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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

R507-2

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, Water, Heritage and the Arts.

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:

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
DIC Australia Pty Ltd (ABN 12 000 079 550)
42 Sunmore Close
HEATHERTON VIC 3202

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical name, Other names, CAS number, Molecular and structural formula, Purity, Impurities, Additives, Import volume, Confidential details of use, Identity of manufacturer.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Partition coefficient, Hydrolysis as a function of pH, Dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Belgium (2008)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
R507-2
SYMULER Red 3120 (Red pigment containing < 20% notified chemical).

MOLECULAR WEIGHT
>500 Da

ANALYTICAL DATA
Reference NMR, IR and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Red powder.

Property	Value	Data Source/Justification
Melting Point	> 400°C	Measured
Density	1790 kg/m^3 at 20°C	Measured
Vapour Pressure	< 1.33 x 10^{-11} kPa at 20°C	Measured
Water Solubility	< 0.07 mg/L at 20°C	Measured. The solubility of the notified chemical was below the detection limit.
Hydrolysis as a Function of pH	Not determined	Test not performed due to the low water solubility. The notified chemical is not expected to hydrolyse given no hydrolysable functional groups exist in the molecule.
Partition Coefficient (n-octanol/water)	Not determined	Test was not possible since the notified chemical is a salt and is ionisable in the environmental pH range of 4-9. Estimation by calculating the quotient of the n-octanol solubility and the water solubility was also not possible due to lack of detection in both water and n-octanol.
Adsorption/Desorption	log K_{oc} < 1.32	Estimated by using HPLC. The result indicates a low absorption of the notified chemical to the organic carbon in soil from water.
Dissociation Constant	Not Measured	The test was not performed due to the low water solubility of < 0.07 mg/L. The notified chemical contains functional groups that would be dissociated in the environmental pH range of 4 – 9, however, the low water solubility prohibits the possible dissociation.
Particle Size	Inhalable fraction (<100 μm): 100% Respirable fraction (<10 μm): 67.7% MMAD* = 5.171 μm	Measured
Flash Point	Not determined.	Not applicable, notified chemical is a solid.
Flammability	Not highly flammable.	Measured
Autoignition Temperature	326°C	Measured
Explosive Properties	Not explosive.	Measured
Oxidising Properties	Not oxidising	Estimated based on chemical structure.

* MMAD = Mass Median Aerodynamic Diameter

DISCUSSION OF PROPERTIES
The notified chemical has a low vapour pressure and low water solubility. All of the notified chemical is inspirable and could be inhaled into the upper respiratory tract, and a large majority (67.7%) is of small enough particle size (<10 μm) to reach the lower respiratory tract

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

Reactivity
Based on the chemical structure and experience in use, the notified chemical is predicted to be stable under normal conditions. It is not flammable, explosive or expected to be oxidising.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia:
1) as a component (up to 20%) of pigments; and
2) as a component (up to 2%) of formulated printing inks.

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

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

PORT OF ENTRY
Sydney and Melbourne.

TRANSPORTATION AND PACKAGING
1) Pigments containing the notified chemical will be transported in 3-ply kraft paper bags with a laminated liner (10 kg), by truck.
2) Inks containing the notified chemical will be transported in cans and drums (18 L and 200 L, respectively), by truck.

USE
The notified chemical is an additive for pigments and will be used in printing inks.

OPERATION DESCRIPTION
Ink formulation
A typical batch size for ink formulation will be 1 tonne, which will be processed over 0.3 days. The imported pigment containing the notified chemical (at up to 20%) will be weighed into a pre-mixing tank, along with varnishes and solvents. After the pre-mixing stage, the components will be passed through a bead-mill and may be filtered. Finished inks containing up to 2% of the notified chemical will then be manually filled into 18 L cans and/or 200 L drums.

Imported inks containing the notified chemical (at up to 2%) will be used without reformulation.

Use of printing inks
The ink containing the notified chemical (at up to 2%) will be drawn automatically into the printing machine from the drum or can by a pipe. The printing operation will be completely automated and largely enclosed.

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)*
Printing ink manufacturers			
Pre-mixing	2	0.25	12
Charging and filling containers	2	2	12
Printer operators	2 per site	0.2	12

EXPOSURE DETAILS

Ink formulation

Workers who may experience the greatest exposure will be those handling the notified chemical at up to 20% concentration during weighing and transfer of the imported pigments to the pre-mixing vessel. As well as dermal and ocular exposure, exposure by the inhalation route by these workers is also possible given airborne dusts may be generated when handling the powdered pigments during weighing and addition to the mixer. These operations are expected to be carried out under LEV, which should reduce the exposure of workers from airborne dusts. Furthermore, workers are expected to wear protective masks while handling the powdered pigments.

