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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Black Dye in LANACRON GREY N-GLN

This Assessment has been compiled in accordance with the provisions of the Industrial Chemicals (Notification and Assessment) Act 1989 (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1410	Huntsman Advanced Materials & Chemiplas Australia Pty Ltd	Black Dye in LANACRON GRAY N-GLN	Yes	≤ 10 tonnes per annum	Textile dye

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

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

R41: Risk of serious eye damage

and

The classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) is presented below.

	Hazard category	Hazard statement
Eye irritation	Category 1	Causes serious eye damage
Environment	Acute 1	Very toxic to aquatic organisms
	Chronic 1	Very toxic to aquatic organisms with long lasting effects

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

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

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

- Use the following risk phrases for products/mixtures containing the notified chemical
  - Conc ≥ 10%: Xi; R41
  - 5% ≤ conc < 10%: Xi; R36

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - Local exhaust ventilation during weighing and pouring the dyestuff containing the notified chemical into the enclosed dyeing vat

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid skin and eye contact
  - Avoid inhalation of dust

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

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

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

- If products and mixtures containing the notified chemical/polymer 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.

Environment

- The following control measures should be implemented by all users to minimise environmental exposure during (manufacture, formulation, use) of the notified chemical:
  - Notified chemical or waste water containing the notified chemical is not to be released, directly or indirectly, to freshwater.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal. Release in concentrated form to the aquatic environment should be prevented.

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/polymer is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
- the concentration of the chemical exceeds or is intended to exceed 20% in imported textile dye preparations;
- the imported textile dye preparations are present in any form other than non-dusting powder;
- the concentration of the chemical exceeds or is intended to exceed 0.1% in the dye solution;
- information associated with the carcinogenicity of the arylamine that may be released through metabolic azo reduction becomes available;
- direct or indirect release to fresh water (river or lake) is proposed.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a textile dye, or is likely to change significantly;
- the amount of chemical being introduced has increased from 10 tonnes per annum, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment;
- the chemical or waste water containing the notified chemical is proposed to be released to sewage treatment plants with effluent outfall that is not to ocean;
- the chemical or waste water containing the chemical is to be released to STP with effluent outfall to ocean where the average daily treatment volume to sewage treatment plant is < 83 ML.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required. Where release to fresh water is proposed, further ecotoxicity testing for fish and algae, and/or supporting data for the removal of the notified chemical in on-site treatment plants may be required.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

Huntsman Advanced Materials Pty Ltd (ABN : 93 091 627 879)
Gate 3, 765 Ballarat Road
Deer Park VIC 3023

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular formula, structural formula, molecular weight, spectral data, purity, residual monomers/impurities,

import volume, and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: flammability, autoignition temperature, acute inhalation toxicity, and bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Black dye in Lanacron Grey N-GLN (product containing the notified chemical at < 20%)

MOLECULAR WEIGHT
> 800 Da

3. COMPOSITION

DEGREE OF PURITY
40-60%

4. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20 ºC and 101.3 kPa: Black powder

Property	Value	Data Source/Justification
Melting Point	Decomposes at >250oC	Measured
Boiling Point	Not determined	Decomposed prior to melting
Particle Density	1692 kg/m3	Measured
Vapour Pressure	3 × 10-30 kPa at 25°C	Estimated
Water Solubility	0.5 g/L at 20oC	Measured
Hydrolysis as a Function of pH	> 1 year at pH 4, 7 and 9 at 25°C	Measured
Partition Coefficient (n-octanol/water)	Log Kow = 1.3 and 1.7 at 20°C	Measured for two components of the notified chemical
Adsorption/Desorption	log Koc < 1.32 at 20°C	Measured
Dissociation Constant	pKa = -5.3	Estimated
Particle Size	Inhalable fraction (< 100 □m): 33.5% Respirable fraction (< 10 □m): 3.1% MMAD = 210 □m	Measured
Flash Point	> 200°C	Based on melting point
Flammability	Not expected to be highly flammable	Based on estimated flash point
Autoignition Temperature	386 °C	MSDS. For imported dye containing < 20% notified chemical
Explosive Properties	Not expected to be explosive	The structural formula contains no explosophers

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

Reactivity
Stable under normal use conditions. No oxidizing properties were observed.

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component (at < 20%) of a powdered textile dye preparation.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	5-10	5-10	5-10	5-10	5-10

PORT OF ENTRY
Melbourne and Sydney

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

USE
The notified chemical will be used as a component (at < 20%) of a textile dye for dyeing carpet and carpet tiles. In future it may find application in dyeing wool and nylon yarns and fabrics.

OPERATION DESCRIPTION
The notified chemical will be imported as a component (at < 20%) of a textile dye as a non-dusting powder or granular solid, which will be dissolved in warm water to produce the dye solutions. Most imported dye will be sold as received, although a small amount (less than 100 kg per year) may be repacked into smaller containers as samples or for use in mill trials. If required, repackaging will take place at the importer's facility.

The dyes will be used in several dyehouses nationally. At the customer facilities, the granular dye (containing the notified chemical at < 20%) will be weighed in a dispensary equipped with local exhaust ventilation, and on average 2.5 kg of dye will be poured through a hatch into the dyeing vat. The dye is mixed with approximately 500 L of water in the enclosed vat to prepare the dye solution (containing 0.5% of the dye stuff, equivalent to < 0.1% notified chemical). Small samples of the dye solution will be removed for quality control testing.

The dye solution containing the notified chemical at < 0.1% will be transferred through an enclosed system to a tank, and then dispensed into an enclosed dyeing machine. The dyeing process is mainly automated with the textile driven by mechanical rollers. The system is mainly enclosed to prevent splashes and spills. After the dyeing process, the wet textiles will be wrapped in plastic film and transported to the wash area where excess dye will be washed off. After washing, the textiles will be dried by hydroextraction followed by heating. During washing and drying, the moist textile will be handled by the operator when wrapping the textile in plastic and during transfer to the hydroextraction and drying units after washing.

