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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

S-500

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, CAS Number, Molecular formula, Structural formula, Molecular weight, Spectral data, Methods of detection and determination, Purity, Information on impurities and additives, Import volume, Use and Identity of sites of reformulation.

NOTIFICATION IN OTHER COUNTRIES
Japan (2008)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
S-500
SYMULLER Fast Yellow 4400NF (≤ 8% notified chemical)

MOLECULAR WEIGHT > 500 Da.

ANALYTICAL DATA
Reference NMR, IR, HPLC, LC/MS, IPC, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Yellow powder

Property	Value	Data Source/Justification
Melting Point	> 300°C	Measured
Boiling Point	> 300°C at 101.3 kPa	Measured
Density	1410 kg/m^3 at 20°C	Measured
Vapour Pressure	< 1.33×10^{-11} kPa at 20°C	Measured
Water Solubility	0.71 mg/L at 20°C	Measured. The water solubility was reported to be 0.71 mg/L at 20°C in the study report. However, further toxicity tests to algae indicated a water solubility of < 0.69×10^{-5} g/L at 23°C. The notified chemical is expected to be insoluble in water based on its hydrophobic structure.
Hydrolysis as a Function of pH	Not Determined.	The test could not be performed due to low water solubility. However, the

		notified chemical contains functional groups that may hydrolyse at the environment pH range of 4-9.
Partition Coefficient (n-octanol/water)	log Pow > 6.5	Measured
Adsorption/Desorption	log K_{oc} > 5.63	Measured. The notified chemical is likely to absorb onto soil (rich in organic carbon) from water.
Dissociation Constant	pKa = 0.36 and 0.96 for component A; pKa = - 4.48, 0.13, 0.66 and 19.4 for component B.	Calculated using pKalc version 5.0.
Particle Size	Inhalable fraction (<100 μm): 100% Respirable fraction (<10 μm): 94.69% MMAD* = 1.452 μm	Measured
Flammability	Not highly flammable	Estimated based on chemical structure
Flammability in contact with water	Not predicted to release flammable gases	Estimated based on chemical structure
Pyrophoric properties	Not predicted to be pyrophoric	Estimated based on chemical structure
Autoignition Temperature	341°C	Measured
Explosive Properties	Not explosive	Measured
Oxidizing Properties	Not predicted to be oxidizing	Estimated based on chemical structure

* MMAD = Mass Median Aerodynamic Diameter

DISCUSSION OF PROPERTIES

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

Reactivity

The notified chemical is not predicted to be reactive under normal environmental conditions. However, it may dissociate at extremely basic or acidic conditions based on calculated pKa values. The generation of airborne dusts may create a dust explosion hazard.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL OVER NEXT 5 YEARS

The notified chemical will be imported in powder form (≤ 8%) as a component of a coloured pigment or as a component of printing inks (0.5%).

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, Melbourne

TRANSPORTATION AND PACKAGING

The notified chemical will be imported in powder form packed in 10 kg 3-ply kraft paper bags with laminated liner or as a component of printing inks in 18 L cans and 200 L drums. The notified chemical will be transported from the wharf to the reformulation sites by road. Once blended, the final ink product will be transported to various customers by road.

USE

The notified chemical will be used as a component in industrial printing inks.

OPERATION DESCRIPTION

The notified chemical in powder form (≤ 8%) will be weighed into a mixing tank and mixed with other ink components. After mixing, the mixture will be poured onto a bead mill for milling into a consistent ink solution. The ink solution will be filtered before being filled into ink containers for sale to customers.
The finished ink product containing the notified chemical will be used in industrial printing machines. The product will be scooped from its container into ink feed ducts on mechanical printing presses, distributed onto rollers, applied to a paper or plastic substrate and laminated with a resin coating.

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 reformulation			
Pre-mixing	2 per site	0.25	150
Transfer of mixtures	2 per site	2	150
End-use in industrial printers			
Printing machine operators	2 per site	0.2	150

EXPOSURE DETAILS
Reformulation into ink products
Inhalation is expected to be the main potential route of exposure when handling, weighing and mixing pigments containing the notified chemical (≤ 8%) in powder form at reformulation sites. Assuming a worst-case scenario involving dry manipulation of the notified chemical in the absence of local exhaust ventilation (LEV), the EASE model predicts an atmospheric particulate concentration of 5-50 mg/m^3. However, the implementation of LEV while handling the notified chemical would lower the predicted atmospheric particulate concentration to 2-5 mg/m^3 according to the EASE model. The notifier supplied an MSDS which recommends the total inhalable dust be monitored to ensure it is present at no more than 4 mg/m^3 and the respirable dust fraction less than 1.5 mg/m^3. The use of respiratory personal protective equipment (PPE) would also help reduce inhalation exposure. Dermal and ocular exposure is also possible during the handling of the notified chemical in powder form. However, it is expected that appropriate personal protective equipment such as impermeable gloves, safety glasses and coveralls will be worn to minimise exposure via these routes.

Once the notified chemical has been mixed to form a wet formulation, it will be poured into a bead mill for milling into a homogenous ink formulation. Dermal and ocular exposure to the notified chemical in the ink formulation at ~ 0.5% may occur from drips, spills and splashes during transfer of the formulation from the mixer to the bead mill and during operation of the mill, cleaning and maintenance of equipment and quality control testing. Exposure is expected to be minimised during these processes by the use of impermeable gloves, safety glasses and coveralls.

No further exposure is expected during the filling and packaging of ink into product containers as this will take place using closed, automated filling and packing equipment.

End use in industrial printing machines
There is potential for dermal and ocular exposure to inks containing the notified chemical during their end use in industrial printing applications. Workers may be exposed to ink containing the notified chemical at ~ 0.5% while transferring ink from the product container to the printing machines. The printing and curing process will be carried out within a closed, automated system, so exposure is expected to be minimal or negligible during these processes. Once cured onto the paper or plastic, the notified chemical will be trapped within the polymer matrix and covered by a resin laminate and therefore unavailable to cause exposure.

Workers are expected to wear safety glasses, protective overalls and safety gloves, during the manual dispensing of inks, washing of rollers and ducts and disposal of empty ink containers to minimize dermal and ocular exposure.

6.1.2. Public exposure

The public will not be exposed to the notified chemical as imported in powder form except in the event of a transport accident.