Workers may experience dermal and/or accidental ocular exposure to the notified chemical at up to 2% during pre-mixing and operation of the beads mill, performing filtration operations, or during manual filling of cans/drums. Where transfer of the finished ink products to the packaging line takes place via a dedicated pipeline (automated filling of cans/drums), worker exposure to the ink containing the notified chemical is not anticipated.

Use of inks

Limited dermal and/or ocular exposures (to up to 2% concentration) may be possible during connection of the printing machine to the ink drums or cans, and during cleaning and maintenance of the printing equipment. Exposure during the printing operations is expected to be minimal given the operation is totally automated.

Exposure to dried inks

All users of inks containing the notified chemical are expected to make dermal contact with dry printed surfaces. However, once the ink is dried, exposure to the notified chemical is not expected, as the notified chemical will be trapped within the dried polymer matrix and is not expected to be bioavailable.

All ink manufacturing workers and printer operators are expected to wear personal protective equipment (PPE) such as overalls, safety glasses, gloves, protective masks and safety boots, and all operations are expected to be conducted under LEV.

6.1.2. Public exposure

The general public may make dermal contact with articles printed with the printing ink containing the notified chemical. However, once the ink has dried, the notified chemical will be trapped within the dried polymer matrix and is not expected to be bioavailable.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 3 mg/L/4 hour; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL ≥ 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test.	non genotoxic

Toxicokinetics
The notified chemical is practically insoluble in water therefore significant transfer across biological membranes is not anticipated. This is supported by the low toxicity observed in the acute oral, dermal and inhalation toxicity studies.

All of the notified chemical is inspirable and could be inhaled into the upper respiratory tract, and a large majority (67.7%) is of small enough particle size (<10 μm) to reach the lower respiratory tract (tracheobronchial and pulmonary regions). Larger particles of inhalable size (<100 μm) are expected to deposit in the nasopharyngeal region and cleared by coughing/sneezing. Due to the low water solubility of the notified chemical smaller particles lodging in the tracheobronchial region are likely to be cleared by the mucociliary mechanism. However, inhaled respirable particulates of the notified chemical lodging in the pulmonary region may not be readily cleared due to its lack of solubility. Hence, higher concentrations of exposure may be expected to result in increased impairment of clearance mechanisms.

Acute toxicity.
The notified chemical was found to be of low acute oral and dermal toxicity in studies conducted on rats (LD50 > 2,000 mg/kg bw). The notified chemical was also determined to be of low acute inhalation toxicity (LC50 > 3 mg/L) in a study where mice were exposed to the nose only for 4 hours to a solid aerosol (particulates) of the notified chemical. The maximum attainable concentration was 3 mg/L and the particulates had an average mass median aerodynamic diameter (MMAD) of 1.2 μm.

Irritation and Sensitisation.

Slight irritation of the conjunctivae was observed in a study conducted on rabbits; however the irritations were resolved in all treated animals within 48 hours. These irritations were not severe enough to warrant hazard classification. The notified chemical was found to be non-irritating to the skin based on no evidence of erythema or oedema in a study conducted on rabbits.

The notified chemical was not a skin sensitiser when tested up to a concentration of 25% in a mouse local lymph node assay. Therefore, the notified chemical is unlikely to cause skin sensitisation in exposed humans.

Repeat dose toxicity
The NOAEL in a 28-day oral repeat dose study in rats was determined to be > 1000 mg/kg bw/day based on the absence of any toxicological significant effects observed at the highest dose level of 1000 mg/kg bw/day.

Mutagenicity.
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 (EC, 2004) or the annexes of EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into arylamine species, although these are unlikely to be mutagenic.

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The notified chemical contains a number of impurities. The impurities have been identified to be mainly isomers of the notified chemical. As such, these impurities are unlikely to contribute to carcinogenicity of the notified chemical. However, there are a small percentage of 4 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*. Furthermore, the notifier also supplied test results showing that the notified chemical was found to be not mutagenic using the modified Ames test for azo dyes (Prival and Mitchell, 1982). The Prival and Mitchell modified Ames test utilises a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out. This modified test is thought to yield a greater detection of mutagenic azo dyes as it is recognised that the standard procedure is not sufficiently sensitive for these chemicals, likely due to their complex metabolism *in vivo* (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998).

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

Health hazard classification
Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
The notified chemical has no identified hazards, and due to its lack of solubility is not expected to be significantly absorbed following occupational exposures. Therefore, the dermal and/or ocular exposure that may occur during most uses of the notified chemical is unlikely to pose a significant risk to the health of workers. Although slight eye irritation is possible based on a study on rabbits, the risk is considered low based on the proposed use of PPE.

