File No: STD/1514

January 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Dye in Cartasol Brown M2RN

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (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 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.

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

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

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1514	Chemcolour Industries Australia Ltd	Dye in Cartasol Brown M2RN	Yes	≤ 100 tonnes per annum	Colouring agent for exterior use on timber products

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Skin sensitisation (Category 1)	H317: May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R43: May cause sensitisation by skin contact

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational setting described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental risk assessment
On the basis of the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin sensitisation (Category 1) - H317: May cause an allergic skin reaction

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

Health Surveillance

- As the notified chemical is a sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - Enclosed, automated processes where possible

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - Coveralls
  - Safety goggles
  - Impervious gloves

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

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- Where reuse or recycling are unavailable or impracticable, dispose of the chemical in an environmentally sound manner in accordance with relevant Commonwealth, State, Territory and local government legislation.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, physical 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 chemical is intended for use as a colouring agent for timber products for use in interior applications, such as furniture;
  - the imported or reformulated products containing the notified chemical are intended to be sold to consumers;

or

(2) Under Section 64(2) of the Act; if

- the function or use of the chemical has changed from a colouring agent for exterior use on timber products, or is likely to change significantly;
- the amount of chemical being introduced has increased, 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 (M)SDS of the product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Chemcolour Industries Australia Pty Ltd (ABN: 70 125 602 271)
Monash Business Park, 20-22 Gardiner Rd
NOTTING HILL VIC 3168

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, degree of purity, impurities, additives/adjuvants, use details, import volume and identity of manufacturer

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: boiling point, vapour pressure, hydrolysis as a function of pH, adsorption/desorption, dissociation constant, particle size and flash point

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU (1990)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Cartasol Brown M2RN (contains the notified chemical at < 50% concentration)

MOLECULAR WEIGHT
> 400 Da

ANALYTICAL DATA
Reference UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: solid

Property	Value	Data Source/Justification
Melting Point*	> 300 °C	Measured
Boiling Point	Not determined	High molecular weight solid with a high melting point
Density*	1,112 kg/m^3 at 20 °C	Measured
Vapour Pressure	Not determined	Expected to be low based on the high molecular weight and melting point
Water Solubility*	> 500 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionalities. However, the notified chemical is not expected to be significantly hydrolysed under the normal environmental pH range of 4 – 9

Partition Coefficient* (n-octanol/water)	log Pow = < 0 at 20 °C	Measured
Surface Tension*	72.3 mN/m at 22 °C	Measured
Adsorption/Desorption	Not determined	Not expected to be significantly adsorbed to soil/sludge based on its high water solubility
Dissociation Constant	Not determined	The notified chemical is a salt. Hence, it is ionised under normal environmental conditions (pH 4 – 9)
Particle Size	Not determined	Imported in an aqueous solution
Flammability*	Not flammable	Measured
Autoignition Temperature*	> 400 °C	Measured
Explosive Properties*	Not explosive	Measured
Oxidising Properties*	Not oxidising	Measured

*Test substance: Notified chemical with a purity of ~52%

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

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as an aqueous solution at a concentration of < 50%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-100	1-100	1-100	1-100	1-100

PORT OF ENTRY
Melbourne, Sydney, Brisbane

IDENTITY OF RECIPIENTS
Chemcolour Industries Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The formulated product containing the notified chemical will be packaged in 1,000 L Intermediate Bulk Containers or 200 L drums and transported by road or rail.

USE
The notified chemical will be used as a colouring agent at < 0.6% concentration for exterior use on timber products, such as decks and fencing.

OPERATION DESCRIPTION
Reformulation
The imported product containing the notified chemical at < 50% concentration will undergo reformulation to form the finished brown colour additive product for timber treatment. The reformulation process will be fully automated and enclosed. The imported product will be mechanically transferred from the import containers to mixing tanks using pumps and lines. The mixing process will be in closed tanks. The final formulated brown additive product containing the notified chemical at < 30% concentration will then be automatically transferred into storage tanks at the industrial timber treatment facility.

Timber treatment
The formulated brown colour additive product containing the notified chemical at < 30% concentration will be combined via a dosing system with the approved wood preservative solution. The resulting solution mixture containing the notified chemical at < 0.6% concentration will then be used to treat the timber using standard vacuum-pressure treatment processes, in an industrial setting. The treatment process will be fully automated and enclosed.

At the end of the treatment process, the timber (which will be effectively dry to the touch at this point) will be removed from the treatment vessel and held within a drip pad. Any drips of treatment fluid will be collected and returned to the treatment plant for re-use. Treated timber will not be removed from the drip pad until it is dry to touch and drip free. Depending on ambient conditions this could take up to 48 hours. The treated timber will then be distributed to retail shops for sale.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport company driver-chemical	0.5	2
Warehouse staff	0.5	6
Reformulation/timber treatment plant staff	4	50
Transport company driver – treated timber	0.5	20
Warehouse/retail staff - timber	0.5	50
Builders	4	6

EXPOSURE DETAILS

Transportation and storage workers
Worker exposure to the notified chemical during transport and storage of the product containing the notified chemical at < 50% concentration is not expected, except in the unlikely event of an accident where packaging may be breached.

Reformulation
During reformulation workers may experience dermal and ocular exposure to the notified chemical at < 50% concentration during sampling, loading and maintenance activities. Inhalation exposure during reformulation is expected to be low given the expected low vapour pressure of the notified chemical and enclosed nature of the reformulation process.

The notifier has stated that workers will be using personal protective equipment (PPE), such as coveralls, gloves and eye protection when carrying out these activities.

Timber treatment
The timber treatment process will be fully automated and enclosed; hence worker exposure to the notified chemical is expected to be low during these activities.

After timber treatment, dermal exposure to the notified chemical at < 0.6% concentration may occur when handling the treated timber if not fully dried.

The notifier has stated that workers will be using personal protective equipment (PPE), such as coveralls, gloves and eye protection when carrying out these activities.

Retail workers and builders
Retail workers and builders may come into contact with exterior timber treated with the notified chemical at < 0.6% concentration. However, once the timber is treated and dried, the notified chemical is not expected to be bioavailable (see Public Exposure below for further details).