The public will be exposed to printed paper or plastic containing the notified chemical. However, once the ink is cured, the notified chemical will be trapped within the polymer matrix, and therefore dermal exposure from contact with the printed media is not expected.

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	oral LD50 > 2000 mg/kg bw low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw low toxicity
Rat, acute inhalation toxicity	LC50 = 1-5 mg/L/4 hour harmful
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	non-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 <chromosome aberration>	non genotoxic

Toxicokinetics, metabolism and distribution

The notified chemical is not expected to be readily absorbed across biological membranes given its low water solubility and high log Pow (6.5). This is supported by tests showing a lack of adverse findings following acute and repeated oral exposure in rats and the observation of yellow-coloured faeces in animals in the acute inhalation study and at higher doses in the repeat dose oral toxicity study. Further supporting information was found in a report on chemicals of the same class as the notified chemical. Although repeated oral exposure of this chemical class produced slight staining of the mucosa of internal organs indicating a potential for absorption.

Airborne dusts of the notified chemical are expected to be inhaled readily given that 94.7% of particles are in the respirable range (< 10 μm). A significant proportion of particles of the notified chemical are ≤ 1 μm (39%), and therefore will likely settle in the tracheobronchial or pulmonary region. Absorption across membranes in the lung is unlikely given its low solubility in water. However, some absorption may have occurred in the acute inhalation study (see below) as evidenced by yellow-stained urine. Due to the poor water solubility of the notified chemical, any particles lodging in the tracheobronchial region will be cleared by the mucociliary mechanism and swallowed. Those particles lodging in the pulmonary region will be phagocytosed by alveolar macrophages. But over time inhalation of high concentrations of the notified chemical may lead to accumulation of particles in the pulmonary region which may overwhelm the alveolar macrophage-mediated lung clearance mechanism.

Acute toxicity

The notified chemical was found to be of low acute oral toxicity in 6 female rats tested according to the method described by OECD TG 423 (Safepharm, 2007). There were no mortalities reported at doses up to 2000 mg/kg bw. Adverse findings reported in the study were limited to pale faeces observed in 3 animals 1 day after treatment. These findings were not considered adverse and the oral LD50 was determined to be > 2000 mg/kg bw.

The notified chemical was found to be of low acute dermal toxicity in 10 rats (5/sex) dosed with 2000 mg/kg bw of the notified chemical according to the OECD TG 402 (NOTOX B.V., 2008b). Chromodacryorrhoea (red-coloured tears) was observed in 2 males and 1 female in the first 2 days following treatment. Yellow staining of the treated area of skin as well as several other body parts was observed in all animals throughout the study. In 1 male and 3 females, scales were also observed on the treated skin area. These findings were considered to be treatment-related, however, post mortem examination did not reveal any macroscopic findings due to treatment with the notified chemical and the LD50 was determined to be > 2000 mg/kg bw.

An acute inhalation study was conducted using the notified chemical at concentrations of 3.2 and 5.7 mg/L

according to OECD TG 403. Three males and 3 females from the groups dosed with 5.7 mg/L died during exposure and 2 males and 1 female were found dead on Day 2 following treatment with 3.2 mg/L. The LC50 was established between 1.0 – 5.0 mg/L (See Appendix B for further details).

Irritation
The notified chemical was found to stain the skin of albino rabbits but was not a skin irritant when tested according to OECD TG 404 (NOTOX B.V. 2008d). However, the application of 2000 mg/kg bw to the skin for 24 hours in the acute dermal toxicity study produced scales in 1 male and 3 females suggesting that it may have some irritant properties (NOTOX B.V., 2008b).

The notified chemical was found to be non-irritating to the eye of rabbits in a test conducted according to OECD TG 405 with slight conjunctival effects observed 1 hour after treatment in all animals resolving within 24 hours (2 animals) or 48 hours (1 animal) (NOTOX B.V., 2008e).

Sensitisation
The notified chemical belongs to a class of compounds which contain known skin sensitisers (Barratt et al, 1994). However, tests on similar chemicals did not show any evidence of sensitisation. A local lymph node assay conducted according to OECD TG 429 which involved application of the notified chemical at concentrations up to 50% did not produce any evidence of sensitisation (NOTOX B.V., 2008f).

Repeated Dose Toxicity
In a 28-day repeat dose oral toxicity study, no significant abnormal clinical or histopathological observations, laboratory findings or effects on the target organs were observed in animals treated with the notified chemical at 50, 250 or 1000 mg/kg bw/day. However, these findings may be indicative of poor absorption from the gastro-intestinal tract. Yellow-coloured faeces were observed in all animals treated with ≥ 250 mg/kg bw/day of the notified chemical for the duration of the study. However, this effect was absent by Day 2 of the recovery phase and was consistent with observations from the acute inhalation toxicity study. This may be indicative of poor oral bioavailability.

The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on the absence of adverse effects at the highest dose level (Chemicals Evaluation Research Institute (Japan), 2008).

In repeated dose inhalation studies using similar chemicals, treatment related effects due to deposition of dust particles in the lungs was seen at all doses, with the lowest dose being 54 mg/m^3 (local LOAEL). However, no systemic effects of toxicological significance were seen at any doses and therefore the systemic NOAEL was considered to be 410 mg/m^3. No repeat dose inhalation toxicity tests were conducted on the notified chemical but its toxicity following repeated inhalation exposure is expected to be similar to that described for the similar chemicals. However as these similar chemicals were found to be less toxic than the notified chemical in acute inhalation studies, it may be expected that the notified chemical will cause local effects after repeated exposure at or below the doses observed in the studies on similar chemicals.

Genotoxicity
The notified chemical was found not to be mutagenic in a bacterial reverse mutation assay modified according to Prival and Mitchell (1984) in order to evaluate the mutagenic potential of metabolites of the notified chemical, as recommended in OECD TG 471 (NOTOX B.V., 2008g). The notified chemical was tested at concentrations up to 333 μg/plate in the absence and presence of metabolic activation. Precipitation occurred at 333 μg/plate and prevented testing at higher concentrations.

The notified chemical was found not to induce an increase in the frequency of structural or numerical chromosome aberrations in chinese hamster lung fibroblasts in a study conducted according to OECD TG 473 (Chemicals Evaluation Research Institute (Japan), 2007).