Repeated inhalation of airborne dusts of the notified chemical, or inhalation of high airborne concentrations, may present some risk of lung overloading effects. This scenario may be possible during the formulation of inks from the imported powdered pigments, where inhalation exposure to airborne particulates of the notified chemical may occur. The Australian recommended exposure standard for nuisance dust is 10 mg/m^3 [NOHSC 3008:(1995)], but the American Conference of Governmental Industrial Hygienists (ACGIH) recommends an exposure limit of 3 mg/m^3 for "respirable (insoluble) particulates (not otherwise regulated)" (ACGIH, 2006). An exposure limit of 1.5 mg/m^3 is recommended on the MSDS provided by the notifier for respirable dust for the notified chemical.

Given the proposed use of dust masks and LEV while handling the imported powdered pigments and low acute inhalation toxicity observed in a study on mice, the risk to the health of workers by the inhalation route is not considered unacceptable.

The risk associated with the use of inks is expected to be low, based on the low hazard that is expected for the concentrations present in these formulations. In addition, the risk to these workers is further reduced through the expected use of PPE.

Worker exposure to dried inks is unlikely to result in exposure to the notified chemical, so the risk presented by the notified chemical in this scenario is expected to be negligible.

6.3.2. Public health

Given the public will not be exposed to the notified chemical in a form that is likely to be bioavailable, the risk to the health of the pubic is not considered unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported to Australia in paper bags for further reformulation.
In the formulation process, it is possible that a small quantity of pigment containing the notified chemical is scattered when it is transferred to pre-mixing tank from paper bags. However the use of local ventilation equipment will keep the release low at this level of supply. The equipment involved will be cleaned by washing with organic solvents. Releases from spills, residues in paper bags and cleaning of equipment are estimated to be less than 0.5% of the imported volume.

RELEASE OF CHEMICAL FROM USE

No significant release is expected from the printing process of the ink containing the notified chemical as the printing is done automatically and the chemical will be covered by resin after being printed to the substrate (e.g. paper, film). Equipment cleaning will be done by washing with organic solvents. Wastes from residues in containers and from rinsings of the printing equipment are estimated to be up to 0.7% of the imported volume.

RELEASE OF CHEMICAL FROM DISPOSAL

Any releases occurring from the reformulation and application will be sent to landfill. The waste organic solvents used for equipment cleaning will be collected by authorized contractors for further re-use, and the solid sediments containing the notified chemical will be separated out and sent to landfill.

7.1.2 Environmental fate

The notified chemical is not considered readily biodegradable according to the provided environmental fate study. It is not expected to have bioaccumulation potential based on its extremely low water solubility. For the details of the environmental fate studies please refer to Appendix C.

In addition to the releases occurred during reformulation and application, most of the notified chemical will share the fate of the substrates to which the ink is applied via printing. Some may be directly sent to landfill, and the majority may undergo waste paper recycling process. During paper recycling, most of the associated notified chemical will either precipitate out from the effluent water due to the low water solubility or be separated out by flocculation, and will finally be sent to landfill.

In landfill, the notified chemical will be slowly degraded via biotic and abiotic pathways to form small molecules of water, salts and oxides of carbon and nitrogen.

7.1.3 Predicted Environmental Concentration (PEC)

The calculation of PEC is not necessary since no significant release of the notified chemical to the aquatic environment is expected based on the reported use pattern of the notified chemical.

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	LL50 > 100 mg/L	Not toxic to fish up to the limit of solubility
Daphnia Toxicity	EL50 > 100 mg/L	Not toxic to daphnia up to the limit of solubility
Algal Toxicity	EC50 > 0.08 mg/L	Not toxic to algae up to the limit of solubility
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not toxic to sludge micro-organisms

The notified chemical is not toxic to the aquatic life up to the limit of the solubility.

7.2.1 Predicted No-Effect Concentration

The PNEC has not been calculated since no significant release of the notified chemical to the environment is expected based on the reported use pattern.

7.3. Environmental risk assessment

The Risk Quotient (PEC/PNEC) has not been calculated since no significant release of the notified chemical to the environment is expected based on the reported use pattern.

