number of PCEs was observed.
Remarks – Results	No alteration in the PCE/NCE ratio with test substance treatment was observed, indicating that the test substance may not have reached the target tissue (bone marrow) despite the relatively high maximum dose administered.

Conclusion	The notified chemical was not clastogenic in this *in vivo* micronucleus assay under the conditions of the test.
Test Facility	RCC (2002d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 A Ready Biodegradability: DOC Die-Away Test.
Inoculum	Mixture of polyvalent bacteria (activated sludge) collected from the aeration tank of a domestic sewage treatment plant.
Exposure Period	28 days
Auxiliary Solvent	None specified
Analytical Monitoring	TOC/DOC Analyser
Remarks - Method	The test item and a reference substance were combined with inoculated mineral media in separate test vessels and had starting dissolved organic carbon (DOC) concentrations of 35.9 mg/L and 37.9 mg/L respectively. The vessels were aerated in dark or diffuse light at ~22°C, with DOC analysis at frequent intervals over 28 days. Biodegradation was calculated from the concentration of DOC removed at each time interval (corrected by the blank inoculum control) as a percentage of the initial DOC concentration. An inhibitory control, containing the inoculated medium with both the test and reference items, was also aerated under the same conditions to determine the effect of the test item on substrate biodegradation.

RESULTS

Test substance		*Glucose*	
Day	*% Degradation*	*Day*	*% Degradation*
1	4	1	58
7	9	7	84
10	0	10	96
14	2	14	96
21	6	21	100
28	0	28	100

Remarks - Results	The test substance was found to degrade by 0% after 28 days. The test was determined to be valid because degradation of the reference item and from inhibitory action exceeded specified levels. The test item was not biodegradable after 28 days of incubation.
CONCLUSION	The notified chemical cannot be classed as readily biodegradable.
TEST FACILITY	Solvias AG (2001a)

C.1.2. Bioaccumulation

The notified chemical is expected to have a low potential to bioaccumulate. This prediction is based on the high water solubility of the notified chemical (92.1 g/L) and its low partition coefficient (log P_{ow} = -2.7).

C.1.3. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD 302B Inherent Biodegradability: Zahn-Wellens/EMPA Test
Inoculum	Mixture of polyvalent bacteria (activated sludge) collected from the aeration tank of a domestic sewage treatment plant.
Exposure Period	28 days

Auxiliary Solvent	None specified
Analytical Monitoring	TOC/DOC Analyser
Remarks – Method	The test item and a reference substance were combined with mineral media and a high concentration of bacterial inoculum (0.2-1 mg/L of suspended solids) in separate test vessels and had starting dissolved organic carbon (DOC) concentrations of ~150 mg/L. The vessels were aerated under diffuse daylight at 20-25°C, with DOC analysis at frequent intervals over 28 days. Biodegradation was calculated from the concentration of DOC removed at each time interval (corrected by the blank inoculum control) as a percentage of the initial DOC concentration.

RESULTS

Test substance		*Diethylene glycol*	
Day	*% Degradation*	*Day*	*% Degradation*
2	0	2	3
7	0	7	85
14	7	14	99
21	11	21	
28	13	28	

Remarks – Results	The test substance was found to degrade by 13% after 28 days. Adsorption after 3 hours was 2%. The test was determined to be valid because degradation of the reference item exceeded 70% after 14 days. The test item was not biodegradable after 28 days of incubation.

CONCLUSION The notified chemical cannot be classed as inherently biodegradable.

TEST FACILITY Solvias AG (2001a)

C.1.4. 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	BOD: Seeding water taken from the aeration tank of a domestic sewage treatment plant.
Exposure Period	BOD: 5 days COD: 2 hours
Auxiliary Solvent	None specified
Analytical Monitoring	BOD: Oxygen sensitive electrode system SYLAND COD: Determination of residual potassium dichromate by potentiometric titration (with a METROHM Titroprocessor 670) with ferrous ammonium sulfate.
Remarks – Method	<u>BOD</u> The test item and a reference substance (D-glucose and L-glutamic acid) were combined with inoculated mineral media in separate test vessels. The test item was diluted to obtain 8 concentrations ranging from 6.2-794.5 mg/L. The oxygen concentration for each dilution vessel was measured, followed by 5 days of incubation at 20°C in the dark, after which the remaining oxygen concentration in the flasks was measured. <u>COD</u> The test item and a reference substance were oxidised by potassium dichromate in a strong sulfuric acid medium with silver sulfate as a catalyst under reflux for 2 hours at ~148°C. After cooling, the residual dichromate was determined by titration with ferrous ammonium sulfate solution.