Carcinogenicity
The notified chemical belongs to a class of chemicals which contains several potential carcinogens. The mode of carcinogenicity is thought to rely upon metabolism of the compounds to metabolites capable of damaging DNA (Sagelsdorff et al., 1996). However, the notified chemical did not cause DNA damage in the *in vitro* genotoxicity assays.

In addition, several long-term studies were conducted on structurally similar chemicals and no increased incidence of tumour was observed. NOAEL for rats was > 630 mg/kg bw/day and > 1960 mg/kg bw/day for

mice. Therefore, the notified chemical is not expected to be carcinogenic.

Toxicity for reproduction
No evidence of teratogenicity, maternal or reproductive toxicity was observed in tests on similar chemicals at doses up to 1000 mg/kg bw/day. Therefore, the notified chemical is expected to have low reproductive toxicity.

Summary of expected human health effects
Based on the deaths in the acute inhalation toxicity study when treated with 5.7 mg/L, the notified chemical is considered to be harmful by inhalation (LC50 between 1-5 mg/L) (NOTOX B.V., 2008c). Due to effects seen with similar chemicals following repeated inhalation the notified chemical may also be a chronic respiratory hazard. Based on the formation of scales observed following dermal exposure to the notified chemical in the acute dermal toxicity study, as well as the potential for slight skin irritation seen in studies using chemicals with structural similarity, the notified chemical may have the potential to be a skin irritant in sensitive individuals.

Health hazard classification

Based on the acute inhalation toxicity the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R20 Harmful by inhalation.

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Inhalation of particles of the notified chemical in the imported product (at ≤ 8%) presents the greatest risk for workers during handling, weighing and mixing at the reformulation site. An acute inhalation toxicity study demonstrated that the notified chemical was harmful to rats (LC50 between 1-5 mg/L) (NOTOX B.V., 2008c) following a 4 hour exposure. No repeat dose inhalation study on the notified chemical was conducted. However, given that repeat dose studies on similar chemicals (which are less acutely toxic than the notified chemical) found that local effects due to deposition in the lungs occurred at the lowest dose of 54 mg/m^3 (0.054 mg/L), and the fact that the notified chemical consists of insoluble particles (94.69% in the respirable range (< 10 μm)), there is potential for accumulation in the lungs leading to injury following repeated inhalation. Therefore in order for the risk of lung effects to be minimised the exposure to respirable particles of the notified chemical must be reduced to the lowest practicable level. This may be achieved through the implementation of low-dust handling techniques and engineering controls such as LEV. In addition PPE such as respirators suitable for particulates would be required. The notifier's MSDS recommends that the respirable dust fraction experienced by workers is reduced to a maximum of 1.5 mg/m^3, and therefore measures to achieve this level would minimise the exposure.

Due to its expected low dermal absorption and its low acute and repeat dose oral toxicity, systemic toxicity is not expected following repeated dermal exposure.

The notified chemical is not considered to pose an unreasonable risk to workers when measures are implemented to reduce inhalation exposure to < 1.5 mg/m^3.

6.3.2. Public health
Members of the public will only be exposed to paper and plastic substrates in which the notified chemical will be trapped within a cured polymer matrix. Therefore, the risk is not considered unacceptable, given that exposure is expected to be negligible.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

During reformulation, a small quantity of the notified chemical may be spilled during transfer to the pre-mixing tank from paper bags. However LEV is expected to be in use and dispersive dust is anticipated to be extremely low. Therefore, it is considered that environmental exposure from reformulation will be low.

RELEASE OF CHEMICAL FROM USE
Releases from industrial printing application are not expected as the printing will be done automatically and the notified chemical will be covered by resin after being printed onto paper or plastic substrates. Wastes from spills, residues in containers and rinsings of the printing equipment are estimated to be a maximum of 0.8% of the imported volume. Any spills will be mechanically collected and sent to landfill. Both the containers and printing equipment will be washed with organic solvents.

RELEASE OF CHEMICAL FROM DISPOSAL
The notified chemical collected by LEV and in empty imported paper bags will be disposed of to landfill.
Any contaminated waste material will also be sent to landfill.
The solvents used for washing and cleaning will be recycled for reuse, and the solid sediment (the notified chemical) thus yielded will be sent to landfill.

7.1.2 Environmental fate

The notified chemical is not considered to be readily biodegradable. It may have some potential for bioaccumulation in the aquatic environment. However, this is not considered to be a concern given no significant release is expected based on the reported use pattern. For the details of the environmental fate studies please refer to Appendix C.
Most of the notified chemical that is applied via printing processes will share the fate of the associated substrate, which may be either sent to landfill or recycled (for paper). Considering the highly hydrophobic property and the high log P_{OW} of the notified chemical, any substrates containing the notified chemical which go to waste paper recycling treatment will eventually end up with landfill in the form of sediment sludge. In landfill, the notified chemical will undergo slow degradation processes via biotic and abiotic pathways, forming small molecules of water, salts and oxides of carbon and nitrogen.

7.1.3 Predicted Environmental Concentration (PEC)

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

7.2. Environmental effects assessment

Test 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	EC50 > limit of the solubility	Not toxic to fish up to the limit of solubility
Daphnia Toxicity	EC50 > limit of the solubility	Not toxic to daphnia up to the limit of solubility
Algal Toxicity	EC50 > limit of the solubility	Not toxic to algae up to the limit of solubility
Inhibition of Bacterial Respiration	EC50 > 100 mg/L (nominal)	Not toxic to active sludge bacteria

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

7.2.1 Predicted No-Effect Concentration

The PNEC was not 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 classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] with the following risk phrase:

R20 Harmful by inhalation

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

	Hazard category	***Hazard statement***
Inhalation	4	Harmful if inhaled

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is considered to pose an unacceptable risk to the health of workers, unless the level of airborne particulates of the notified chemical is kept to the lowest practicable level. Therefore the use of engineering controls such as LEV and PPE such as particulate respirators will be required when handling the imported powder containing the notified chemical.