Cleaning and maintenance will be performed by the machine operators. This involves flushing the holding and mixing tanks with water.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

Number and Category of Workers

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

Exposure Details

Transport and storage

Worker exposure to the textile dye containing the notified chemical at < 20% during importation, transportation and storage is not expected, except in the unlikely event of an accident where the packaging may be breached.

Preparation of dye solution and end use application

Dermal, ocular and inhalation exposure to the notified chemical may occur during weighing and pouring of the powdered textile dye containing the notified chemical at < 20% into the enclosed dyeing vat. However, exposure should be minimised by the use of an anti-dusting formulation of the dyestuff, the use of purpose-designed dispensary, local exhaust ventilation and PPE such as elbow-length PVC gloves, safety glasses/face shield and protective coveralls when handling the dyestuff containing the notified chemical.

Exposure will also be minimised by the use of an enclosed system to transfer the prepared dye solution containing the notified chemical at < 0.1% to the tank and also by the use of an enclosed dyeing machine. Exposure during manual handling of the dyed wet cloth during transportation to the wash off batch on a pin chain and during further washing and drying processes will be minimised by the use of plastic film to wrap up the wet cloth. Workers are expected to wear gloves, safety glasses and protective coveralls while handling the moist textile, to further reduce exposure.

During cleaning and maintenance processes, dermal, ocular and inhalation (to aerosols) exposure could occur to the notified chemical at concentrations at < 0.1%. Workers are expected to wear an organic vapour cartridge respirator, gloves, safety glasses and coveralls to minimise exposure.

6.1.2. Public exposure

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

The notifier has stated that 98% of the dye is bound covalently to the substrate (cloth). In this regard, the notifier has provided fixation/exhaustion curves and fastness results. The excess dye will be washed off, and the textile will be dried by hydroextraction followed by heating. During washing and drying, unfixed dye will be washed off. Although no leaching/bleeding study has been provided by the notifier, considering almost 100% fixation and low concentration of the notified chemical in the dye solution (< 0.1%), a significant amount of the notified chemical is not expected to be released from the dyed textile over time.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	irritating with persistent colouration of the eye
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 50 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Chromosome Aberration in Chinese Hamster V79 cells	non genotoxic

Toxicokinetics.
Given the high molecular weight of the notified chemical (> 800 Da) dermal absorption is not expected. However, absorption across the gastrointestinal tract by passive diffusion cannot be ruled out given the notified chemical is moderately lipophilic. This is supported by evidence of systemic toxicity in the 28 day repeat dose oral toxicity study.

Acute toxicity.
The notified chemical has low acute oral (LD50 > 2000 mg/kg bw) and dermal toxicity (LD50 > 2000 mg/kg bw) in rats. Although no acute inhalation study has been conducted, exposure is expected to be limited by its low vapour pressure and the non-dusting form of the imported textile dye containing the notified chemical (< 20%).

Irritation and Sensitisation.
The notified chemical is non-irritating to the skin but is an irritant to the eyes of rabbits.

In the eye irritation study provided, the notified chemical caused moderate conjunctival irritation, slight corneal opacity and a short-term reduction or absence of the iridic light reflex. All irritation effects were resolved at the end of the observation period (7 days) apart from pale black staining of the peripheral cornea observed in one animal. However this observation was not noted with this animal at other observation points. As the observation period was not extended to determine if the staining of the cornea was reversible, the notified chemical should be considered as being classified as R41 “Risk of serious eye damage” according to the Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004).

The notified chemical is not a skin sensitiser in guinea pigs.

Repeated dose toxicity.
The NOEL in a 28-day oral repeat dose study in rats was 50 mg/kg bw/day on the basis of the treatment related changes observed in the spleen at 1000 mg/kg bw/day and, to a lesser extent, at 200 mg/kg bw. These findings were accompanied by increased methaemoglobinaemia and increased haemopoietic activity. These effects were mostly reversed in the 14-day recovery group.

Mutagenicity and Carcinogenicity
Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. The azo linkage is the most labile portion of an azo dye molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecule into component amines. Some metabolism may also occur in the cells of the bladder wall, and during percutaneous absorption. Anaerobic intestinal bacteria are also capable of reductive cleavage of the azo linkage.

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

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 or the annexes of EU SCCNFP/0495/01 (SCCNFP,

2002). However, the notified chemical can be broken by azo reduction to release an arylamine species. A number of in vitro genotoxicity studies have been reported in the literature for the arylamine which have provided conflicting results in regards to its mutagenicity potential. However, it is not classified as a carcinogen on Safe Work Austalia's Hazardous Substances Information System (HSIS) and is classified as Group 3 'not classifiable as to carcinogenicity in humans' by the International Agency for Research on Cancer (IARC).

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard et al 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. HPLC analysis provided by the notifier indicated that the notified chemical contains a number of impurities. About half of the impurities are structurally similar to the notified chemical. As such, these impurities are unlikely to contribute to carcinogenicity of the notified chemical. However, there are a small percentage of unidentified impurities that may be free amine species. Free amines may exhibit a higher risk of toxicity as they are likely to be more readily absorbed across the skin.

The notifier supplied test results showing that the notified chemical was found to be not mutagenic in bacteria (under the conditions of the Ames test used), and did not induce chromosomal aberrations in mammalian cells in vitro.

Overall, on the basis of weight of evidence the notified chemical is not expected to be genotoxic.

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

R41: Risk of serious eye damage

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical is expected to have low acute oral and dermal toxicity. It is not a skin sensitiser and is not expected to be genotoxic. The notified chemical is not a skin irritant but is irritating to the eyes and there is the possibility of persistent staining of the eye. There is evidence of systemic toxicity by the oral route based on a 28-day repeat dose oral toxicity study (NOEL = 50 mg/kg bw/day), however based on the high molecular weight (> 800 Da) of the notified chemical, systemic toxicity via the dermal route is not expected.