The notified chemical is not considered to pose an unacceptable risk to the aquatic ecosystem based on its reported use pattern and structural features.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is not classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

Human health risk assessment

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

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

Environmental risk assessment

On the basis of the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced in the powdered pigment:
  - Local exhaust ventilation wherever weighing and addition to mixers occurs

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in the powdered pigment:
  - Avoid the formation of airborne dusts
  - The level of atmospheric dust should be maintained as low as possible. The Australian recommended exposure standard for nuisance dust is 10 mg/m^3 [NOHSC 3008:(1995)]. However, the ACGIH exposure standard for atmospheric dust is 3 mg/m^3.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced in the powdered pigment:
  - Dust mask sufficient for respirable particulates (where high airborne concentrations occur)

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

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

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

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from as an additive for pigments used in printing inks, or is likely to change significantly;
  - the amount of chemical being introduced has increased from one tonne 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 Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point > 400°C

Method OECD TG 102 Melting Point
Remarks No melting was observed within the measuring range of the experiment (up to 400°C). An endothermic peak was observed during the Differential Scanning Calorimetry experiment, but this was considered to be due to the evaporation of water of crystallisation.
Test Facility NOTOX (2008a)

Density 1790 kg/m^3 at 20°C

Method OECD TG 109 Density of Liquids and Solids.
Remarks Gas comparison pycnometer method.
Test Facility NOTOX (2008a)

Vapour Pressure < 1.33 × 10^{-11} kPa at 20°C

Method OECD TG 104: Vapour Pressure.
EEC directive 92/69 EEC, A, Part A.4: Vapour Pressure.
Remarks Isothermal thermogravimetric effusion method was chosen for the determination of the vapour pressure. The validity of the method was verified using a suspension of hexachlorobenzene in chloroform as a reference substance. Temperature program was selected and carried out at 200-240°C for the measurement, since the compound was found not molten or boiled at a temperature below 400°C.

The weight loss of the notified chemical at 210°C was lower than that of benzo(ghi)perylene (vapour pressure is 1.33 × 10^{-8} Pa at 20°C) at the same temperature. From this, it was concluded that the vapour pressure of the notified chemical is < 1.33 × 10^{-11} kPa.
Test Facility NOTOX (2008a)

Water Solubility < 0.07 mg/L at 20°C

Method OECD TG 105: Water Solubility.
EC Directive 92/69/EEC A.6: Water Solubility.
Remarks Column Elution Method was chosen for the main test after a preliminary test. Samples were taken for analysis at flow rates of 12 mL/hour and 24 mL/hour. HPLC-UV was used for the determination of concentrations.

n-Hexane was used as a solvent to prepare the carrier material. An experiment was also performed using a column filled with blank carrier material with the identical procedure except for the loading of the notified chemical.

After completion of the main study, test substance was detected in the column material.

No undissolved particles were detected in the elute samples from the main test using Tyndall effect (light scattering). No test substance was detected in the samples from the blank column. The limit of detection (LOD) in the end solution was determined to be 0.07 mg/L.

No notified chemical was detected in any of the aqueous fractions collected at both flow rates of 12 and 24 mL/h, indicating that the solubility of the notified chemical in water is < 0.07 mg/L at 19.9 ± 0.6°C and pH of 7.85 - 7.87.
Test Facility NOTOX (2008a)

Adsorption/Desorption – screening test log K_{oc} < 1.32

Method OECD TG 121: Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage

Sludge using High Performance Liquid Chromatography (HPLC);
EC directive 2001/59 EC, C.19: Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks The test was conducted at a column temperature of 35°C.

Since it was not possible to elute the test substance from the column at neutral pH, a mobile phase consisting of 55/45 (v/v) methanol/citric acid buffer pH 6.0 was used.

In the chromatogram of the test substance solution, one test substance peak was observed at both a wavelength of 210 nm and the test substance specific wavelength of 516 nm. The test substance eluted before the reference substance phenol (with a log Koc of 1.32), indicating that the log K_{OC} for the notified chemical is < 1.32.

Test Facility NOTOX (2008a)

Particle Size

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

Range (μm)	*Mass (%)*
< 100	100
< 10	67.7

Remarks Dispersant: Acetone. Particle size measured using the Malvern Mastersizer 2000 laser Diffraction Analyser.

Under microscopic observation the particle size range was approximately 3-82 μm, and under 400x magnification was observed to be made up of agglomerated particles. In the laser diffraction analysis the particle range was found to be 0.2 μm to 100 μm.

Test Facility Chilworth (2007)

Flammability Not highly flammable.

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The flame of a gas burner could ignite the test substance pile. The test substance burned with a yellow flame, developed smoke and turned into a grey/black ash residue. After removal of the ignition source, propagation of combustion by smouldering was observed. The burning time over a distance of 200 mm was 34 minutes and 19 seconds.

As no burning with flame or smouldering over 200 mm within 4 minutes was observed, the notified chemical is considered not highly flammable.

Test Facility NOTOX (2008a)

Autoignition Temperature 326°C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks After the test, the sample cube contained a small amount of grey/black, charred remains.
Test Facility NOTOX (2008)

Explosive Properties Not explosive.