RESULTS

BOD (5 days) g/g	*COD g/g*	*BOD/COD*
0	0.897	0
0.187	0.197	

Remarks – Results

The BOD_5 of the test substance was calculated to be 0 mg O_2/g. The test was considered valid because the BOD_5 of the blank did not exceed 1 mg O_2/g, and the BOD_5 of the reference substance was between 180-230 mg O_2/g.

The COD of the test item was 897 mg O_2/g, and was considered valid since the COD of the test item was determined to be ~200 mg O_2/g.

CONCLUSION

The notified chemical had low oxidation potential by bacteria under neutral pH conditions. The chemical oxidation potential is high.

TEST FACILITY

Solvias AG (2001c), Solvias AG (2001d)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 203 Fish, Acute Toxicity Test – Rainbow Trout under Static Conditions.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – Rainbow Trout under Static Conditions.

Species: Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period: 96 h
Auxiliary Solvent: None specified
Water Hardness: 142 mg $CaCO_3$/L
Analytical Monitoring: Liquid chromatography
Remarks – Method: The nominal concentration of 100 mg/L was selected based on a static range-finding study using the same concentration. The effects of toxicity were observed at 2-4, 24, 48, 72 and 96 hours following exposure. Control fish were observed under the same conditions, but were not exposed to the test substance. During the exposure period the temperature was maintained between 13.4-14°C, the oxygen content at saturation, and the pH 7.8-8.3.

RESULTS

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

LC_{50}: >100 mg/L at 96 hours.
NOEC: 100 mg/L at 96 hours.

Remarks – Results

The test substance appeared homogeneously distributed in the test vessel and no precipitation was observed during the test period. Liquid chromatography confirmed >95% recovery (relative to the nominal concentration) of the test item at the start of the exposure period and at 96 hours for all concentrations. No mortality or sublethal effects were observed in the control or test concentration of 100 mg/L. The LC_{50} (96 hours) of the notified chemical was determined to be greater than 100 mg/L based on nominal concentrations, the highest concentration with no sublethal or lethal effects (NOEC) corresponds to 100 mg/L nominal, and the LC_{100} (96 h) was determined to be >100 mg/L.

CONCLUSION

The notified chemical is non-toxic to rainbow trout.

TEST FACILITY Solvias AG (2001e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – *Daphnia magna*.
EC Directive 92/69/EEC C.2 Acute Toxicity for *Daphnia* – Immobilisation test.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None specified
Water Hardness	214 mg $CaCO_3$/L
Analytical Monitoring	Liquid chromatography
Remarks - Method	Prior to exposure, *Daphnia* were sieved through a 1,000 µm sieve to select young *Daphnia* with an age of less than 24 hours. A range finding study was performed by subjecting 2 replicates of 10 *Daphnia* to 0.01, 0.1, 1, 10 and 100 mg/L of the test substance and to a blank control without the test substance. Based on results from the rang finding study, the main test was performed by subjecting duplicate samples of 10 *Daphnia* to nominal test substance concentrations of 4.3, 9.4, 21, 45 and 100 mg/L and *Daphnia* immobilisation was examined at 24 and 48 hours following exposure. During the exposure period, the temperature was 20.1-21°C, the oxygen content 96-98%, and the pH 8.1-8.5.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Blank		20	0	0
4.3		20	0	0
9.4		20	0	0
21		20	0	0
45		20	0	0
100		20	0	0

LC50	>100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	Liquid chromatography confirmed >85% recovery (relative to the nominal concentration) of the test item at the start of the exposure period and at 96 hours for all concentrations. No immobilisation or sublethal effects of *Daphnia* was observed in the control or at any of the test substance concentrations after 24 and 48 hours. The EC_{50} (96 h) of the notified chemical was determined to be >100 mg/L (nominal conc.), the highest concentration with no sublethal or lethal effects (NOEC) corresponds to 100 mg/L (nominal conc.).