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

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following risk phrase for health hazard classification for the notified chemical:
  - R20 Harmful by inhalation

- Use the following risk phrase for products/mixtures containing the notified chemical:
  - ≥ 25%: R20 Harmful by inhalation

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 product SYMULER Fast Yellow 4400NF:
  - Local exhaust ventilation

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in the product SYMULER Fast Yellow 4400NF:
  - Use low dust techniques during direct handling

- 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 product SYMULER Fast Yellow 4400NF:

- Face mask or respirator suitable for respirable airborne particulates

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 component of a colour pigment for use in industrial printing inks, or is likely to change 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/Freezing Point > 300ºC

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Decomposition or reaction occurred at > 300ºC. Measured with a differential scanning calorimeter.
Test Facility	NOTOX B.V. (2008a)

Boiling Point > 300ºC at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Decomposition or reaction occurred at > 300ºC. Measured with a differential scanning calorimeter.
Test Facility	NOTOX B.V. (2008a)

Density 1410 kg/m^3 at 20ºC

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Measured with a gas comparison stereopycnometer.
Test Facility	NOTOX B.V. (2008a)

Vapour Pressure < $1.33x10^{-11}$ kPa at 20ºC

Method	OECD TG 104: Vapour Pressure. EEC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Measured using the isothermal thermogravimetric effusion method.
Test Facility	NOTOX B.V. (2008a)

Water Solubility 0.71 mg/L

Method	OECD TG 105: Water Solubility. EEC Directive 92/69/EEC A.6: Water Solubility.
Remarks	Flask Method was used. TOC analysis was performed to determine the content of test substance dissolved in the water samples from this test. The pH of the aqueous samples after 72 hours stirring was 5.37. In the preliminary test the water solubility of the test substance was < 10^{-2} g/L. Based on this result, the column elution method should be used for the determination of the water solubility. However, the test substance was not soluble in a volatile solvent, such as hexane or acetone at a concentration required for the column elution method. Since dry-mixing of the test substance with the column material was also not considered to result in a homogeneous mixture, column material for the column elution method could not be prepared, and hence the flask method was the alternative used even though it is not recommended in the test guideline. The value was determined to be 0.71 mg/L at 20ºC. However, HPLC analysis of the test solution in the ecotoxicity study to algae could not detect the test substance above the limit of detection (0.69×10^{-5} g/L), which is far lower than the result determined in this study.
Test Facility	NOTOX B.V. (2008a)

Partition Coefficient (n-octanol/water) log Pow > 6.5

Method	OECD TG 117: Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method. EEC directive 92/69 EEC A.8: Partition Coefficient.
Remarks	HPLC method was used at neutral pH and a column temperature of 23 ± 1.0°C.

In the chromatogram of the test solution, no notified chemical peak was observed. The notified chemical eluted during the column rinse (gradient) from the column, which is after 2,4-DDT (log Pow 6.5). Therefore, the log P_{OW} of the notified chemical is estimated to be > 6.5.
Using the Reckker calculation method, the log P_{OW} of component A and B of the notified chemical were calculated to be 9.44 and 5.72, respectively, which is consistent with the test results.
A high P_{OW} of the notified chemical is expected based on its highly hydrophobic feature of the structure.

Test Facility NOTOX B.V. (2008a)

Adsorption/Desorption – screening test log K_{OC} > 5.63

Method OECD TG 121: Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
EC directive 2001/59 EC, C.19 – Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).

Remarks HPLC method was used at a column temperature of 35°C.
In the chromatogram of the test substance solution no test substance peak was observed during isocratic elution with 55/45 (v/v) methanol/water. The notified chemical eluted during the column rinse (gradient) from the column, which is after 2,4-DDT (log K_{OC} 5.63). Hence, it was estimated that the log K_{OC} of the notified chemical was > 5.63 (K_{OC} > 4.27×10^5).
A high K_{OC} is expected based on the highly hydrophobic structure and high log P_{OW} of the notified chemical.

Test Facility NOTOX B.V. (2008a)

Dissociation Constant pK_a = 0.36 and 0.96 for component A;
pK_a = - 4.48, 0.13, 0.66 and 19.4 for component B.

Method OECD TG 112: Dissociation Constants in Water.

Remarks It is impossible to determine the dissociation constants of the notified chemical experimentally due to its low water solubility in combination with its complexity. As an alternative, the pK_a values were calculated using the pK_{alc} version 5.0 computer program.
For the major component A, two pK_a values were calculated: 0.36 and 0.96. It was assumed that these pK_a values derive from the subsequent protonation of the amide groups.
For the neutral species of component B, four pK_a values were calculated: - 4.48, 0.13, 0.66 and 19.4. It was assumed that these pK_a values derive from the subsequent protonation or deprotonation of the amide groups as well as deprotonation of the strong acid group.

Test Facility NOTOX B.V. (2008a)

Particle Size MMAD = 1.452 µm

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

Range (µm)	*Mass (%)*
< 0.5	10
0.5 – 1.223	50
> 1.223 – 4.343	90

Remarks 94.69% of the notified chemical has particle size < 10 µm.

Test Facility Chilworth (2008)

Flammability in contact with water Not predicted to evolve a dangerous amount of flammable gases.

Method EC Directive 92/69/EEC A.12 Flammability (contact with water).

Remarks Estimated based on chemical structure.
Test Facility NOTOX B.V. (2008a)

Flammability Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks No flame or smouldering was observed during the test.
Test Facility NOTOX B.V. (2008a)

Autoignition Temperature 341°C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Test Facility NOTOX B.V. (2008a)

Pyrophoric Properties Not predicted to be pyrophoric.

Method EC Directive 92/69/EEC A.13 Pyrophoric Properties of Solids and Liquids.
Remarks Estimated based on chemical structure.
Test Facility NOTOX B.V. (2008a)

Explosive Properties Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks Thermal and mechanical explosivity were tested.
Test Facility NOTOX B.V. (2008a)

Oxidizing Properties Not predicted to be oxidizing

Method EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks Estimated based on chemical structure.
Test Facility NOTOX B.V. (2008a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical (> 90%)
METHOD	OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rat: Crl:WI (Han)
Vehicle	Aerosols generated by a stream of pressurised air
Method of Exposure	Oro-nasal exposure.
Exposure Period	4 hours
Physical Form	solid aerosol (particulate).
Particle Size	3.7 – 3.1 μm (at 5.7 mg/L) and 3.2 – 2.3 μm (at 3.2 mg/L)
Remarks - Method	The recommended number of dose groups according to OECD TG is 3. However, only 2 were used in this study. The study authors followed guidance by the globally harmonized system (GHS) of classification of chemicals. After more than 50% of animals died in each sex after dosing with 5 mg/L, a second study was conducted at a lower concentration.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
I	5M	50.9	5.7	3 (between 1-3 hrs of exposure)
II	5F	50.9	5.7	3 (between 2-3 hrs of exposure
III	5M	44.8	3.2	2 (Day 2)
IV	5F	44.8	3.2	1 (Day 2