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks *Thermal sensitivity*: None of the six sheet steel tubes showed a deformation or had burst into fragments after exposure to intense heat for 5 minutes.

Mechanical sensitivity with respect to shock: None of the six cylinders had burst open spontaneously when a 10 kg mass had dropped from 0.4 m height.

Mechanical sensitivity with respect to friction: No fire, smoke, crepitation or other (traces of) vigorous reactions were observed in each of the six friction tests (friction being a porcelain peg (loaded to 360 N) and a porcelain plate).

Test Facility NOTOX (2008a)

Oxidizing Properties Not oxidising.

Method EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks Based on the chemical structure, the result of any testing for oxidising properties was predicted to be negative.
Test Facility NOTOX (2008a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar Crl:WI (Han)
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	Hunched posture and/or uncoordinated movements were noted in all animals on Day 1. In the first set of animals, red staining of the back was observed on Day 1 and red faeces were observed between Days 2 and 4. These findings were considered to be related to staining properties of the test substance and to be of no toxicological significance. The body weight gain shown by the animals over the study period was considered to be normal.
Effects in Organs	No treatment related effects observed.
Remarks - Results	No deaths occurred during the 14-day study.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	NOTOX (2008b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Rat/Wistar Crl:WI (Han)
Vehicle	Propylene glycol
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Scales and/or scabs were observed in the treated skin area of the majority of females. Red staining of the treated skin area and/or other body parts of the animals was observed and was considered to be related to staining properties of the test substance.

Signs of Toxicity - Systemic	Hunched posture, piloerection and/or chromodacryorrhoea were noted among both males and females. In addition, lethargy, flat posture, uncoordinated movements, quick breathing, shallow respiration, ptosis and/or hypothermia were noted in all males. The animals had recovered from the symptoms between Days 2 and 3. Based on their mild nature and short duration, these symptoms were considered to be of no toxicological relevance.
Effects in Organs	At macroscopic post mortem examination, isolated grey/white foci, enlargement and dark red discolouration were found in the papillary process in the liver of one male. No abnormalities were observed in the other animals.
Remarks - Results	The changes noted in body weight gain in males and females were within the range expected.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	NOTOX (2008c)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rats/Wistar Crl:WI
Vehicle	None
Method of Exposure	Oro-nasal exposure.
Exposure Period	4 hours
Physical Form	solid aerosol (particulate).
Particle Size	The mean mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined twice. The MMAD was 1.2 μm and 1.0 μm respectively and the GSD was 2.4 and 3.3 respectively.
Remarks - Method	Limit Test. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
1	5F/5M	14.9	3.0	0/10

LC50	> 3 mg/L/4 hours
Signs of Toxicity	No mortalities and no signs of systemic toxicity were observed.
Effects in Organs	Dark red discolouration of the caecum in one male and two females and reddish discolouration of the tail in all animals.
Remarks - Results	Slight body weight loss was shown by the males between Days 1 and 8. This body weight loss was considered minor based on the absence of any corroborative findings in these animals. Red staining of the fur due to disposition of test substance on the snout of the animals during exposure was noted in all animals throughout the entire observation period. Red faeces were noted in all animals from Day 2 to Day 5. This finding is considered to be due to grooming of the fur and subsequent ingestion of the test substance. An average maximum attainable exposure concentration of 3.0 mg/L was attained instead of the target concentration of 5 mg/L. Based on the maximum attainable concentration in air, the minor,

temporary decrease in body weight, the absence of mortality and signs of toxicity, the notified chemical is considered to be of low toxicity.

CONCLUSION The notified chemical is of low toxicity via inhalation.

TEST FACILITY NOTOX (2008d)

B.4. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 Males
Vehicle 50% ethanol in water
Observation Period 72 hours
Type of Dressing Semi-occlusive.
Remarks - Method Red staining of the skin prevented scoring for erythema at 1, 24 and 48 hours after exposure.

RESULTS

Remarks - Results There was no evidence of a corrosive effect on the skin.
No oedema was observed throughout the observation period. No erythema was noted at 72 hours after exposure. Given these results, it was considered by the study authors that no severe erythema was present during the first 48 hours after exposure.

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

TEST FACILITY NOTOX (2008e)

B.5. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 Males
Observation Period 72 hours
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	0.3	0.3	0.3	1	< 48 hrs	0
Conjunctiva: chemosis	0	0	0	2	< 24 hrs	0
Conjunctiva: discharge	0	0	0	1	< 24 hrs	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results Instillation of the test substance resulted in irritation of the conjunctivae, which consisted of redness, chemosis and discharge. The irritation had

completely resolved within 48 hours.