No acute inhalation toxicity study was submitted for the notified chemical. A significant proportion (~34%) of the powdered notified chemical is inspirable and could be inhaled into the upper respiratory tract. However, only a small fraction (< 4%) is of small enough particle sizes to reach the lower respiratory tract (< 10 µm). The imported product containing the notified chemical will contain anti-dusting agents and is in the form of granules with a size range of 150-350 microns. This, coupled with the notified chemical having a negligible vapour pressure and the use of local exhaust ventilation when handling the powdered textile dye indicates that inhalation exposure will be minimal.

The greatest risk from exposure to the notified chemical is irritation or permanent staining of the eyes. Workers most at risk of eye irritation effects will be those handling the notified chemical as introduced at concentrations up to 20% during weighing and addition of the textile dye to the dyeing vats. This risk should be minimised by the expected use of PPE (elbow-length PVC gloves, safety glasses/face shield and protective coveralls), local exhaust ventilation and purpose-designed dispensary during these processes.

The risk of irritation is not expected during other operations as workers will only be exposed to low concentrations (0.1%) of the notified chemical in solution.

Overall, under the conditions of the occupational settings described, the risk presented by the notified chemical to the health and safety of workers is not expected to be unreasonable.

6.3.2. Public Health

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

Although no leaching/bleeding study has been provided by the notifier, the notifier states that almost 100% of the notified chemical will be bound covalently to the substrate (cloth), as shown in fixation/exhaustion curves and fastness study. It is also noted that dyed textile material will be dried by hydroextraction followed by heating and it is expected that unfixed dye will be washed off during washing and heating cycles. Therefore, a significant amount of the notified chemical is not expected to be released from the dyed textile over time.

Therefore, considering the above and the hazard profile of the notified chemical, the risk to the public from exposure to the notified chemical, given its proposed uses, is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

Since the notified chemical will not be manufactured locally, there will be no environmental exposure associated with this process in Australia. Repacking of the dye (less than 100 kg per year) may take place, however, release from the process is expected to be negligible.

RELEASE OF CHEMICAL FROM USE

The dye containing the notified chemical will be used at a single dyehouse in Australia. During use, releases due to spills are expected to account for < 0.2% of the notified chemical. Dissolution of the dye containing the notified chemical takes place in an enclosed vat and the dye is pumped through a closed system to the dyeing machine. The dye will be used to colour textiles by the exhaust dyeing method. Once the dye has diffused into the fibre matrix it is expected to react with the material. The excess dye will be washed off and the fabric is then dried and steamed to fix the dye to the material. The notifier indicated that the fixation rate of the notified chemical to textiles would be 98%. It was indicated that during dyeing processes water containing the notified chemical would be recovered for re-use and treated by ultra filtration and reverse osmosis, however no indication was given how wastes containing the notified chemical would be disposed. Hence it was assumed that 2% of the total imported notified chemical will be discharged to the dyehouse effluent system. Cationic flocculation was indicated by the notifier to be used to remove the notified chemical from dyehouse waste water. However, information regarding the efficiency of removal of the notified chemical from waste water was not provided. Solid waste containing the notified chemical is expected to be collected and disposed of according to State/Territory regulations. The treated effluent containing the notified chemical will be disposed of to the sewer.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of notified chemical will share the fate of articles in which it is incorporated. These articles are expected to be disposed of to landfill at the end of their useful life. Solid waste generated at the dyehouse including the residue in empty import containers (up to 0.2%) will be disposed of according State/Territory regulations. The treated effluent containing the notified chemical will be disposed of to the sewer.

7.1.2. Environmental Fate

A hydrolysis study on the notified chemical indicated that it is hydrolytically stable in water. Studies submitted on the notified chemical indicate it is not readily biodegradable nor inherently biodegradable. Therefore the notified chemical has the potential to be persistent in the environment. Notified chemical released to sewer is not likely to be removed from the water column during sewage treatment plant (STP) processes as it has a low soil absorption coefficient (Koc) and is not expected to degrade rapidly. The notified chemical released to STPs is therefore expected to reach surface waters. The notified chemical is not expected to bioaccumulate based on its high water solubility, high molecular weight and charge.

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

For details of the fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A Predicted Environmental Concentration (PEC) has been determined based on the notifier's information for the operational procedures at the dyehouse. It was assumed that a maximum of 42 kg of notified chemical (i.e. the maximum annual import volume (10,000 kg) ÷ 240 days used per year) would be used at the dyehouse per day with a total of 2% released to sewer based on a 98% fixation rate. The notified chemical concentration entering the STP was calculated (i.e. mass of unfixed notified chemical released to sewer per day ÷ sewage treatment plant (STP) average daily flow rate). The average daily flow rate of the STP receiving the dyehouse effluent was calculated by dividing the annual STP volume (30,326 ML) by 365 days. No removal of the notified chemical during STP processes was predicted by SimpleTreat (EC, 2003). The calculations and resulting riverine and marine PECs are summarised in the table below.

Calculation Factor	Value
Maximum daily use of notified chemical	42 kg
Chemical in dyehouse wastewater released to STP per day (assuming 2% released to sewer)	0.84 kg
Average daily flow rate of receiving STP	83 ML
Removal of notified chemical in STP	0%
PECriver = estimated concentration of notified chemical in diluted STP effluent per day (dilution factor = 1)	10.1 µg/L
PECocean = estimated concentration of notified chemical in diluted STP effluent per day (dilution factor = 10)	1.01 µg/L

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LC50 > 0.31 to ≤ 0.4 mg/L	Very toxic
Daphnia Toxicity (48 h)	EC50 > 100 mg/L	Not harmful
Algal Toxicity (72 h)	ErC50 ≥ 35.6 mg/L	Not toxic, potentially harmful
Inhibition of Bacterial Respiration (0.5 h)	IC50 > 100 mg/L	Not expected to be inhibitory to bacterial respiration at concentrations ≤ 100 mg/L

Specific guidance is provided for the hazard classification of metals under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009). However, the notified chemical is expected to be a substitutionally inert metal complex which is not expected to rapidly transform in aquatic environments. The ecotoxicity endpoints for the notified chemical above are therefore suitable to use for classification under the GHS without the inclusion of effects due to dissolved metal ions released from the notified chemical.