CONCLUSION The notified chemical is non-toxic to *Daphnia magna*.

TEST FACILITY Solvias AG (2001f)

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: 1-100 mg/L Actual: 10.4-98.5 mg/L (<10 mg/L not analytically tested)
Auxiliary Solvent	None specified
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Coulter counter; HPLC analysis
Remarks - Method	The test was necessarily conducted in two parts to separate the algicidal effect of the test item from growth inhibition caused by reduced light intensity in the coloured test solutions. In Experiment A, ~1×10^4 cells/mL algal cells were subjected to nominal test substance concentrations of 1.0, 3.2, 10, 32 and 100 mg/L (determined from a range finding study). Glass dishes containing purified water were placed above the flasks of each of the 3 replicates for each concentration and 6 control replicates. In Experiment B, the same concentration of algal cells were grown in test medium without the test substance, however the glass dishes above the flasks contained 4 mm depth of test substance at the same nominal test concentrations as for Experiment A (3 replicates per concentration and 6 control replicates). The depth of the test substance in the glass dish is based on the statistical mean depth of algae in the flask. For both experimental parts, the flasks were continuously stirred with magnetic stirrers. Each flask was placed in a black cylinder coated inside with aluminium foil. In addition, all flasks were incubated in a temperature controlled water bath at 22°C and continuously illuminated at ~7900 Lux by placing fluorescent tubes over the flasks. The pH was maintained between 7.8-8.3. Small volumes (~1-2 mL) of the test media and control were taken from all test and control flasks after 24, 48 and 72 hours of exposure, and algal densities measured with an electronic particle counter.

RESULTS

Experiment A (in direct contact and unshaded)

Biomass	*Growth*
E_bC50 (mg/L at 72 h)	*E_bC50 (mg/L at 72 h)*
>100	>100

Experiment B (shaded only)

Biomass	*Growth*
E_bC50 (mg/L at 72 h)	*E_bC50 (mg/L at 72 h)*
100	>100

Remarks - Results	HPLC analysis on a separate series of test (nominal concentrations between 10-100 mg/L) and control samples was performed in duplicate at 0 and 72 hours, with recoveries ranging from 96-108% of the nominal concentrations. The LOEC for Experiment A was 32 mg/L, however, the differences between growth rate inhibition in experiments A and B are lower than 10% and the inhibition curves are essentially the same (µA/µB = 1.0-1.2). AUC inhibition was actually greater in Experiment B at 49% compared to 29% in Experiment A at a test substance concentration of 100 mg/L. This modified algal test has clearly demonstrated that the observed inhibition effect of the notified chemical on *Scenedesmus subspicatus* was caused only by the indirect effect, the light absorption in the coloured test solutions. Thus, a real toxic effect of the test item on the growth of *Scenedesmus subspicatus* can be excluded up to the highest test concentration of 100 mg/L.

CONCLUSION	The notified chemical is non-toxic to *Scenedesmus subspicatus*.
TEST FACILITY	RCC (2001w)

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 obtained from a communal wastewater plant
Exposure Period	3 hours
Concentration Range	25.6-1000 mg/L (nominal)
Remarks – Method	Sewage sludge was subject to nominal concentrations of 25.6, 64.0, 160, 400 and 100 mg/L test substance, and to 3.2, 10 and 32 mg/L of a reference substance (3,5-dichlorophenol). Two control replicates were also run. The respiration rate was calculated as a percentage of the mean of the 2 control respiration rates.
RESULTS	
IC50	>1,000 mg/L
NOEC	1,000 mg/L
Remarks – Results	The test was considered valid because the respiration rates were within 5% of each other, and the IC_{50} of the reference item was 7.7 mg/L (i.e. with the expected range of 5-30 mg/L). A maximum inhibition of 25% was observed at 1,000 mg/L.
CONCLUSION	The notified chemical is slightly inhibitory to sewage sludge micro-organisms.
TEST FACILITY	Solvias AG (2001g)

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