6.1.2. Public Exposure

The public may come into contact with exterior timber treated with the notified chemical at < 0.6% concentration. Once the timber is treated and dried, it is stated by the notifier that the notified chemical will be strongly bound to the wood components and is not expected to leach. This was supported by test results provided by the notifier of the dye on wood pulp which showed that ~99% of the dye was retained on the substrate. Under the industrial application process, which involves a combination of vacuum and high pressure to force the dye and other components deep into the timber matrix, it is suggested by the notifier that an even greater retention of the notified chemical is expected. Therefore public exposure to the notified chemical in a bioavailable form is not expected.

The formulated brown colour additive product containing the notified chemical at < 30% concentration will not be available for sale to consumers.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical with a purity of ~52% are summarised in the following table. The impurities present in the notified chemical are not expected to have a significant contribution to the toxicity of the notified chemical. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 5,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	evidence of sensitisation
Guinea pig, skin sensitisation – non-adjuvant test	no evidence of sensitisation
Human, skin sensitisation – RIPT (dyed paper)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 200 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Mutagenicity – bacterial reverse mutation (incorporating Prival and Mitchell modification for azo dyes)	mutagenic
Genotoxicity – in vivo mammalian erythrocyte micronucleus test	non genotoxic
Developmental toxicity	NOEL 100 mg/kg bw/day (maternal) NOAEL 1000 mg/kg bw/day (developmental)

Toxicokinetics

Absorption of the notified chemical across biological membranes is likely to be limited, based on the relatively high molecular weight (> 400 Da).

The notified chemical is an azo compound. Azo compounds may break down to their component amines. The azo linkage is the most labile portion of an azo colourant molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecules into component amines. Some metabolism of azo colourants may also occur in the cells of the bladder wall, and during percutaneous absorption. Intestinal bacteria are also capable of catalysing reductive cleavage of the azo bond. Based on its chemical structure, the notified chemical may be broken down to an aromatic amine which is a suspected mutagen. Given the low bioavailability of the notified chemical reductive cleavage is not expected to occur via the dermal route. However, there is potential for formation of the aromatic amine in the GI tract, which is expected to be strongly absorbed.

Acute toxicity

The notified chemical was found to be of low acute oral and dermal toxicity in rats.

No acute inhalation toxicity study was provided. Given the expected low vapour pressure of the notified chemical, inhalation exposure is not expected unless aerosols or mists are formed.

Irritation

The notified chemical was non-irritating to the skin and slightly irritating to the eyes in studies conducted in rabbits.

Sensitisation
The notified chemical was sensitising in a guinea pig maximisation test. However, the notified chemical was not sensitising in a non-adjuvant guinea pig test (modified Buehler test). In a human repeat insult patch test, paper dyed with the notified chemical (concentration not reported) was non-sensitising.

Repeated dose toxicity
In a 28-day repeat dose toxicity study, rats were administered the notified chemical by gavage at 0, 50, 200 or 1,000 mg/kg bw/day. Liver weights (after adjustment for body weight) of females receiving 1,000 mg/kg were statistically significantly greater when compared to controls. Liver weights of males receiving 1,000 mg/kg were also greater but did not achieve a level of statistical significance in comparison with controls. The No Observed Effect Level (NOEL) was established as 200 mg/kg bw/day, based on biological effects observed for animals treated at 1,000 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was negative in a standard bacterial reverse mutation assay but was positive in a modified bacterial reverse mutation assay for azo dyes (Prival and Mitchell, 1982). The modified test is thought to yield a greater detection of mutagenic azo dyes as it utilises a reductive pre-incubation (during which the azo dye is reduced to amine species) before the test is carried out. This positive result is consistent with the potential formation of an aromatic amine break down product from the notified chemical which is a suspected mutagen. The notified chemical was not genotoxic in an *in vivo* mammalian erythrocyte micronucleus test.

Overall, due to the results of the modified bacterial reverse mutation test, the potential for the notified chemical to be mutagenic cannot be ruled out.

Developmental toxicity
In a prenatal developmental toxicity study, rats were administered the notified chemical by gavage at 0, 100, 300 or 1000 mg/kg bw/day. The No Observed Effect Level (NOEL) for maternal toxicity was established as 100 mg/kg bw/day based on lower body-weight gain in the mid- and high dose groups, and the No Observed Adverse Effect Level (NOAEL) for developmental toxicity was established as 1,000 mg/kg bw/day.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Skin sensitisation (Category 1)	H317: May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Toxicological studies on the notified chemical indicate that it is a skin sensitiser. Furthermore, the potential for the notified chemical to cause mutagenic effects cannot be ruled out. However, the relatively high molecular weight and low partition coefficient of the notified chemical are expected to limit is absorption through the skin.

During reformulation and timber treatment, the processes will be largely enclosed and automated. Furthermore the notifier has stated that workers will wear PPE, therefore potential for exposure during these operations is expected to be limited. Use of the notified chemical is therefore only considered to be reasonable when sufficient engineering controls, safe work practices and personal protective equipment (PPE) are used to greatly reduce the potential for exposure.

Once the timber is treated and dried, the notified chemical is expected to be strongly bound to the timber matrix and hence is not expected to be bioavailable.

Therefore, provided the stated control measures are in place to limit exposure, the risk to workers from use of the notified chemical is not considered unreasonable.

6.3.2. Public Health

The public may come into contact with exterior timber treated with the notified chemical at < 0.6% concentration. Once the timber is treated and dried, the notified chemical is expected to be strongly bound to the timber matrix and hence is not expected to be bioavailable. Furthermore, the public is expected to have limited, at most occasional, direct dermal contact with treated external timber surfaces. Therefore the risk to the public is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. Release to the environment during shipping, transport and warehousing will only occur in the unlikely event of accidental spills or leaks.

Reformulation of the product containing the notified chemical will occur by mixing with other ingredients to prepare treatment solutions, which will take place in an industrial setting. This process is expected to be performed under controlled conditions and no significant release of the notified chemical from this process is expected.