The results obtained for algal growth inhibition are concluded to be very likely influenced by the light absorbing properties of the notified chemical, but it cannot be concluded that algal growth has been inhibited solely as a result of a reduction in light intensity (EC 2006, and references therein). The ErC50 for algae is therefore classified as not toxic to algae but potentially harmful to algae.

Under the GHS the notified chemical is considered to be very toxic to fish, not toxic but potentially harmful to algae and not harmful to aquatic invertebrates. Based on the acute toxicity to fish the notified chemical is formally classified under the GHS as "Acute category 1; Very toxic to aquatic life". The notified chemical is acutely very toxic to aquatic life and is demonstrated to be not rapidly degradable. It is therefore formally classified as "Chronic category 1; Very toxic to aquatic life with long lasting effect" under the GHS.

Inhibitory or toxic effects were noted in the 28 day biodegradation tests at notified chemical concentrations ≥ 30 mg/L. The sludge respiration inhibition test EC50 after 30 minutes was >100 mg/L, although inhibition was noted at all test concentrations. A no-effect concentration (NOEC) or EC10 were not reported.

7.2.1. Predicted No-Effect Concentration

The endpoint range of the most sensitive species, 96 h fish LC50, determined from ecotoxicological studies submitted for the notified chemical was used to calculate the Predicted No-Effect Concentration range (PNECfish). An assessment factor of 100 was used as acute toxicity endpoints are available for the effects of the notified chemical on aquatic species from three trophic levels. See the study summary in Appendix C for discussion of the endpoint range.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (fish, 96 h)	> 0.31 to ≤ 0.4	mg/L
Assessment Factor	100	
PNECfish:	> 3.1 to ≤ 4.0	µg/L

When the notified chemical is expected to be released through a sewage treatment plant (STP) and there is evidence for toxicity of the chemical towards STP microorganisms a Predicted No-Effect Concentration for micro-organisms (PNECmicro-organisms) should be considered (EPHC, 2009). In lieu of a NOEC or EC10, the lowest test concentration with an observed inhibitory effect (3.2 mg/L) was used with an assessment factor of 50. A conservative assessment factor was used since the NOEC (or EC10) was not available. This resulted in a PNECmicro-organisms = 64µg/L. The PNECfish was < PNECmicro-organisms, hence PNECfish was utilised to determine the risk quotients in Section 7.3 below.

7.3. Environmental Risk Assessment

The Risk Quotients (Q = PEC/PNEC) for the riverine and marine environments and are presented below. These are based on the diluted discharge of effluent from the sewerage treatment plant (STP) that is receiving waste water from the dyehouse.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	10.1	> 3.1 to ≤ 4.0	≥ 2.53 to < 3.26
Q - Ocean	1.01	> 3.1 to ≤ 4.0	≥ 0.253 to < 0.326

Based on the above calculations, there is potentially an unreasonable risk to freshwater compartments (Q > 1) if dyehouse effluents are directly or indirectly released to these environments. However, the notifier indicated the notified chemical will only be used at a dyehouse that releases effluent to an STP with ocean outfall. Based on the assessed use pattern the notified chemical is not expected to pose an unreasonable risk to the marine environment (Q < 1). The above risk quotients are upper limits as there is expected to be some removal of the notified chemical from dyehouse effluent by further waste water treatment systems prior to release to the STP. However information on the efficiency of these procedures was not provided.

Whilst the notified chemical may have potential toxicity or inhibitory effects on sewage sludge microorganisms, the notified chemical is not expected to pose an unreasonable risk to STP processes based on the results of sludge inhibition test provided and the assessed use pattern.

If the notified chemical is proposed to be used at a site where released to an STP with ocean outfall and average of flow rate less than 83 ML/day or released to an STP without ocean outfall, the notified chemical will require reassessment. If release to fresh water is proposed, further ecotoxicity testing for fish and algae, and/or supporting data for the removal of the notified chemical in on-site treatment plants may be required.

Appendix A: Physical and Chemical Properties

Melting Point/Freezing Point Decomposes at > 250oC

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks OECD TG 102 Melting Point/Melting Range. In this test, no melting was observed up to the maximum temperature (300 oC) for the test method. However in the Vapour Pressure report the notified chemical was observed to decompose at > 250oC in a melting point test.
TEST FACILITY Ciba-Geigy (1995a)

Boiling Point Not determined

METHOD Calculated boiling point is approximately 800 oC (refer to Vapour Pressure report)
Remarks Expected to decompose at >250 oC prior to boiling point reached (refer to Vapour Pressure report).

Particle Density 1692 kg/m3

METHOD Helium- density measuring appartus
TEST FACILITY RWYÜV (1995)

Vapour Pressure 3 × 10-30 kPa at 25oC

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The vapour pressure was estimated using the Modified Watson Correlation method based on an estimated boiling point at 250oC. The estimated boiling point was chosen on the basis that an exothermic reaction was observed at 250oC when conducting a melting point determination. Thus the boiling point is > 250 oC. The calculated boiling point was 800 oC using Meissnor's method.
TEST FACILITY RCC (1995a)

Water Solubility 0.5 g/L at 20 oC

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method with spectrophotometric analysis. The pH of the solution at 20°C was 9.4.
TEST FACILITY Ciba-Geigy (1995b)

Hydrolysis as a Function of pH

METHOD EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*t½ (years)*
4	25	>1
7	25	>1
9	25	>1

Remarks A preliminary test was conducted at pH 4, 7, 9 and at 50°C for up to 5 days. The test substance was stable (i.e. <10% degradation) at pH 7 and 9. Thus, a higher tier test was conducted at pH 4. Concentrations were determined by HPLC.
TEST FACILITY Ciba-Geigy (1995c)