No iridial irritation or corneal opacity were observed.

Remnants of the test substance were present in the eye on Day 1 and on the outside of the eyelids during the observation period. Red staining of the fur on the head and paws, caused by the test substance, was noted throughout the observation period.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY NOTOX (2008f)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/CBA strain
Vehicle DMF
Remarks - Method No significant protocol deviations. The highest concentration (25%) was chosen as this was the highest that could be prepared homogeneously.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	410	1.0
5	609	1.5
10	433	1.1
25	630	1.5
Positive Control[1]		
0 (vehicle control[2])	462	1.0
5	445	1.0
10	930	2.0
25	462	5.7

[1]Hexylcinnamaldehyde
[2]Acetone:Olive oil (4:1)

Remarks - Results Red staining by the test substance prevented scoring for erythema. No oedema was observed in any of the animals examined.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical. The positive control induced an appropriate response, confirming the validity of the test system.

TEST FACILITY NOTOX (2008g)

B.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain Rat/ Wistar Crl:WI(Han)
Route of Administration Oral – gavage

Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations other than there was no recovery period.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5F/5M	0	0
low dose	5F/5M	50	0
mid dose	5F/5M	150	0
high dose	5F/5M	1000	0

Mortality and Time to Death

No mortalities were observed.

Clinical Observations

There were no toxicological significant changes observed in functional performance, food consumption and body weight gains. Red faeces were noted among all dose groups.

Incidental findings that were noted included rales, chromodacryorrhea and a dark red eye. These were considered of no toxicological significance based on these findings being occasionally noted in rats of this age and strain under previous studies.

Laboratory Findings – Clinical Chemistry, Haematology

Clinical Chemistry

No toxicologically relevant changes.

All statistically significant changes were considered to be of no toxicological significance as they occurred in the absence of a treatment related distribution and were of a very slight nature (i.e. remained within the range considered normal for rats of this age and strain). These changes included lower inorganic phosphate levels in males at 50 mg/kg bw/day, while in females at 1000 mg/kg bw/day these changes consisted of higher total bilirubin and calcium levels and lower chloride levels.

Haematology

Statistically significant higher red blood cell and relative basophil counts in females at 1000 mg/kg bw/day were considered to be of no toxicological significance as these changes occurred in the absence of supportive morphological or haematological changes.

Effects in Organs

Reddish contents of the gastro-intestinal tract were noted in 1/5 males at 50 mg/kg bw/day, 3/5 males and females at 150 mg/kg bw/day (one additional male showing reddish contents of the stomach only) and in all animals at 1000 mg/kg bw/day.

No toxicologically significant changes were noted in organ weights.

Remarks – Results

The red faeces noted among all groups treated with the test substance was confirmed by a dose related incidence of reddish contents of the gastro-intestinal tract/stomach. No histopathological correlates in the gastro-intestinal tract were noted. These findings were considered to be due to passive staining properties of the test substance (a red powder) and to be of no toxicological significance.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as > 1000 mg/kg bw/day in this study, based on no adverse effects observed at the highest dose level tested.

TEST FACILITY NOTOX (2008h)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE

METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Test 1 - Plate incorporation procedure Test 2 - Pre-incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Plate Assay (Test 1): Rat liver S9-mix induced by phenobarbital/β-naphthoflavone. Pre-incubation Assay (Test 2): uninduced male Golden Syrian Hamster liver S9-mix with flavin mononucleotide.
Concentration Range in Main Test	a) With metabolic activation: 10-1000 µg/plate b) Without metabolic activation: 10-1000 µg/plate
Vehicle	DMSO
Remarks - Method	The pre-incubation procedure incorporated the Prival and Mitchell modification for azo dyes (Prival and Mitchell, 1982).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5000			
Test 1		> 1000	1000	negative
Test 2		> 1000	333*	negative
Present	> 5000			
Test 1		> 1000	1000	negative
Test 2		> 1000	1000	negative

* Only slight precipitation was observed at 333 µg/plate.

Remarks - Results	No increase in the number of revertants was observed under all conditions tested with or without metabolic activation in both the plate and pre-incubation procedure.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the standard Ames test and modified Prival and Mitchell method for azo dyes.
TEST FACILITY	NOTOX (2008i)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Chinese hamster
Cell Type/Cell Line	Chinese hamster lung fibroblasts/(CHL/IU) cells
Metabolic Activation System	Rat liver S9-mix induced with phenobarbital/5,6-benzoflavone
Vehicle	5 w/v% carboxymethyl cellulose sodium salt (CMC) in distilled water
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			

Test 1	625, 1250*, 2500*, 5000*	6 hr	24 hr
Test 2	78.1, 156, 313*, 625*, 1250*, 5000	24 hr	24 hr
Present			
Test 1	625, 1250*, 2500*, 5000*	6 hr	24 hr

*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	> 5000	> 5000	625	negative
Test 2	> 1250	1250	78.1	negative
Present				
Test 1	> 5000	> 5000	625	negative

Remarks - Results

Precipitation was observed at all doses.
The frequencies of cells with structural aberration and numerical aberration (polyploid) cells showed less than 5% at all doses of the test substance in the short-term treatments with and without S9 mix and 24 hours continuous treatment.