RELEASE OF CHEMICAL FROM USE

Treatment of timber with formulations containing the notified chemical (< 0.6%) will take place in fully contained industrial treatment facilities. Based on the data provided by the notifier, the notified chemical is expected to be bound with the treated timber at a level of above 98.8%. According to the notifier, during the colouring process with pressure treatment, the notified chemical penetrates the wood deeply and evenly, and bleeding of the chemical is very unlikely. The notified chemical is expected to be tightly bound to wood fibres. Hence the notified chemical is not expected to be released from the timber articles in significant amounts during service life. The treatment solution is in aqueous form and, after treatment processes, it is expected to be pumped back into a storage tank. In the tank, the volume absorbed by the wood is replaced with fresh treatment solution. It is then used to treat the next batch of wood and the cycle is repeated. After application, the notified chemical will share the fate of the associated timber articles.

RELEASE OF CHEMICAL FROM DISPOSAL

When the timber treated with formulations containing the notified chemical reaches the end of its service life, it will be disposed of under controlled conditions, e.g. to a suitable hazardous waste treatment facility. Any wastes generated at timber treatment facilities including spills, residues, and cleaning washings are expected to be disposed of to a hazardous waste treatment facility. No significant release to the aquatic environment is expected.

7.1.2. Environmental Fate

The notified chemical is not expected to be readily biodegradable according to the provided fate study. For the details of the environmental fate studies please refer to Appendix C. It is not expected to have bioaccumulative potential given the presence of cationic functional groups and the determined low partition coefficient.

Most of the notified chemical is expected to share the fate of the timber treated with formulations containing the notified chemical. The timber is expected to be treated under controlled conditions, and is most likely to be sent to hazardous landfill at the end of its service life. Wastes of the notified chemical generated during timber treatment application are expected to be treated through a hazardous waste treatment facility. The notified chemical is expected to have a high adsorption/desorption (K_{OC}) due to the high density of cationic functional groups. Therefore, in the case of any release to sewage or waste water treatment plants, most of the notified chemical is expected to be removed from the water phase by adsorption to sludge/sediment. Therefore, no

significant release to the aquatic environment is expected. In landfill, the notified chemical is expected to decompose slowly by biotic and/or abiotic degradation processes to form water, oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

As significant release of the notified chemical is not expected at any time under the proposed use patterns, a PEC in the aquatic compartment has not been be derived.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical with a purity of ~52% (unless otherwise stated) are summarised in the table below. The impurities present in the notified chemical are not expected to have a significant contribution to the toxicity of the notified chemical. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EC50 = 143 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 = 156.8 mg/L	Not harmful to aquatic invertebrates
Duckweed Toxicity*	7 d EC50 = 105 mg/L	Not applicable
Inhibition of Bacterial Respiration	3 h EC50 > 100 mg/L	Not inhibitory to bacteria respiration
Earthworm	14 d LC50 >1,000 mg/kg dry soil	Very slightly toxic to sediment dwellers

*Test substance: Notified chemical with a purity ~89%

The notified chemical is not considered to be harmful to aquatic organisms based on the above endpoints. Therefore, the notified chemical is not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic effects.

7.2.1. Predicted No-Effect Concentration (PNEC)

No significant adverse effects were observed in any of the ecotoxicity tests submitted. In addition, no significant release of the notified chemical to the aquatic environment is expected based on the reported use pattern. Therefore, it is not appropriate to derive the PNEC, as this concentration is not expected to reach ecotoxicologically significant levels.

7.3. Environmental Risk Assessment

A risk Quotient (Q = PEC/PNEC) value was not calculated since neither PEC nor PNEC were derived. The notified chemical is not expected to pose an unreasonable risk to the environment based on the assessed use pattern and the reported low adverse ecotoxicological effects to aquatic organisms.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point > 300 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	The measurements were performed at ambient temperature using the capillary method. The test substance did not melt below 300 °C.
Test Facility	RCC NOTOX B.V. (1989a)

Density 1,112 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	A pycnometer was used.
Test Facility	RCC NOTOX B.V. (1989b)

Water Solubility > 500 g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method. The notified chemical was miscible with water at 1:1 ratio (w/v) in a primary test. Therefore, no further test was conducted.
Test Facility	RCC (1989c)

Partition Coefficient (n-octanol/water) log Pow < 0

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method. The temperature of the mobile phase was 28 ± 1 °C. The partition coefficient of the notified chemical was determined to be < 1, or the log P_{OW} < 0.
Test Facility	RCC (1989d)

Surface Tension 72.3 mN/m at 22 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration: 528 mg/L A solution of the test substance in water does not lower the surface tension of water.
Test Facility	RCC NOTOX B.V. (1989c)

Flammability Not flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Test Facility	RCC NOTOX B.V. (1989d)

Autoignition Temperature > 400 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks	The test substance was determined to be not auto-flammable between room temperature and 400 °C, under the test conditions.
Test Facility	RCC NOTOX B. V. (1989e)

Explosive Properties Not explosive under the conditions of the test

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	The test substance was exposed to thermal and mechanical stress. No positive reaction was observed during the test.
Test Facility	RCC NOTOX B. V. (1989f)

Oxidizing Properties Not oxidising

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).
Remarks	In the only preliminary test performed, the reference mixture (a barium nitrate/cellulose mixture at 2/1 w/w) burned with a flame, emitting a white smoke. The burning time was approximately 41 s. The test substance/cellulose (2/1, w/w) pile did not burn.
Test Facility	RCC NOTOX B.V. (1989g)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (~52% purity)
METHOD	OECD TG 401 Acute Oral Toxicity.
Species/Strain	Rat/Wistar
Vehicle	Water
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	5,000	1

LD50	> 5,000 mg/kg bw
Signs of Toxicity	The animal that died showed lethargy, tachypnoea and diarrhoea on day 1 of the study. All animals were noted to be drinking a lot after dosing. The bedding was noted as discoloured red from the urines on day 1. All animals were noted as having diarrhoea on day 2 of the study, with the bedding material stained dark with faeces and urine. The faeces were again noted as dark on day 3.
Effects in Organs	Macroscopic examination of the animal that died revealed black residual substances around the mouth and anus and all the organs in the thoracic and abdominal cavities to be black/blue in colour making more detailed examination difficult. Macroscopic examination of all surviving animals at termination did not reveal any abnormalities.
Remarks - Results	All surviving animals showed normal body weight gain during the study.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	RCC NOTOX B.V. (1989h)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical (~52% purity)
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Rat/Wistar
Vehicle	Water
Type of dressing	Occlusive
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2,000	0

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	The treated skin of all animals had a brown mottled appearance on day 2, 5 and 8, and in all males and in 4/5 females on day 15. The 4/5 females also

had red spots on day 15.
Signs of Toxicity - Systemic: No clinical signs of toxicity were observed.
Effects in Organs: No abnormalities were noted.
Remarks - Results: All surviving animals showed normal body weight gain during the study.