LC50	1.0 – 5.0 mg/L/4 hours
Signs of Toxicity	After exposure to 5.7 mg/L: One male was found dead after 1 hour of exposure, 2 females were found dead after 2 hours and 2 males and 1 female were found dead after approximately 3 hours exposure. Slightly increased breathing rate, hunched posture, lethargy, shallow respiration, rales, ptosis and marked yellow urine were observed in all surviving males and females following treatment. The observations in males occurred between days 1 and 3. Laboured respiration, hypothermia and uncoordinated movement were also observed among the surviving females following treatment. Observations in females occurred between days 1 and 15. Yellow staining of fur was observed throughout the observation period. Yellow faeces and marked yellow urine were also observed in animals between days 4 and 6. In the first week following exposure, males showed reduced body weight gain while females showed body weight loss. In the second week, body weight gain was considered similar to that of healthy animals of the same age and strain. Piloerection was also observed but considered to be a result of restraint for inhalation exposure and deposition of the notified chemical on the fur. After exposure to 3.2 mg/L: Two males and 1 female were found dead on Day 2. Hunched posture was observed in 1 male and 1 female on days 2 and 3. Laboured respiration was observed until day 3 in 1 male and 2 females and in 1 male decedent and 1 female decedent until death. Slightly decreased breathing was observed in 1 female. Lethargy was also observed in 1 female. Slightly yellow faeces were observed in 3 males and 4 females on day 3.

	Yellow stained fur was observed in all animals. In the first week following exposure, both males and females showed body weight loss. In the second week, body weight gain was considered similar to that of healthy animals of the same age and strain.
Effects in Organs	Post mortem examination of all decedents found yellowish granular contents in the larynx and yellowish contents in the stomach. Yellow faeces and marked yellow urine were considered to be due to grooming of the fur and subsequent ingestion of the test substance.
Remarks - Results	The clinical signs observed in animals of both sexes after exposure to 5.7 mg/L and 3.2 mg/L were considered to be adverse effects as a result of exposure to the notified chemical.
CONCLUSION	The LC_{50} of the notified chemical was established within 1-5 mg/L. The notified chemical is harmful via inhalation.
TEST FACILITY	NOTOX B.V. (2008c)

B.2. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (> 90%)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat: CRL:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	0.5% carboxymethyl cellulose sodium salt in water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0
low dose	5 per sex	50	0
mid dose	5 per sex	250	0
high dose	5 per sex	1000	0
control recovery	5 per sex	0	0
high dose recovery	5 per sex	1000	0

Mortality and Time to Death

No mortalities were observed during the study.

Clinical Observations

No statistically significant differences in mean food consumption, body weight gain or sensorimotor function were observed between treated and control animals throughout the study.

Yellow-coloured faeces were observed in both sexes treated with ≥ 250 mg/kg bw/day. The stool colour returned to normal in both sexes, 2 days following the last treatment in the 1000 mg/kg bw/day recovery group.

Other minor clinical signs observed included soft stool observed in 1 male dosed with 1000 mg/kg bw/day, loss of hair and scab formation on the neck of females in the 50 mg/kg bw/day group. These were not considered treatment-related effects.

Haematology

A statistically significant decrease in haemoglobin concentration was observed in males treated with 1000

mg/kg bw/day and the ratio of large unstained cells was increased to statistical significance in females treated with 1000 mg/kg bw/day. However these values were within the range of historical values for those parameters and were not considered to be of toxicological significance.

Clinical chemistry

A statistically significant increase in sodium levels was observed in males treated with 1000 mg/kg bw/day. However these values were within the historical range for those parameters and were not considered to be of toxicological significance.

Effects in Organs

Statistically significant increases in the weight of the spleen and liver of males in the recovery group treated with 1000 mg/kg bw/day were observed compared to control males. However, no abnormalities were observed in these organs during examination at post mortem, and the increases were within the ranges of historical data. Therefore, these were not considered to be of toxicological significance.

A few isolated abnormalities were noted at post mortem examination: diverticulum of the jejunum in 1 female from the recovery control group, and 1 female treated with 1000 mg/kg bw/day; pelvic dilation of the kidney in 1 male from the recovery control group and recessed region of the kidney in 1 female from the 1000 mg/kg bw/day recovery group; smaller testis in 1 male from the vehicle control group; cysts in 1 male treated with 1000 mg/kg bw/day and decreased size of the left lobe of the thyroid in 1 female treated with 250 mg/kg bw/day. However, none of these effects were considered attributable to treatment with the notified chemical.

The effects noted during histopathological examination did not show a dose-response relationship and were not considered related to treatment.

Remarks – Results

The abnormalities noted in the faeces of animals of both sexes treated with ≥ 250 mg/kg bw/day, the variations in a small number of haematology and clinical chemistry parameters and the effects seen in organs were not considered to be of toxicological significance.

CONCLUSION

The No Observed Adeverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day in this study, based on the lack of adverse findings at the highest dose.

TEST FACILITY Chemicals Evaluation and Research Institute, Japan (2008a)

B.3. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (> 90%)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test modified according to Prival et al. (1984) EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP_2uvrA.
Metabolic Activation System	S9 fraction derived from uninduced Golden Syrian hamster liver
Concentration Range in Main Test	a) With metabolic activation: 0 - 333 µg/plate b) Without metabolic activation: 0 - 333 µg/plate
Vehicle	DMSO
Remarks - Method	The method was modified according to Prival et al. (1984). The modification involved incubation prior to the assay to test the mutagenicity of metabolites of the notified chemical.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*

Absent				
Test 1	-	-	333	None
Test 2	-	-	333	None
Present				
Test 1	-	-	333	None
Test 2	-	-	333	None

Remarks - Results

In the first experiment, an increase in the number of revertant colonies in the TA100 strain treated with 3300 and 5000 µg/plate in the absence and presence of metabolic activation was reported in comparison to solvent controls. In the TA1537 strain, increases in the number of revertant colonies was observed at 33 and 333 µg/plate with metabolic activation in comparison to solvent controls. However, the increases observed were not dose-dependent and all values remained within the laboratory historical ranges for the TA100 and TA1537 strains.

In the second experiment, an increase in the number of revertant colonies was observed in the TA1537 strain treated with 33 µg/plate of the notified chemical without metabolic activation. However, the increases observed were not dose-dependent and all values remained within the laboratory historical range for the TA1537 strains.