The validity of the results was confirmed from the results of the negative and positive controls.

CONCLUSION

The notified chemical was not clastogenic to Chinese hamster lung fibroblasts
treated in vitro under the conditions of the test.

TEST FACILITY

Chemicals Evaluation and Research Institute (2008)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 B: Ready Biodegradability: CO2 Evolution Test; EC 92/69/EEC, C.4-C: Carbon dioxide (CO2) evolution test (Modified Sturm Test); ISO Standard 9439 "Water Quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium - carbon dioxide evolution test (1999).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Titration was used to determine the amount of CO_2 produced during the test.
Remarks - Method	The test substance was tested at a nominal concentration of 26.5 mg/L in duplicates, corresponding to 12 mg TOC/L, and at temperature of 21 – 22.1˚C and pH of 7.8 – 7.8. To prepare the test solutions, weighed amounts of test substance were added to the test bottles (2 litres) containing medium with microbial organisms and mineral components. To this end, 10 mL of Milli-RO water was added to each weighing bottle containing the test substance. After vigorous mixing (vortex) the resulting solution was added quantitatively to the test medium. The test solutions were continuously stirred during the test. Furthermore, the test medium was daily swirled around to ensure optimal contact between the test substance and test medium. In addition to the duplicate blank control tests containing only inoculum, a positive control (containing reference substance sodium acetate and inoculum) and toxicity control (containing test substance, reference substance and inoculum) were carried out.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	1	5	32
14	3	14	76
29	6	29	86

Remarks - Results	The criteria for test validity were met. A mean degradation of 6% was reached after 29 days, indicating that the notified chemical is not readily biodegradable.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	NOTOX (2008j)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203: Fish, Acute Toxicity Test – static; EC Directive 92/69/EEC C.1: Acute Toxicity for Fish – static; ISO International Standard 7346-1: Water quality - Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static method; Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment number 23, December 14, 2000.
Species	*Cyprinus carpio* (Carp)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	HPLC for determination of test contents.
Remarks – Method	Filtrated water accommodation fractions (WAFs) were used as the test solutions. The preparation of test solution started with loading rates of 100 mg/L followed by 5 – 6 minutes of ultrasonication, 3 days of magnetic stirring, and a filtration through a 0.45 µm membrane filter. A solution of lower test concentration was prepared by ten-fold dilution of the filtrate with the test medium. All the final test solutions were clear and colourless. Given the low water solubility of the test material, test was conducted directly without any preliminary test. The number of fish used in the tests was 7 for the control and the filtrate test, 3 for the diluted filtrate test. All tests were conducted at 22.1 – 22.8˚C and pH of 7.6 – 7.9. Test concentrations were analyzed at 0, 24, and 96 hours. Pentachlorophenol was used for the reference test at concentrations of 0.10, 0.22 and 0.46 mg/L to check the sensitivity of the test system.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1h*	*24h*	*48h*	*72h*	*96h*
0	0	7	0	0	0	0	0
10	-	3	0	0	0	0	0
100	< 0.02 (detection limit)	7	0	0	0	0	0

LL50	> 100 mg/L at 96 hours.
NOEL	Limit of solubility at 96 hours.
Remarks – Results	Neither mortality nor sub-lethal effect was observed in the study and the reference tests. HPLC analysis showed that the measured concentration was below the limit of detection, i.e. < 0.02 mg/L. The filter residue was identified as the notified chemical.
CONCLUSION	The notified chemical is not toxic up to the limit of solubility.
TEST FACILITY	NOTOX (2008k)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 202: Daphnia sp. Acute Immobilisation Test and Reproduction Test - static. EC Directive 92/69/EEC C.2: Acute Toxicity for Daphnia - static. ISO International Standard 6341: "Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus - Acute toxicity test, Third edition, 1996-04- 01. Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment number 23, December 14, 2000.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	HPLC for determination of test contents
Remarks - Method	WAFs were used for the test and prepared in the same way as that for the fish test (filtrate and diluted filtrate). The final test solutions were all clear and colourless. Both the control test (without the notified chemical) and the filtrate test were conducted in 4 replicates, and the diluted filtrate test was conducted in duplicate, each of all the tests used 5 organisms. All tests were conducted at 18 – 22˚C and pH of 7.7 – 7.9. Potassium dichromate was used in the reference test at concentrations of 1.10, 0.18, 0.32, 0.56, 1.0 and 1.8 mg/L (and a blank control) to check the sensitivity of the test system.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	20	0	0
10	-	10 (5*)	0	0
100	< 0.02 (detection limit)	20 (1*)	0	0

* Number of daphnia observed trapped at the surface of the test solution. These organisms were reimmersed into the respective solution before recording of mortality.