CONCLUSION: The test substance is of low toxicity via the dermal route.

TEST FACILITY: RCC NOTOX B.V. (1989i)

B.3. Irritation – skin

TEST SUBSTANCE: Notified chemical (purity ~52%)

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 F
Vehicle: Moistened with water
Observation Period: 72 hours
Type of Dressing: Semi-occlusive
Remarks - Method: No protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	0	1	< 24 hours	0
Oedema	0	0	0	0	-	0

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

Remarks - Results: No symptoms of systemic toxicity were observed in the animals during the study. No corrosive effect was evident on the skin.

In the area of application brown staining of the treated skin by the test substance was observed.

The observed skin irritation consisted of very slight erythema in 2/3 animals. The irritation was reversible within 24 hours after exposure.

CONCLUSION: The test substance is non-irritating to the skin.

TEST FACILITY: RCC NOTOX B.V. (1989j)

B.4. Irritation – eye

TEST SUBSTANCE: Notified chemical (< 60%)

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 F
Observation Period: 14 days
Remarks - Method: Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			

Conjunctiva: redness	2	1.7	1.7	2	< 14 days	0
Conjunctiva: chemosis	1	0.7	0.7	1	< 7 days	0
Conjunctiva: discharge	0.7	0.3	0.7	1	< 72 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results

No corrosion or signs of systemic toxicity was observed. No mortality occurred.

Brown staining of eyelids and the fur on the head by the test substance was observed on day 1. Conjunctivae of the treated eye of all three animals were covered with a film of the test substance.

Treatment of the eyes with fluorescein 2%, 24 hours after test substance instillation revealed no corneal epithelial damage in any of the animals.

CONCLUSION

The test substance is slightly irritating to the eye.

TEST FACILITY

RCC NOTOX B.V. (1989k)

B.5. Skin sensitisation

TEST SUBSTANCE

Notified chemical (purity ~52%)

METHOD

OECD TG 406 Skin Sensitisation - Magnusson and Kligman test.

Species/Strain

Guinea pig/ Dunkin-Hartley albino

PRELIMINARY STUDY

Test Concentration:
intradermal: 0.5% (based on deaths at 2.5% and 5%)
topical: 5% (slight irritation at 10% and 50% but not at 25%)

MAIN STUDY

Number of Animals

Test Group: 20 Control Group: 10

INDUCTION PHASE

Induction Concentration:
intradermal: 0.5%
topical: 25%

Signs of Irritation

All experimental animals showed skin irritation after the 48 hour occluded induction exposure.

CHALLENGE PHASE

challenge

topical: 0, 5, 10, 25%

Remarks - Method

Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

In the preliminary study, the two animals treated by intradermal injection with the test substance at 5% and 2.5% concentration died within a few hours of dosing. The 0.5% test substance concentration was therefore chosen for intradermal induction in the main study. The 25% test substance concentration was chosen as the maximum tolerated concentration.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: challenge*	
		24 h	*48 h*
Test Group	0	0	0
	5	5/20	10/20
	10	11/20	17/20
	25	17/20	20/20
Control Group	0	0/10	0/10

5	0/10	0/10
10	0/10	0/10
25	0/10	0/10

Remarks - Results: No symptoms of system toxicity were observed. No mortality occurred.

The reactions observed in experimental group were characterised by crust formation, swelling and scaliness.

The average body weight gain of experimental and control animals was similar.

CONCLUSION: There was evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.

TEST FACILITY: RCC NOTOX B.V. (1989l)

B.6. Skin sensitisation

TEST SUBSTANCE: Notified chemical (purity ~52%)

METHOD: OECD TG 406 Skin Sensitisation - modified Buehler method.

Species/Strain: Guinea pig/Ibm: GOHI, Albino

PRELIMINARY STUDY: Maximum Non-irritating Concentration:
topical: 25%

MAIN STUDY

Number of Animals: Test Group: 20 Control Group: 10

INDUCTION PHASE: Induction Concentration:
topical: 25%

Signs of Irritation: None

CHALLENGE PHASE

challenge: topical: 25%

Remarks - Method: Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: challenge*	
		24 h	*48 h*
Test Group	25%	0/20	0/20
Control Group	25%	0/10	0/10

Remarks - Results: No symptoms of system toxicity were observed. No mortality occurred.

The average body weight gain of experimental and control animals was similar.

Topical application area was found to show staining on days 30 and 31 in the control group while in the test groups the staining was shown from day 2 to 31 (termination of the study).

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

TEST FACILITY: RCC (1990)

B.7. Skin sensitisation – human volunteers

TEST SUBSTANCE	Paper (~4 cm^2) dyed with test substance (concentration not reported)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: Patches were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed after 24 h of each application and the test sites were evaluated prior to each re-application. Rest Period: 14 days Challenge Procedure: A challenge patch was applied to a naïve site. The patch was removed after 24 h and the site was evaluated 24 h and 48 h post-application.
Study Group	97 F, 17 M; age range 21-81 years; 104 subjects completed the test
Vehicle	None
Remarks - Method	The test substance was applied under an occlusive patch (2 cm x 2 cm). Ten subjects discontinued participation for non-test substance related reasons. Undyed paper served as the control.
RESULTS	
Remarks - Results	There was no evidence of sensitisation to the test substance tested.
CONCLUSION	The test substance was non-sensitising under the conditions of the test.
TEST FACILITY	TKL Research Inc. (1990)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Sprague-Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	Water
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

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	200	0
high dose	5 per sex	1,000	0

Mortality and Time to Death

There was no mortality during the treatment.