The negative control was within normal limits and the positive controls (2-aminoanthracene and congo red) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

CONCLUSION

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

TEST FACILITY

NOTOX B.V. (2008g)

B.4. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical (> 90%)

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain: Chinese hamster

Cell Type/Cell Line: Lung fibroblasts (CHL/IU)

Metabolic Activation System: Phenobarbital, 5,6-benzoflavone-induced rat liver S9 fraction

Vehicle: 0.5% carboxymethyl cellulose sodium salt solution

Remarks - Method: In the 24 hours continuous treatment test, analysis of chromosomes was considered too difficult at ≥ 156 µg/mL due to precipitate of the notified chemical. Therefore the medium was exchanged and demecolcine solution was added 2 hours before the end of the treatment.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	78.1, 156, 313*, 625*, 1250*, 2500, 5000	6	24
Test 2	9.77, 19.5, 39.1*, 78.1*, 156*, 313, 625, 1250, 2500	24	24
Present			
Test 1	78.1, 156, 313, 625*, 1250*, 2500*, 5000	6	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic	*Test Substance Concentration (µg/mL) Resulting in:*

Activation	*Cytotoxicity in Preliminary Test (IC_{50})*	*Cytotoxicity in Main Test (IC_{50})*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	2500	4600	156	None
Test 2	590	1200	9.77	None
Present				
Test 1	4900	> 5000	78.1	None

Remarks - Results

Precipitation prevented analysis at concentrations of 2500 µg/mL or more during Test 1. In Test 2, conducted only in the absence of metabolic activation, analysis was not possible at concentrations of 78.1 µg/mL or greater due to precipitation. No more than 2% of cells treated with the notified chemical contained numerical or structural aberrations at any of the doses analysed.

The percentage of numerical and structural aberrations observed in the negative control was within normal limits and the positive controls (Mitomycin (-S9); Cyclophosphamide monohydrate (+S9)) demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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, Japan (2007)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified Chemical (> 90%)
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Measurement of biochemical oxygen demand (BOD) with a close system oxygen consumption measuring apparatus; Determination of test item by HPLC; Determination of aluminium and calcium by atomic absorption (AA) spectrophotometry.
Remarks - Method	On-site sampling for sludge, surface water and surface soil was carried out in ten locations in Japan. The test was conducted in triplicate at a loading rate of 100 mg/L at 25˚C. Aniline was used as the reference item to confirm that the sludge was sufficiently active.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation (BOD)*
28	0*	7	64
		14	78

* The percentage biodegradation was regarded as 0 since the calculated average value was -2% for BOD and -1% for HPLC.

Remarks - Results	All criteria for the test validity were met. The test substance is a mixture, which includes organic aluminium salts and organic calcium salts. Under the conditions of this study, some of the aluminium and calcium salts were eliminated. Organic compounds left after the elimination were unchanged test substance. The notified chemical is not considered to be readily biodegradable since no degradation was detected in the study.
CONCLUSION	The notified chemical is not readily degradable.
TEST FACILITY	Kurume Laboratory Chemicals Evaluation and Research Institute, Japan (2008b)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified Chemical (> 90%)
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test.
Species	*Cyprinus carpio* (Carp)
Exposure Period	Exposure: 28 days Depuration: 0 days
Auxiliary Solvent	Dispersants: - Sugar candy - HCO-40 (hydrogenated castor oil) - HCO-100 (hydrogenated castor oil) - Polyvinyl alcohol (degree of polymerization ~ 500)
Concentration Range	Nominal: 10 and 100 µg/L; Actual: Level 1: 91.5 µg/L (peak 3); 101 µg/L (peak 4) Level 2: 9.68 µg/L (peak 3); 10.1 µg/L (peak 4)

Analytical Monitoring	HPLC for determination of the test concentration.
Remarks - Method	The test was conducted in duplicate. Four dispersants were used together in each single test, with the concentrations being 10-30 times higher than that of the notified chemical. A control test was conducted under identical conditions except for the absence of the notified chemical. External disinfection of the fish was carried out in an aqueous solution of 50 mg/L oxytetracycline hydrochloride for fisheries and 7 g/L sodium chloride for 24 hours after acclimatizing. The number of the fish for each test was 28 for the main and 12 for the control.

RESULTS

Bioconcentration Factor	Level 1: ≤ 1.9 (peak 3); ≤ 0.82 -1.1 (peak 4) Level 2: ≤ 18 (peak 3); ≤ 8.3 (peak 4)
CT50	Not applicable (BCF < 10)
Remarks - Results	Steady-state was reached after 28 days. Four peaks were detected with HPLC analysis of the test item corresponding to the various components of the notified chemical. Peak 1 and 2 (minor components of the notified chemical) were excluded from quantitative analyses due to the small size of the peaks. The bioconcentration potentials of the major components, component A (peak 4) and component B (peak 3), were found to be low. Caution should be taken when interpreting the test results because the presence of the dispersant at concentrations much higher than the notified chemical may emulsify and actually shield the notified chemical from water, and therefore, prevent the permeating of the chemical into the fish organisms. As a result of this effect, the concentration detected in test fish would not be reliable and may lead to underestimation of the extent of bioaccumulation for the notified chemical. However, based on the extremely low water solubility and the molecular weight of higher than 600 (Connell, 1989), the notified chemical is not expected to bioaccumulate in aquatic organisms. The depuration phase was not carried out during the study given the calculated BCF being < 10.