LL50	> 100 mg/L at 48 hours
LOEL	100 mg/L at 48 hours
Remarks - Results	No mortality was observed in all the tests. A few daphnids were observed trapped at the surface of the test solutions. This may be considered as sub-lethal effect, given the fact that test solutions were clear and colorless and therefore, no physical effects from undissolved particles would be expected. Given the concentration of the test material was below the detection limit, it is considered that the notified chemical is not toxic to invertebrates up to the limit of its solubility.
CONCLUSION	The notified chemical is not toxic to *Daphnia magna* up to the limit of the solubility.
TEST FACILITY	NOTOX (2008l)

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. ISO International Standard 8692: Water quality - Freshwater algal growth inhibition test with unicellular green algae, Second edition, 01 October 2004. Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment number 23, December 14, 2000.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	48 hours, static
Concentration Range	Nominal: 100 mg/L Actual: 0.08 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	HPLC for determination of test contents
Remarks - Method	Based on a range-finding test, a limit test was conducted at a nominal concentration of 100 mg/L (filtrated WAF) with an initial algal cell density of 1×10^4 cells/mL and pH of 7.8. The WAFs used were prepared in the same way as that for the fish test (filtrate). The final test solutions were all clear and colourless. A blank control test without the test material was also conducted. Both groups of tests were conducted in 6 replicates plus an extra replicate for sampling purpose. Analyses for actual exposure concentrations were performed after 24 and 48 hours of exposure. A Reference control test (72-hours, including a blank test) was conducted using potassium dichromate at concentrations of 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 48 h*	*NOEC* *mg/L*	*E_rC50* *mg/L at 48 h*	*NOEC* *mg/L*
> 0.08	≥ 0.08	> 0.08	≥ 0.08

Remarks - Results	The 72-h E_rC50 for potassium dichromate was determined to be 1.0 mg/L with a 95% confidence interval of 0.70 – 1.5 mg/L, which was within the historical range of 0.82 – 2.3 mg/L. The 72-h E_bC50 for the reference substance was determined to be 0.43 mg/L with a 95% confidence interval of 0.32 – 0.57 mg/L. No historical record for this endpoint was available. No reduction of growth rate or inhibition of yield was recorded in both the range-finding and the limit tests. Decreasing of the concentration throughout the exposure test was detected, with the concentration of 0.180 mg/L at the start being decreased to 0.070 mg/L after 24 hours and further to ca. 0.036 mg/L after 48 hours. The average exposure concentration was calculated to correspond to 0.08 mg/L. Considering the low water solubility, the notified chemical is considered not toxic to algae up to the limit of solubility.
CONCLUSION	The notified chemical is not toxic to algae up to the limit of solubility.

TEST FACILITY	NOTOX (2008m)

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. ISO Standard 8192, Water Quality - Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation (2007).
Inoculum	Micro-organisms in activated sludge (Municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands)
Exposure Period	3 hours
Concentration Range	100 mg/L (nominal)
Remarks – Method	The test was conducted in duplicate at a nominal loading rate of 100 mg/L and temperature of 18.1 – 18.5˚C and pH of 8.1 – 8.2. Given the low water solubility of the notified chemical, the test suspensions were prepared separately in Milli-RO water. Initial loading rates of 200 mg/L were stirred for 24 hours in the test bottles. Subsequently, synthetic sewage feed and sludge were added resulting in a loading rate of 100 mg/L. Optimal contact between the test substance and test medium was ensured applying continuous aeration and stirring. A reference control test was conducted using 3,5-dichlorophenol at concentrations of 5.0, 12 and 30 mg/L.
RESULTS	
IC50	> 100 mg/L
NOEC	100 mg/L
Remarks – Results	IC50 for the reference substance was determined to be 8.8 mg/L with a 95% confidence interval ranging between 0.3 – 296.3 mg/L. No inhibition of respiration rate of the sludge was detected under the test conditions.
CONCLUSION	The notified chemical is not toxic to sludge micro-organisms.
TEST FACILITY	NOTOX (2008n)

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