Clinical Observations

Dark faeces, which may be attributed to the colour of the test formulation, were noted in animals receiving 1,000 mg/kg. The sign was noted from day 9 in both sexes until termination. Regurgitation of the test substance was noted in males receiving 1,000 mg/kg on day 10. In females receiving 1,000 mg/kg, regurgitation was noted intermittently between days 7-14. It was also noted on day 10 for one female receiving 50 mg/kg.

Other clinical signs that were noted but not considered treatment-related by study authors included one incidence of diarrhoea, rough appearance of the coat and a swelling on a hind limb.

Although there were no statistically significant differences in the body weights of treated rats compared to controls, the body weight gain by females receiving 1,000 mg/kg was consistently statistically significantly decreased compared to controls.

Food consumption by females receiving 1,000 mg/kg/day was marginally decreased compared with controls (as may be expected following the slightly low body weight gain by these rats). However, after adjustment for differences in body weight, relative food consumption by females receiving 1,000 mg/kg/day was still marginally low compared to the controls.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no differences in haematology and clinical chemistry that were considered to have arisen as a result of treatment between control and treated rats.

Statistically significantly high creatinine values noted in males receiving 1,000 mg/kg were within the normal range, but with the difference from control being exacerbated by one high value of 59 µmol/L. Statistically significant differences in urea values between controls and males receiving 50 or 1,000 mg/kg/day were, in the absence of a treatment related distribution, considered by study authors to have arisen by chance. Treated females had statistically significantly decreased alanine aminotransferase (ALAT/GPT) levels when compared to controls, but this difference was attributed to a slightly abnormally high control value and of no biological significance.

Effects in Organs

Macroscopic observations that were noted at necropsy (eg. pelvic dilation of the kidneys) were not considered related to treatment but within the normal background range.

Liver weights (after adjustment for body weight) of females receiving 1,000 mg/kg were statistically significantly greater when compared to controls. Liver weights of males receiving 1,000 mg/kg were also greater but did not achieve a level of statistical significance in comparison with controls.

There was an apparent increase in interstitial nephritis seen in high and intermediate dose males. As the increase of this relatively frequent spontaneous background lesion was unilateral and absent in treated females, this effect was considered by study authors to be of no toxicological significance.

Remarks – Results

There were no signs of a toxic reaction to treatment in animals receiving 50 or 200 mg/kg/day test substance.

Animals receiving 1,000 mg/kg showed increased liver weights (the effects were not statistically significant in males). However, as there were no changes in serum liver enzyme levels or pathological evidence to confirm this, the toxicological significance of this change was considered doubtful by study authors.

The decreased food consumption by females receiving 1,000 mg/kg/day after adjustment for body weight suggested a loss of appetite for these females rather than any impairment of metabolic function.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 200 mg/kg bw/day in this study, based on slight biological effects observed for animals treated at 1,000 mg/kg bw/day.

TEST FACILITY RCC NOTOX B.V. (1989m)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test incorporating the Prival and Mitchell modification for azo dyes (Prival and Mitchell, 1982) Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100
Metabolic Activation System	S9 fraction from un-induced hamster liver
Concentration Range in	a) With metabolic activation: 0, 10, 100, 333.3, 1,000 and 5,000 µg/plate

Main Test	b) Without metabolic activation: 0, 10, 100, 333.3, 1,000 and 5,000 µg/plate
Vehicle	Water
Remarks - Method	*E. coli* was not used.

RESULTS

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

Remarks - Results	The test substance induced a slight increase in the number of revertants in strain TA1535 at 333.3 µg/plate and above in Test 1 and in strain TA100 at 1000 µg/plate in Test 2, with metabolic activation. These effects were not considered relevant by the study authors as they were not reproducible in the independent experiment. A distinct increase in the reversion rate was found in TA98 at 5,000 µg/plate in Test 1 without metabolic activation. This result was also shown not to be reproducible in the independent experiment. In strains TA1537 and TA98 a significant and reproducible dose-dependent increase in revertant colony numbers was obtained up to 1,000 µg/plate with metabolic activation. At the highest investigated dose the reversion rate decreased in these strains due to toxic effects of the test substance. All criteria for a valid study were met as described in the protocol.
CONCLUSION	The test substance was mutagenic to bacteria under the conditions of the test.
TEST FACILITY	CCR (1989)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0, 1, 3.3, 10, 33.3, 100 µg/plate b) Without metabolic activation: 0, 1, 3.3, 10, 33.3, 100, 333 µg/plate
Vehicle	Water
Remarks - Method	*E. coli* was not used. In the preliminary test, in the absence of S9-mix the survival of strain TA100 was severely reduced at the concentrations of 333 µg/plate upwards. Both in the absence and presence of S9-mix the survival was eliminated from 1,000 µg/plate upwards. Based on these data, the test substance was tested up to a concentration of 100 µg/plate in the absence of S9-mix and up to 333 µg/plate in the presence of S9-mix in the main test.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 333			
Test 1		> 100	> 100	negative
Test 2		> 100	> 100	negative
Present	≥ 333			
Test 1		> 333	> 333	negative
Test 2		> 333	> 333	negative

Remarks - Results: All bacterial strains showed negative responses over the entire dose range of the test substance.

All criteria for a valid study were met as described in the protocol.

CONCLUSION: The test substance was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY: RCC NOTOX B.V. (1989n)

B.11. Genotoxicity – in vivo

TEST SUBSTANCE: Notified chemical (purity ~52%)

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain: Swiss mice/CD-1
Route of Administration: Oral – intubation
Vehicle: Water
Remarks - Method: In a preliminary study 24 animals (3 M/3 F per group) were dosed orally with 5000, 4000, 3000 and 2000 mg/kg bw. In the 5000 mg/kg group all animals died within 2 hours post-dosing. In the 3000 mg/kg and 4000 mg/kg group five animals died within 3 hours post-dosing. No signs of toxicity were observed in 2000 mg/kg group.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 per sex	0	24
		0	48
		0	72
II (test dose)	5 per sex	2,000	24
		2,000	48
		2,000	72
III (positive control, CP)	5 per sex	50	48

CP=cyclophosphamide.