CONCLUSION	The notified chemical is expected to have a low bioaccumulation based on its low water solubility and molecular features.
TEST FACILITY	Kurume Laboratory Chemicals Evaluation and Research Institute, Japan (2008c)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical (> 90%)
METHOD	OECD TG 203 Fish, Acute Toxicity Test – semi-static (renewal after 48 hours).
Species	*Oryzias latipes* (Medaka)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	37 mg $CaCO_3$ /L
Analytical Monitoring	HPLC for analysis of the test concentrations
Remarks – Method	Based on the result of the preliminary test, a limit test was conducted in duplicate with four fish each at a nominal concentration of 100 mg/L at 23.7-24.2˚C and in the pH range of 7.8-8.0. Test solutions were prepared by mixing the notified chemical and dilution water, followed by stirring for 48 hours by a magnetic stirrer. Dechlorinated tap water was used as

dilution water with controlled temperature after being sufficiently aerated.
A 96-hour acute test of $CuSO_4$ $5H_2O$ was conducted to confirm the reproducibility of the test system. A control test without the notified chemical was also conducted.
Concentrations of the notified chemical were measured at the start and the end of the exposure test, and also before and after the renewal of the test medium. Equal volumes of the test solution were taken out from the middle layer of the test solution for each vessel and mixed for analysis by HPLC.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	0	8	0	0	0	0
100	< detection limit	8	0	0	0	0

LC50	> Limit of solubility at 96 hours.
NOEC (or LOEC)	Limit of solubility at 96 hours.
Remarks – Results	Validity criteria for the study were met. The 96 hour LC50 of reference item was determined to be 0.54 mg/L for $CuSO_4$, which is within the stipulated range (mean ± 2S.D.: 0.13-0.98 mg/L) [mean ± S.D.: 0.55 ± 0.21 mg/L (n=40)]. The concentrations of the notified chemical during the test were below the detection limit, indicating that the water solubility of the notified chemical is < 0.0073 mg/L. This is reasonable considering the samples were taken out at the middle of the solutions, while most of the notified chemical was floating on top of the solution. Neither mortality nor sub-lethal response was observed in both the limit and the control tests.
CONCLUSION	The notified chemical is not toxic to fish up to the limit of its solubility in water.
TEST FACILITY	Kurume Laboratory Chemicals Evaluation and Research Institute, Japan (2008d)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified Chemical (> 90%)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	none
Water Hardness	37 mg $CaCO_3$/L
Analytical Monitoring	HPLC for analysis of test concentrations.
Remarks - Method	Based on the result of the preliminary test, a limit test was conducted in four replicates with five daphnids each at a nominal concentration of 100 mg/L at 20˚C and in the pH range of 7.9-8.8. Test solutions were prepared by mixing the notified chemical and dilution water, stirring for 48 hours by a magnetic stirrer, and followed by filtration with a glass fiber filter (ADVANTEC, GB-140, pore size: 0.4 μm) to produce the test solution. Dechlorinated tap water was used as dilution water with controlled temperature after being sufficiently aerated. A 48-hour acute immobilization test of $K_2Cr_2O_7$ was conducted to confirm the reproducibility of the test conditions. A control test without

the notified chemical was also conducted.
Concentrations of the notified chemical were measured at the start and the end of the test. Equal volumes of the test solutions were taken out from the start solution and from the middle layer of the end solution for analysis by HPLC.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	20	0	0
100	< detection limit	20	0	0

LC50	> solubility at 48 hours
NOEC (or LOEC)	Limit of solubility at 48 hours
Remarks - Results	Criteria for the test validity were met. The 48-hour EC50 for reference item $K_2Cr_2O_7$ was determined to be 0.28 mg/L, which is within the normal range in the laboratory (mean ± 2S.D.: 0.13-0.35 mg/L) [mean ± S.D.: 0.24 ± 0.057 mg/L (n=61)]. The concentrations of the notified chemical during the exposure test were below the detection limit, indicating that the water solubility of the notified chemical is < 0.0073 mg/L. This is reasonable considering the most of the notified chemical was floating on top of the test solution owing to the low solubility. No immobilization of the test *Daphnia magna* was observed in both the limit and the control tests.

CONCLUSION — The notified chemical is not toxic to *Daphnia magna* up to the limit of its solubility.

TEST FACILITY — Kurume Laboratory Chemicals Evaluation and Research Institute, Japan (2008e)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE — Notified Chemical (> 90%)

METHOD — OECD TG 201: Freshwater Alga and Cyanobateria, Growth Inhibition Test.

Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	0-100 mg/L
Auxiliary Solvent	None
Water Hardness	Not mentioned (test medium according to the composition mentioned in OECD guideline 201)
Analytical Monitoring	HPLC
Remarks - Method	Based on the result of the preliminary test, a limit test was conducted in six replicates at a nominal concentration of 100 mg/L at 23.0-23.2˚C and in the pH range of 7.9-8.0. Test solutions were prepared by mixing the notified chemical and dilution water, stirring for 48 hours by a magnetic stirrer, and followed by filtration with a glass fiber filter (ADVANTEC, GB-140, pore size: 0.4 μm) and a suction filtration with a membrane filter (ADVANTEC, Hydrophilic PTFE, 0.2 μm filter unit) to produce the test solution. The solutions were colourless and clear at start of the exposure test, and were green at end of the exposure due to the algae growth. Concentrations of the notified chemical were measured at the start and the end of test.

RESULTS

Biomass		*Growth*	
ErC_{50} mg/L at 72 h	*NOEC mg/L*	*ErC_{50} mg/L at 72 h*	*NOEC mg/L*
> solubility in test medium	solubility in test medium	> solubility in test medium	solubility in test medium

Remarks - Results	All criteria of validity for the test were met. No adverse effects on the growth of the test species were observed at the test level. The concentrations of the notified chemical in the test solutions were under the detection limit, indicating that the water solubility of the notified chemical in water is < 0.0069 mg/L.
CONCLUSION	The notified chemical is not considered toxic to algae up to the limit of the solubility.
TEST FACILITY	Kurume Laboratory Chemicals Evaluation and Research Institute, Japan (2008f)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified Chemical (> 90%)
METHOD	OECD TG 209: Activated Sludge, Respiration Inhibition Test. EC Directive 87/302/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
Inoculum	Micro-organisms in activated sludge (Municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands)
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L
Remarks – Method	Test was conducted in duplicate at a loading rate of 100 mg/L at 18.5-18.9˚C and a pH of 8.3. Control tests were conducted by using 3,5-dichlorophenyl as the reference at concentrations of 5.0, 12 and 30 mg/L.
RESULTS	
IC50	> 100 mg/L (nominal)
NOEC	100 mg/L (nominal)
Remarks – Results	All criteria for test validity were met. The percentages of inhibition respiration of the notified chemical to the sludge microbial at a loading rate of 100 mg/L were detected as 1% and 8%, respectively for the duplicate tests.
CONCLUSION	The notified chemical is not toxic to the active sludge bacteria up to the limit of its solubility.
TEST FACILITY	NOTOX B.V. (2008h)

BIBLIOGRAPHY

Barratt, M., Basketter, D., Chamberlain, M., Admans, G., Langowski, J. (1994) An Expert System Rulebase For Identifying Contact Allergens. *Toxic. in vitro.* 8(5):1053-1060.