Partition Coefficient (n-octanol/water) log Pow = 1.3 at 20oC (first component)
log Pow = 1.7 at 20oC (second component)

METHOD OECD TG 117 Partition Coefficient (n-octanol/water).
EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Flask Method. The partition coefficient was determined for each of the two isomers of the notified chemical.
TEST FACILITY RCC (1995b)

Adsorption/Desorption – screening test log Koc = <1.32 at 20°C

METHOD OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
Remarks The log Koc of the reference substances was in the range of 1.32 – 5.63. Since the log Koc of the test substance was below the lower end of the reference range (phenol), the actual log Koc could not be determined using this method.
TEST FACILITY RCC (2003)

Dissociation Constant pKa = -5.3

METHOD OECD TG 112 Dissociation Constants in Water.
Remarks The molecular structure of the free acid of the notified chemical was used for the estimation of the dissociation behaviour. The estimated pKa value for the notified chemical indicates that the reaction centre will be completely dissociated in the environmental pH range of 4 – 9.
TEST FACILITY RCC (1995c)

Particle Size 210 μm (MMAD)

METHOD OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*	
< 10	3.1	
≥ 63-100	9.01	< 63 24.55%
≥ 100-200	14.18	≥ 200-500 29.06 %
≥ 500-710	12.24	
≥ 710-1000	9.33	
> 1000	*1.63*	
Remarks	Multistage impactor (Range 0.5-63 μm) and sieving (> 63 μm) methods were used. Particle density was 1.692 g/cm3.	

TEST FACILITY RWYÜV (1995)

Appendix B: Toxicological Investigations

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity. EC Directive 92/69/EEC B.1 Acute Toxicity (Oral).
Species/Strain	Rat/HanIbm: WIST (SPF)
Vehicle	Bi-distilled water
Remarks - Method	Oral gavage to 5 male and 5 female rats at one dose only, 2000 mg/kg bw.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M	2000	0
2	5 F	2000	0

LD50	>2000 mg /kg bw
Signs of Toxicity	One male had dyspnoea, ruffled fur and emaciation; this same animal had slightly lower body weight gain during the first week compared to the other animals. No clinical signs of toxicity were observed in any of the other animals and there were no deaths.
Effects in Organs	Nil in any animal
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	RCC (1995d)

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/Han \Ibm: WIST (SPF)
Vehicle	Bi-distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	5 male and 5 female rats treated with one dose only, 2000 mg/kg bw.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M	2000	0
2	5 F	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Black discolouration on the application site was observed in all animals from test day 2 and persisted until test day 6 (1 male ,1 female), day 7 (2 males) and day 11 (2 males, 4 females).
Signs of Toxicity - Systemic	Nil except reduced body weight gain in 2/5 females in week 1 believed by the authors to be due to the semi-occlusive dressing.
Effects in Organs	No macroscopic signs. Microscopic not conducted.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	RCC (1995e)

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 (2 female, 1 male)
Vehicle	Moistened with bi-distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol.deviations.
Remarks - Results	There were no signs of erythema or oedema observed in any test animal. Pale blue staining of the skin caused by the test substance was observed in all animals at 1and 24 hours after removal of the dressing. The staining persisted to 48 and 72 hours for the 2 females.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	RCC (1995f)

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 (2 female, 1 male)
Observation Period	7 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	2	2	2	3	< 7days	0
Conjunctiva: chemosis	2	2.33	2.33	4	< 7days	0
Corneal opacity	1	1	0.5	1	< 7days	0
Iridial inflammation	2	0.67	0.5	2	< 7days	0

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

Remarks - Results	Reversible corneal opacities, slight in degree and moderate conjunctival irritation and watery discharge were observed in all animals up to and including the 72 hour observation period. A short-term reduction or absence of the iridic light reflex was evident in all animals (in one rabbit, up to 72 hours). After 7 days, no signs of irritation were evident. Pale black staining of the cornea was noted in one rabbit at the 7 day observation period. No staining was noted at other observation points for this animal.
CONCLUSION	The notified chemical is irritating to the eye.
TEST FACILITY	RCC (1995g)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation - Maximization Test. EC Directive 92/69/EEC B.6 Acute Toxicity - Skin Sensitisation - Maximization Test.
Species/Strain	Guinea pig/
preliminary study	Maximum Non-irritating Concentration: intradermal: 5% topical: 50% (in vaselinum album)
main study	
Number of Animals	Test Group: 20 Control Group: 10
induction phase	Induction Concentration: intradermal: 5% topical: 50% (in vaselinum album)
Signs of Irritation	Erythema could not be assessed due to black discolouration of test site. Oedema was not present in any test animal.
challenge phase	
1st challenge	intradermal: Not performed topical: 50%
Remarks - Method	Intradermal induction was conducted on day 1, topical induction on day 8 and topical challenge on day 22. Following challenge the test sites were treated with depilatory cream to remove staining caused by the notified chemical and allow for the reading of any skin reactions. All animals at the control and test group were pretreated with 10% SLS in paraffinum per liquidum on test day 7 (24 hours prior to induction).

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge**	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50%	*0/20*	*0/20*	-	-
	0%	0/10	0/10	-	-

* 2nd challenge was not performed

Remarks - Results

CONCLUSION	No irritation was observed for any animals in both the test and control groups. There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	RCC (1995h)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Wistar HanIbm (SPF)
Route of Administration	Oral – gavage/diet/drinking water
Exposure Information	Total exposure days: 28 days Dose regimen: once daily, 7 days per week Post-exposure observation period: 14 days
Vehicle	Bi-distilled water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10/sex	0	0
low dose	5/sex	50	0
mid dose	5/sex	200	0
high dose	10/sex	1000	0
control recovery	5/sex	0	0
high dose recovery	5/sex	1000	0

Mortality and Time to Death

There was no mortality in the test group during the course of the study. One control female died after blood sampling, one day after the last administration.