RESULTS
Doses Producing Toxicity
Genotoxic Effects: No increase in the frequency of micronuclei was observed.
Remarks - Results: The positive control induced a statistically significant increase in the number of micronuclei in both male and female mice. The incidence of micronuclei in the vehicle control groups did not exceed the range of historical data. Based upon this, the test was considered valid.

CONCLUSION: The test substance was not clastogenic under the conditions of this in vivo mouse micronucleus test.

TEST FACILITY: RCC NOTOX B.V. (1989o)

B.12. Developmental toxicity

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 414 Prenatal Developmental Toxicity Study.
Species/Strain	Rat/Sprague Dawley
Route of Administration	Oral – gavage
Exposure Information	Exposure days: once daily, from day 6 to 19 post coitum Post-exposure observation period: none
Vehicle	Water
Remarks - Method	Minor protocol deviations occurring during the study were not considered to have compromised the validity or integrity of the study.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
1	22 F	0	0
2	22 F	100	0
3	24 F	300	0
4	22 F	1,000	0

Mortality and Time to Death
All mated females survived the treatment.

Effects on Dams
Treatment with the test substance caused lower body-weight gain (%) in the females treated with 300 mg/kg and 1000 mg/kg from day 7 of pregnancy onwards.

In the observations derived from hysterectomy, no test substance related embryofetal toxicity was observed at any of the administered doses.

Effects on Foetus
No treatment-related abnormal findings were recorded in the external examination of the foetuses.

One foetus from 100 mg/kg group from one litter showed exencephaly as an abnormality. This abnormality was regarded by study authors as spontaneous and was not considered treatment-related.

The skeletal examination of the foetuses did not reveal any toxicologically relevant alterations.

A slight delay of ossification was recorded among some litters from 1,000 mg/kg group compared with the control group. This finding was considered by the study author as a variation as this effect did not have pathological meaning.

The visceral examination of the foetuses confirmed the presence of exencephaly, already recorded in one foetus from 100 mg/kg group in the external examination.

Remarks - Results
The remaining visceral findings recorded in the control and test substance treated groups were simple variations mainly involving urogenital morphology (dilated renal pelvis, and dilated and or convoluted ureter, malpositioned kidney, absent renal papilla, malpositioned cranial testis) in addition to others findings (dilated stomach, left-sided umbilical artery, additional small lobe in the liver and bilateral azygos vein). These alterations were regarded by study authors as spontaneous variations of limited pathological relevance.

CONCLUSION

The No Observed Effect Level (NOEL) for maternal toxicity was established as 100 mg/kg bw/day in this study, based on lower body-weight gain in the 300 and 1,000 mg/kg groups.

The No Observed Adverse Effect Level (NOAEL) for developmental toxicity was established as 1000 mg/kg bw/day in this study.

TEST FACILITY Harlan Laboratories S. A. (2014)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 301 E Ready Biodegradability: Modified OECD Screening Test
Inoculum	Micro-organisms from a domestic waste water sewage plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved organic carbon (DOC) was measured for determination of biodegradability.
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The study was performed at a test level of 150 mg/L and 108 mg/L in duplicates, which was equivalent to 30.8 and 21.4 mg carbon/L, respectively.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation**	*Day*	*% Degradation*
7	16.9	7	98.4
14	20.3	28	98.6
28	5.3		

* Mean value of the test results from the two tested levels

Remarks - Results	No information regarding test validity criteria is available. The reference compound, aniline, reached greater than 60% pass level by day 7 indicating the suitability of the inoculum. No toxicity control was performed according to the study. The test substance attained the maximum of biodegradation degree by day 14. After day 14, the degree of degradation was decreased. No explanation has been provided in the study report.
CONCLUSION	The test substance is not readily biodegradable
TEST FACILITY	RCC (1989a)

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 302 B: "Zahn-Wellens/EMPA(1) Test"
Inoculum	Micro-organisms from a domestic waste water sewage plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved organic carbon (DOC) was measured for determination of biodegradability.
Remarks – Method	The test was conducted following the test guideline and good laboratory practice (GLP) principles. The study was performed at a test level of 358 mg/L and 365 mg/L in duplicates, which was equivalent to 47.5 and 56.5 mg carbon/L, respectively.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	-3	7	96
28	-17	28	99

Remarks – Results	No information regarding test validity criteria is available. The reference

compound, aniline, reached greater than 60% pass level by day 7 indicating the suitability of the inoculum. No toxicity control was performed according to the study. It is unclear if the no biodegradation degree outcome is due to the test substance's toxicity to bacteria. The notified chemical may be not inherently biodegradable based on the above test outcome.

CONCLUSION The test substance may not be inherently biodegradable

TEST FACILITY RCC (1989b)

C.1. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical (purity ~52%)

METHOD OECD TG 203 Fish, Acute Toxicity Test-Static.

Species	Carp (*Cyprinus carpio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	2.33 mmol/L
Analytical Monitoring	Duplicate samples were taken at 0, 2, 24 and 96 hours for stability test.
Remarks – Method	The test was conducted following the test guidelines and good laboratory practice (GLP) principles. Following a range-finding test, the definitive study was performed at nominal concentrations of 0, 32, 56, 100, 180, 320 and 560 mg/L.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*				
Nominal		*6.5 h*	*24 h*	*48 h*	*72 h*	*96 h*
Blank control	10	0	0	0	0	0
32	10	0	0	0	0	0
56	10	0	0	0	0	0
100	10	0	0	0	0	1
180	10	0	6	1	0	0 *
320	10	9	1	-	-	-
560	10	10	-	-	-	-

* All surviving fish were hypoactive compared to the fish in the blank control.