Chemicals Evaluation and Research Institute, Japan (2007). Chromosomal Aberration Test of S-500 Using Cultured Mammalian Cells (Study Code: K06-1208). Kurume Chemicals Evaluation and Research Institute, Oita, Japan. (Unpublished report submitted by notifier).

Chemicals Evaluation and Research Institute, Japan (2008a). Twenty-Eight Day Repeated-Dose Oral Toxicity Study of S-500 in Rats (Study Code: B11-0843). Chemicals Evaluation and Research Institute, Oita, Japan. (Unpublished report submitted by notifier).

Chemicals Evaluation and Research Institute, Japan (2008b) Biodegradation study of S-500 by microorganisms. Kurume Laboratory Chemicals Evaluation and Research Institute (Study Number: 14997), Fukuoka, Japan (Unpublished report submitted by notifier).

Chemicals Evaluation and Research Institute, Japan (2008c) Biconcentration study of S-500 in carp. Kurume Laboratory Chemicals Evaluation and Research Institute (Study Number: 44603), Fukuoka, Japan (Unpublished report submitted by notifier).

Chemicals Evaluation and Research Institute, Japan (2008d) A 96-hour Acute Toxicity Study of S-500 with Medaka. Kurume Laboratory Chemicals Evaluation and Research Institute (Study Number: 94003), Fukuoka, Japan (Unpublished report submitted by notifier).

Chemicals Evaluation and Research Institute, Japan (2008e) A 48-hour Acute Immobilization Study of S-500 with *Daphnia Magna*. Kurume Laboratory Chemicals Evaluation and Research Institute (Study Number: 94002), Fukuoka, Japan (Unpublished report submitted by notifier).

Chemicals Evaluation and Research Institute, Japan (2008f) Algae Growth Inhibition Study of S-500 with *Pseudokirchneriella subcapitata*. Kurume Laboratory Chemicals Evaluation and Research Institute (Study Number: 94001), Fukuoka, Japan (Unpublished report submitted by notifier).

Chilworth (2008) Particle Size Analysis on a Sample of S-500 (Report Number: GLP101075AR1V1/08). Chilworth Technology Limited. Southampton, UK (Unpublished report submitted by notifier).

Connell, D. (1989), Bioaccumulation of Xenobiotic Compounds, CRC Press, Inc.

DIC Corporation, Japan (2005) Analysis of MF1456-2345 (SYN500) (Receipt Number: 05115B) (Original report in Japanese). Analysis Center (Kashima Plant), Ibaraki, Japan (Unpublished report submitted by notifier).

DIC Corporation, Japan (2006a) Analysis of SYN500 (Receipt Number: 05187B) (Original report in Japanese). Analysis Center (Kashima Plant), Ibaraki, Japan (Unpublished report submitted by notifier).

DIC Corporation, Japan (2006b) Trace Ingredient Weight Analysis of SYN500 by LC/MS (ESI) (Receipt Number: 06112B) (Original report in Japanese). Analysis Center (Kashima Plant), Ibaraki, Japan (Unpublished report submitted by notifier).

DIC Corporation, Japan (2006c) S-500 Lot No. MF-1456-2345 Ca and Fe percentage-weight content analysis (Receipt Number: 2006217K) (Original report in Japanese). Analysis Center (Kashima Plant), Ibaraki, Japan (Unpublished report submitted by notifier).

DIC Corporation, Japan (2007) NMR Analysis of (S-500 LOT No. MF1456-2345, Y-513 LOT No.F-1570) for the purpose of European Union New Chemical Substances Registration (REACH 1-10t/annual) (Receipt Number: G-07-043) (Original report in Japanese). Central Research Laboratories, Chiba, Japan (Unpublished report submitted by notifier).

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NOTOX B.V. (2007) Acute Oral Toxicity with S-500 in the Rat – Acute Toxic Class Method (SPL Project Number: 0606/0196). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008a) Determination of Physico-Chemical Properties of S-500 (NOTOX Project No. 486815). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008b) Assessment of Acute Dermal Toxicity with S-500 in the Rat (NOTOX Project No. 486834). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008c) Assessment of Acute Inhalatory Toxicity with S-500 in the Rat (NOTOX Project No. 487878). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008d) Primary Skin Irritation/Corrosion Study with S-500 in the Rabbit (4-Hour Semi-Occlusive Application) (NOTOX Project No. 486835). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008e) Acute Eye Irritation/Corrosion Study with S-500 in the Rabbit (NOTOX Project No. 486836). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008f) Assessment of Contact Hypesensitivity to S-500 in the Mouse (Local Lymph Node Assay) (NOTOX Project No. 486837). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B.V. (2008g) Evaluation of the Mutagenic Activity of S-500 in the *Salmonella Typhimurium* Reverse Mutation Assay and the *Escherichia Coli* Reverse Mutation Assay (with Independent Repeat) (NOTOX Project No. 486840). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NOTOX B. V. (2008h) Active Sludge Respiration Inhibition Test with S-500 (Project Number: 486838). NOTOX B.V., 's-Hertogenbosch, The Netherlands (Unpublished report submitted by notifier).

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia.

Prival, M., Bell, S., Mitchell, V., Reipert, M., Vaughn, V. (1984) Mutagenicity of Benzidine and Benzidine-Congener Dyes and Selected Monoazo Dyes in a Modified Salmonella Assay. *Mutation Res*., 136, 33-47.

Safepharm (2007) S-500: Acute Oral Toxicity in the Rat – Acute Toxic Class Method. Safepharm Laboratories Limited. Derbyshire, United Kingdom. (Unpublished report submitted by notifier).

Sagelsdorff, P., Haenggi, R., Heuberger, B., Joppich-Kuh, R., Jung, R., Weideli, H., Joppich, M. (1996) Lack of bioavailability of dichlorobenzidine from diarylide azo pigments: molecular dosimetry for hemoglobin and DNA adducts. *Carcinogenesis* 17 (3), 507-514 Available online: [18 March 2009] http://carcin.oxfordjournals.org/cgi/reprint/17/3/507]

SCCP (2006) The SCCP's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 6th Revision. SCCP. Adopted by the SCCP during the 10th plenary meeting of 19 December 2006 (SCCP/1005/06).

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.