Clinical Observations

All test animals were of normal appearance and behaviour throughout the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Some statistically significant but not toxicologically significant elevations in uric acid, total cholesterol concentration, triglyceride concentration and alanine aminotransferase activity were seen in mid and high dose animals. All elevations in clinical biochemistry returned to normal after the recovery period.

Changes in haematological parameters, such as increased number of reticulocytes and increases in HFR reticulocyte fluorescence were seen in mid and high dose animals of both sexes and considered of test-article related.

There were no changes in urinalysis data of toxicological significance at termination of treatment. Light brown urine discolouration was observed in 4/10 females in the high dose group. Bilirubin scores were increased in both sexes in mid and high doses and these returned to normal after the recovery period. Increased bilirubin scores were considered due to interference of the test article or its metabolites with the reagent test-strip reaction as there were no supporting changes in plasma biochemistry or underlying morphology.

Effects in Organs

Gross pathology:

Organ weights (absolute and relative weights of spleen, kidney and liver) were increased dose-dependently in female animals during the treatment period. Increase in liver and kidney weights were considered due to metabolic adaptations. The increase in spleen weights was considered due to extramedullary haemopoisis. Both extramedullary haemopoiesis and haemosiderosis were seen histopathologically in the mid and high dose spleens of both sexes.

Bluish discolouration of the gastrointestinal tract was seen in both mid and high dose animals of both sexes. This was considered due to the passive colouration of the tissues by the test article.

Histopathology:

The severity of splenic haemopoiesis and haemosiderosis was increased in female and male rats at mid and high doses. This effect persisted in high dose males until after the recovery period.

Remarks – Results

Toxicologically relevant changes occurred in the spleen in both sexes of the mid and high-dose groups.

CONCLUSION

The Lowest Observed Adverse Effect Level (LOAEL) was established as 200 mg/kg bw/day and the No Effect Level (NOEL) was 50 mg/kg bw/day in this study, based on methemoglobinemia with an increase in haemopoietic activity in both sexes of animals at 1000 mg/kg bw and also, but to a lesser extent, at 200 mg/kg bw.

TEST FACILITY RCC (1995i)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure (Test 1) and Pre incubation procedure (Test 2)
Species/Strain	S. typhimurium: TA1535, TA1537, TA98, TA100 E. coli: WP2uvrA, WP2
Metabolic Activation System	S9 fraction Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 33.3-5000 µg/plate b) Without metabolic activation: 33.3-5000 µg/plate
Vehicle	DMSO
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*
Absent				
Test 1	None	5000	None	None
Test 2		None	None	Negative
Present				
Test 1	None	None	None	Negative
Test 2		None	None	Negative

Remarks - Results	No treatment related increase in numbers of revertant colonies was seen in any of the tester strains. Slight toxic effects were seen in the reduction in the number of revertants in Test 1, TA 1537 treated with 5000 µg/plate without metabolic activation.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	RCC (1995j)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Cytogenetic Test. EC Directive 92/69/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Chinese Hamster
Cell Type/Cell Line	V79 cell line
Metabolic Activation System	S9 fraction Araclor 1254 induced rat liver
Vehicle	DMSO
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	3,5,10*, 30,60*, 110*	18 h	18 h	110
Test 2	10,30, 60, 110*	28 h	28 h	110
Present				
Test 1	3,5,10*, 30,60*, 110*	4 h	18 h	110
Test 2	10, 30,60, 110*	4 h	28 h	110

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	*Cytotoxicity in Preliminary Test**	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	None*	None	None	Negative
Test 2		None	None	Negative
Present				
Test 1	None*	None	110	Negative
Test 2		None	110	Negative

*Pretest concentrations ranged from 0.3 to 110 µg/mL

Remarks - Results

In both experiments, in the presence or absence of S9, no statistically significant or biologically relevant increase in the frequency of cells with chromosomal aberrations was noted.

There was no biologically relevant increase in the occurrence of polyploid metaphases.

EMS (Ethylmethanesulfonate) and CPA (Cyclophosphamide) were used as positive controls and showed distinct increases in cells with structural chromosomal aberrations.

CONCLUSION

The notified chemical was not clastogenic to Chinese Hampster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY

RCC (1995k)

Appendix C: Environmental Fate and Ecotoxicological Investigations

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (>70% purity)
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Microorganisms from a domestic waste water treatment plant
Exposure Period	26 Days
Auxiliary Solvent	None
Analytical Monitoring	Electro-chemical analysis by electrode type manometer
Remarks - Method	The test was conducted according to the guidelines above and in compliance with good laboratory practice (GLP) regulations. The biodegradation of the test substance was investigated by determining the biological oxygen demand (BOD) for two solutions comprising test media, test substance (100 mg/L) and inoculums (30 mg/L) over a period of 26 days. An abiotic control, an inoculum blank, two procedure controls (aniline, 100 mg/L) and a toxicity control (test substance, 100 mg/L, and aniline, 100 mg/L) were run in parallel. Test conditions: 22 ± 1°C, darkness, pH 7.4 – 7.7 (pH 10.8 for the toxicity control at the end of the test). The chemical oxygen demand (COD) of the test substance was determined to be 86.6 mg ThOD/100 mg test substance. The theoretical oxygen demand (ThOD) of aniline was calculated as 241 mg O2/100 mg aniline.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
		18	64.9
26	5.2	26	71.9