LC50	143 mg/L at 96 hours (nominal)
NOEC	56 mg/L at 96 hours (nominal)
Remarks – Results	All validity criteria for the test were satisfied. Differences between the reported actual and nominal concentrations during the test appear to have been concentration dependent. It is considered plausible that these differences may be due to uptake and elimination of the test substance by the fish exposed. The test end points were calculated based on the nominal concentrations by using the maximum likelihood estimation method. A statistical analysis called Probit was used. The test substance contains < 60% of the notified chemical and other chemical constituents. Consequently, the resultant toxicity in this experiment may have been contributed to by all of the chemical constituents, including the notified chemical, in the product. Therefore, the observed effect (toxicity) of the notified chemical (< 60% concentration in the product) has not been adjusted to reflect the actual 100% concentration of the notified chemical.

CONCLUSION	The test substance is not harmful to fish.
TEST FACILITY	RCC (1989e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	11.7 mg $CaCO_3$/L
Analytical Monitoring	Not applicable
Remarks - Method	The test was conducted following the test guidelines and good laboratory practice (GLP) principles. A stock solution was prepared by adding the test substance directly into dilution water. The test media with lower concentrations of the test substance were prepared by serial dilution of the stock solution. The test substance appeared to be fully dissolved in the test media at each of the test concentrations from 32-1000 mg/L with a factor of 1.8.

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
0	20	0	0
32	20	0	0
56	20	1	1
100	20	2	2
180	20	5	10
320	20	14	20
560	20	18	20
1,000	20	19	20

LC50	156.8 (196.3 – 306.2) mg/L at 48 hours (nominal)
NOEC	56 mg/L at 48 hours (nominal)
Remarks - Results	All validity criteria for the test were satisfied. The actual concentrations of the test substance in the treatments were not measured. The test end points were calculated based on the nominal concentrations by using the maximum likelihood estimation method. A statistical analysis called Probit was used. The test substance contains < 60% of the notified chemical and other chemical constituents. Consequently, the resultant toxicity in this experiment may have been contributed to by all of the chemical constituents, including the notified chemical, in the product. Therefore, the observed effect (toxicity) of the notified chemical (< 60% concentration in the product) has not been adjusted to reflect the actual 100% concentration of the notified chemical.
CONCLUSION	The test substance is not harmful to aquatic invertebrates
TEST FACILITY	RCC (1989f)

C.2.3. *Lemna gibba* test

TEST SUBSTANCE	Notified chemical (purity ~89%)

METHOD

OECD Guidelines for the Testing of Chemicals, No. 221, *Lemna sp.* Growth Inhibition Test, 2006.

Commission Regulation (EC) No *761 /2009* laying down test methods pursuant to Regulation (EC) No *190712006* of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), 2009, C.26: Lemna sp. Growth Inhibition Test.

US EPA OPPTS 850.4400: Ecological Effects Test Guidelines, Aquatic Plant Toxicity Test Using *Lemna spp.;* Public Draft, Apri l 1996.

Species	Duckweed (*Lemna gibba)*
Exposure Period	7 days
Concentration Range	Nominal: Control and 0.1, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L Measured: Control and 0.035, 0.14, 0.51, 2.3, 9.0, 32 and 105 mg/L
Auxiliary Solvent	None
Water Hardness	300 mg $CaCO_3$/L
Analytical Monitoring	The test concentrations were analysed using HPLC/UV at the start of the test (Day 0) and at the renewal on Day 2.
Remarks - Method	The test was conducted following the test guidelines and good laboratory practice (GLP) principles.

The test was to evaluate the impact of the notified chemical on the growth of the freshwater aquatic plant *Lemna gibba* (duckweed). The plants were exposed to the test item for seven days in a semi-static test. The treatments were renewed every 48 hours. At the test medium renewal dates, the test plants were transferred under aseptic conditions to clean test vessels with freshly prepared test medium of the corresponding concentration.

On Day 3, 5 and 7, the number of fronds and colonies of the *Lemna gibba* plants were counted. At the same dates, the plants were inspected for changes in appearance (e.g. discoloration, sinking, root length, or other abnormalities). The dry weights of the plants at the start and end of the test were determined.

RESULTS

Remarks - Results

All validity criteria for the test were satisfied. At the mean measured concentration of 0.51 mg/L and all lower test item concentrations, the growth rate and yield based on dry weight were not statistically significantly lower than in the control after the exposure period of 7 days.

No abnormalities were recorded in appearance of the test plants in the control and the mean measured concentrations of 0.035 and 0.14 mg/L. At the mean measured concentration of 0.51 mg/L the roots turned brown throughout the test. No mortality of fronds was observed during the entire test duration.

The 7 day NOEC was determined to be 0.035 mg/L since the growth of the plants was not inhibited and no abnormalities in appearance of the plants was observed. The 7 day EC50 was determined to be > 105 mg/L based on frond mortality and frond number growth rate.

The test substance contains < 90% of the notified chemical and other chemical constituents. Consequently, the resultant toxicity in this experiment may have been contributed to by all of the chemical constituents, including the notified chemical, in the product. Therefore, the observed effect (toxicity) of the notified chemical (< 90% concentration in the product) has not been adjusted to reflect the actual 100% concentration of the notified chemical.

CONCLUSION

The 7 day EC50 was > 105 mg/L based on frond mortality and frond

	number growth rate.
TEST FACILITY	Harlan (2012)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Aerobic activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 3.2, 10, 32, 50, and 100 mg/L
Remarks – Method	The test was conducted following the test guidelines and good laboratory practice (GLP) principles. In addition to a blank control test, a reference control test using 3,5-dichlorophenol was conducted at concentrations of 1, 3.2, 10, 32 and 50 mg/L.
RESULTS	
IC50	> 100 mg/L
NOEC	100 mg/L
Remarks – Results	All validity criteria for the test were satisfied. No inhibition of the bacteria respiration was observed during the 3-hour test at all the test concentrations.
CONCLUSION	The test substance is not inhibitory to the sludge bacteria respiration up to 100 mg/L.
TEST FACILITY	RCC (1989c)