Remarks - Results	At the end of the test, the BOD of the test substance suspensions (BOD = 0 and 11 mg O2/L) was below or slightly above the BOD of the mean inoculums blank (2 mg O2/L). The BOD of the test substance was below the BOD observed in the abiotic control (14 mg O2/L). Expressed as a percentage, these values correspond to an average of 5.2% degradation. Therefore, the test substance was determined to be not readily biodegradable under the conditions of the test. The reference compound, aniline, degraded by 64.9% at the end of the 10-day window (Day 18). By day 26, the reference compound had degraded by 71.9%. Thus the suitability of the inoculums for this test was demonstrated. The test was terminated on day 26 due to a malfunction of the agitating/incubating equipment. The test was considered valid as all validity criteria were fulfilled. No degradation was observed in the toxicity control (BOD = 7 mg O2/L), indicating an inhibitory or toxic effect of the test substance to the test microorganisms under the conditions of the test.
CONCLUSION	The test substance is not readily biodegradable
TEST FACILITY	RCC (1995l)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical (>70% purity)
METHOD	OECD TG 302C Inherent Biodegradability: Modified MITI-Test(II).
Inoculum	Aerobic activated sludge from domestic waste water treatment plant
Exposure Period	28 Days
Auxiliary Solvent	None
Analytical Monitoring	Electro-chemical analysis by electrode type manometer
Remarks – Method	The test was conducted according to the guidelines above and in compliance with good laboratory practice (GLP) regulations. The inherent biodegradability of the test substance was investigated by determining the biological oxygen demand (BOD) for three solutions comprising test media, test substance (30 mg/L) and inoculum (100 mg/L) over a period of 28 days. An abiotic control, an inoculum blank, two procedure controls (aniline, 100 mg/L) and a toxicity control (test substance, 30 mg/L, and aniline, 100 mg/L) were run in parallel. Test conditions: 25°C, darkness, pH 6.8 – 7.6. The theoretical oxygen demand (ThOD) of the test substance was determined to be 140 mg ThOD/100 mg test substance , or 200 mg ThOD/100 mg test substance when accounting for nitrification. The theoretical oxygen demand (ThOD) of aniline was calculated as 241 mg O2/100 mg aniline.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
		7	73.5
28 Days	0	14	82.3

Remarks – Results	The BOD of the test substance suspensions (BOD = 8, 8 and 3 mg O2/L) was below the BOD of the inoculum blank (26 mg O2/L) after 28 days of exposure. Therefore, the test substance was determined to be not inherently biodegradable under the conditions of the test. The reference compound, aniline, degraded by 73.5% after seven days of exposure. Thus the suitability of the inoculums for this test was demonstrated. The test was considered valid as all validity criteria were fulfilled. No degradation was observed in the toxicity control (BOD = 0 mg O2/L), indicating an inhibitory or toxic effect of the test substance to the test microorganisms under the conditions of the test.
CONCLUSION	The test substance is not inherently biodegradable.
TEST FACILITY	RCC (1995m)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical (>70% purity)
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Semi-static EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Semi-static.
Species	Rainbow trout (Oncorhynchus mykiss)
Exposure Period	96 hours
Auxiliary Solvent	None

Water Hardness	215 mg CaCO3/L
Analytical Monitoring	HPLC and UV/VIS-detection (as the metal complex)
Remarks – Method	The test was conducted according to the guidelines above and in compliance with good laboratory practice (GLP) regulations. The test concentrations were based on the results of a range finding test (non-GLP). A semi-static test system was used with daily changes of test medium. A flow-through test was not used as a homogenous stock solution of test substance could not be prepared. No significant deviations to protocol were reported. Test conditions: 13.1-15.2°C, pH 7.9-8.7, 16-h light to 8-h darkness photoperiod, dissolved oxygen concentration was 8.2 mg or higher.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0 (Control)	-	7	0	0	0	0	0
0.46	0.31	7	0	0	0	0	0
1.0	0.45	7	0	0	7	-	-
2.1	1.4	7	0	7	-	-	-
4.6	3.4	7	1	7	-	-	-
10	3.7	7	1	7	-	-	-

LC50	> 0.31 to ≤ 0.4 mg/L at 96 hours
NOEC	0.31 mg/L at 96 hours.
Remarks – Results	Mortalities were observed in the two highest doses from 1 hour post treatment. Sublethal effects including extreme mucous secretion, changed body colour, swimming at surface, strong ventilation and apathy were observed after 2 hours in the 3 highest test concentrations. The gills of the fish at these test concentrations were covered with test substance particles. The test media showed a strong colouration caused by the test substance, and a part of the test substance settled on the bottom of the test vessels. There were no mortalities in the control. Analysis of the test media showed the test substance concentration to be between 26% and 78% of the nominal value, or 37% to 73% when calculated as the average over all measurements per test concentration. Therefore, the results are based on the mean measured concentrations. The deviation between nominal and actual test substance concentrations increase with concentration. This was interpreted by the study author to be due to the low solubility of the test substance leading to increasing precipitate with the progression of the test. Due to the very steep concentration effect relationship, the median lethal concentration (LC50) was determined as the geometric mean of the two consecutive concentrations with 0% and 100% mortality. The LC50 was reported to be at 96 hours, however this is inconsistent with 100% mortality of fish at 48 hours. Hence the 96 hour LC50 is expected to lie between the NOEC and the reported value. The no-observed effect concentration (NOEC) was determined directly from the raw data. Excess undissolved test substance was not reported for the test concentration used to derive the LC50. However, as fish gills were observed to be covered with test substance particles at the three highest test concentrations these results should be treated with caution.
CONCLUSION	The notified chemical is very toxic to fish
TEST FACILITY	RCC (1995n)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (>70% purity)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static
Species	Daphnia magna
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg CaCO3/L
Analytical Monitoring	HPLC and UV/VIS-detection
Remarks - Method	The test was conducted according to the guidelines above and in compliance with good laboratory practice (GLP) regulations. The test concentrations were based on the results of a range finding test (non-GLP). Test conditions: 21.6-21.8°C, pH 7.8 - 8.1, 16-h light to 8-h darkness photoperiod, dissolved oxygen concentration was 8.3-11.9 mg/L in all test concentrations.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0 (Control)	-	20	0	0
2.1	1.5	20	0	0
4.6	4.4	20	0	4
10	9.8	20	0	7
21	21.7	20	0	7
46	49.8	20	0	6
100	111.2	20	0	7

EC50	>100 mg/L at 48 hours
NOEC	1.5 mg/L (2.1 mg/L nominal) at 48 hours
Remarks - Results	No mortalities or immobilisation of the test animals were seen in the control or at the lowest test concentration (1.5 mg/L) after 48 hours but were seen in all higher test concentrations (from 4.6 to 100 mg/L). There was no immobilisation observed at 24 hours and the dose response curve of the effect at 48 hours was very shallow indicating that the test substance is a low toxicant in Daphnia magna. The test media showed a strong colouration caused by the test substance, and a part of the test substance settled on the bottom of the test vessels. Analysis of the test media showed the test substance concentration to be between 58 to 112% of the nominal value or 95 to 111% when calculated as the average over all measurements per test concentration, except for the 2.1 mg/L test concentration (72% of nominal). Thus, the test concentrations were reported as nominal, except for the 2.1 mg/L test concentration (mean measured value 1.5 mg/L). The median effect concentration (EC50) could not be determined from the concentrations tested. The no-observed effect concentration (NOEC) was determined directly from the raw data.
CONCLUSION	The notified chemical is not harmful to Daphnia magna.
TEST FACILITY	RCC (1995p)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical (>70% purity)
METHOD	OECD TG 201 Alga, Growth Inhibition Test - Static EC Directive 92/69/EEC C.3 Algal Inhibition Test - Static
Species	Scenedesmus subspicatus
Exposure Period	72 hours
Concentration Range Nominal	0 (Control), 0.32, 1, 3.2, 10, 32, 100 mg/L (selected based on preliminary test results)
Concentration Range Actual	Lowest doses not analysed (i.e. control, and test concentrations of 0.32 and 1mg/L); 2.8, 9.3, 28, 92 mg/L (88%, 93%, 88%, 92% of nominal) for the higher doses.
Auxiliary Solvent	None
Water Hardness	24 mg CaCO3/L
Analytical Monitoring	Electronic particle counter
Remarks - Method	The test was conducted according to the guidelines above, with modifications for coloured substances, and in compliance with good laboratory practice (GLP) regulations. As the test substance is a dye which results in coloured media, the test method was modified to differentiate between a reduced growth of algae due to real toxic effects of the test substance and the algal cells or due to an indirect effect, a reduced algal growth by light absorption in coloured test solutions. Three experiment parts were used: Part A used the usual algal toxicity test protocol. Erlenmeyer flasks containing test substance and algae were covered with glass dishes containing untreated test water. Algal growth inhibition in these vessels would be due to any toxic effects in addition to reduced light intensity. Part B used the same procedure but replaced the contents of the glass dishes with the coloured test substance. The Erlenmeyer flasks contained algae but no test substance. Thus Part B results show the algal growth inhibition due to light absorption only. Part C was conducted for the correction of algal cell density which was determined by the particle counter to be significant in a range-finding test. Erlenmeyer flasks contained test substance alone. Test concentrations were based on the results of a range finding test (non-GLP). Test conditions: 24-24.2°C, pH 8.0 to 10.3, continuous illumination.

RESULTS

	Biomass		*Growth*	
	EbC50 (95% CI) mg/L at 72 h	*NOEC mg/L*	*ErC50 (95% IL) mg/L at 72 h*	*NOEC mg/L*
Part A-Test solutions (coloured)	10.5 (3.7-29.9)	3.2	35.6 (11.3-112.3)	3.2
Part B-No test substance	8 (2.9-22.5)	3.2	23.8 (9.1-62.5)	3.2

Remarks - Results	All test media down to the test concentration of 1.0 mg test substance were slightly to strongly coloured by the test substance.

As the NOECs are identical, and no significant differences were observed between the EC50s, in Parts A and B tests, the modified algal test demonstrates that the observed algal growth inhibition effect of the test substance is caused by an indirect effect, namely the light absorption in the coloured test solution. Therefore, the authors of the study conclude that a real toxic effect of the test substance on the algal cells can be excluded up to at least the highest concentration tested. However, this method is too simplistic to allow evaluation of both toxic and light absorption effects of coloured substances (EC, 2006a). Therefore, as it cannot be concluded that algal growth has been inhibited solely as a result of a reduction in light intensity (EC, 2006b) the endpoint can be classified. It is likely that the coloured solution did contribute to the observed growth inhibition to a large extent if not completely.

Microscopic examination of the algal cells indicated that algal cell shape did not differ between algae grown in Part A and Part B conditions.

The median effective concentrations (EC50s) and 95% confidence intervals were determined by Probit analysis and the no-observed effect concentrations (NOECs) were determined by Dunnet-tests.

CONCLUSION The notified chemical is not toxic to algae, but may potentially be harmful to algae.

TEST FACILITY RCC (1995q)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical (purity > 70%)

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
- Inoculum: Activated sewage sludge
- Exposure Period: 30 minutes
- Concentration Range: Nominal: 3.2, 10, 32, 50, 100 mg/L; Actual: Not reported
- Remarks – Method: A definitive test was conducted according to the guidelines above at test substance concentrations of 3.2 – 100 mg/L. A blank control and reference (3,5-dichlorophenol) control were run in parallel. No significant deviations to the test protocol were reported. Test conditions were: 21.5°C, pH 7.8 – 7.9.

RESULTS
- IC50: > 100 mg/L (30 min)
- NOEC: Not reported
- Remarks – Results: All validity criteria for the guidelines were satisfied. Slight inhibition of respiration rates (6.3% and 14.7%) was observed at the two lowest test concentrations, respectively. At 32, 50 and 100 mg/L the inhibition was 25.9%, 29.4% and 26.2% respectively.

CONCLUSION The test substance is not inhibitory to bacterial respiration at concentrations > 100 mg/L under the conditions of the test.

TEST FACILITY RCC (1995r)

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