C.2.5. Earthworm

TEST SUBSTANCE	Notified chemical (purity ~52%)
METHOD	OECD TG 207: Earthworm, Acute Toxicity Tests. EEC Directive 87/302, No. L 133.
Remarks - Method	The test was conducted following the test guidelines and good laboratory practice (GLP) principles.
RESULTS	
Remarks - Results	All validity criteria for the test were satisfied. No mortality was observed after 14 days of exposure in the control and at test concentrations of 1.0, 10 and 100 mg/kg dry soil. In comparison to the control group, no statistically significant inhibitory effect on the average body weight of worms was observed at the lowest concentration of 62.5 mg/kg. The LC50 after 14 days of exposure was > 1,000 mg/kg dry soil. The body weight data were evaluated by the Dunnett's-test. The test substance contains < 60% of the notified chemical and other chemical constituents. Consequently, the resultant toxicity in this experiment may have been contributed to by all of the chemical constituents, including the notified chemical, in the product. Therefore, the observed effect (toxicity) of the notified chemical (< 60% concentration in the product) has not been adjusted to reflect the actual 100% concentration of the notified chemical.
CONCLUSION	The test substance is considered to be very slightly toxic to earthworms.
TEST FACILITY	RCC (1989d)

BIBLIOGRAPHY

CCR (1989) [Notified chemical]: Salmonella Typhimurium Reverse Mutation Assay for Azo Dyes (Project Number 154607, July, 1989). D-6100 Darmstadt, F.R.G, Germany Cytotest Cell Research GMBH & CO. KG (Unpublished report provided by the notifier).

Clariant (undated) Exhaustion Value for Cartasol Brown M-2R liquid (Unpublished report provided by the notifier).

Finney, D.J. (1971) Probit analysis, Cambridge University Press; Cambridge, U.K., 3rd edition.

Harlan (2012) [Notified chemical]: Toxicity to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test (Study Number D35037, November, 2012). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report provided by the notifier).

Harlan Laboratories S. A. (2014) [Notified chemical]: Development Toxicity Study (Oral Gavage) in the Rat (Study Number S41301, June, 2014). Barcelona, Spain, Harlan Laboratories S. A. (Unpublished report provided by the notifier).

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

Prival MJ and Mitchell VD (1982) Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S9. *Mutat Res*. 97(2): 103-16.

RCC (1989a) Ready Biodegradability: "Modified OECD Screening Test" for the [Notified chemical] (Project Number 229781, June, 1989). Itingen/BL, Switzerland, RCC Umweltchemie AG (Unpublished report provided by the notifier).

RCC (1989b) [Notified chemical]: Inherent Biodegradability: "Modified Zahn-Wellens Test" (Project Number 244438, December, 1989). Itingen/BL, Switzerland, RCC Umweltchemie AG (Unpublished report provided by the notifier).

RCC (1989c) [Notified chemical]: Assessment of the Acute Toxicity of the [Notified chemical] on Aerobic Waste-Water Bacteria (Project Number 229792, July, 1989). Itingen/BL, Switzerland, RCC Umweltchemie AG (Unpublished report provided by the notifier).

RCC (1989c) [Notified chemical]: Determination of the Water Solubility of the [Notified chemical] (Project Number 29972, December, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC (1989d) Acute Toxicity (LC50) of the [Notified chemical] to Earthworms (Project Number 368010, June 1994). Itingen/BL, Switzerland, RCC Umweltchemie AG (Unpublished report provided by the notifier).

RCC (1989d) [Notified chemical]: Determination of the Partition Coefficient (N-Octanol/Water), by High Performance Liquid Chromatography (HPLC), of the [Notified chemical] (Project Number 229623, September, 1989). Hambakenwetering 7, 5231 DD's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC (1989e) [Notified chemical]: 96-Hour Acute Toxicity Study (LC50) (Project Number 019293, October, 1989). Hambakenwetering 7, 5231 DD's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC (1989f) 24/48-Hour Acute Toxicity Study of [Notified chemical] to *Daphnia magna* (Project Number 007425, September, 1989). Hambakenwetering 7, 5231 DD's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC (1990) [Notified chemical]: Contact Hypersensitivity in Albino Guinea Pigs (Modified Buehler Method) (Project Number 260728, May, 1990). P.O. Box Ch-4452 Itingen, Switzerland, RCC, Research & Consulting Company AG (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989a) [Notified chemical]: Determination of the Melting Point/Melting Range (Project Number 229588, May, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989b) [Notified chemical]: Determination of the Density (Project Number 229590, July, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989c) [Notified chemical]: Determination of the Surface Tension of an Aqueous Solution (Project Number 229601, August, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989d) [Notified chemical]: Determination of the Flammability (Project Number 229634, August, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989e) [Notified chemical]: Determination of Auto-Flammability (Project Number 229656, September, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989f) [Notified chemical]: Determination of Explosive Properties (Project Number 229645, October, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989g) [Notified chemical]: Determination of Oxidising Properties (Project Number 229667, August, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989h) [Notified chemical]: Assessment of Acute Oral Toxicity (Project Number 229680, October, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989i) [Notified chemical]: Assessment of Acute Dermal Toxicity (Project Number 229691, August, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989j) [Notified chemical]: Primary Skin Irritation/Corrosion Study (Project Number 229702, July, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989k) [Notified chemical]: Acute Eye Irritation/Corrosion Study (Project Number 229713, August, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989l) [Notified chemical]: Skin Sensitisation in the Albino Guinea Pig (Maximization Test) (Project Number 229724, October, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989m) [Notified chemical]: Subacute 28-day Oral Toxicity by Daily Gavage (Project Number 229735, September, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989n) [Notified chemical]: Evaluation of the Mutagenic Activity in the AMES Salmonella/Microsome Test (with Independent Repeat) (Project Number 007403, May, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

RCC NOTOX B.V. (1989o) [Notified chemical]: Micronucleus Test in Bone Marrow Cells of the Mouse (Project Number 007392, July, 1989). Hambakenwetering 7, 5231 DD 's-Hertogenbosch The Netherlands, RCC NOTOX B.V. (Unpublished report provided by the notifier).

SCCNFP (2002) The Safety Review Of The Use Of Certain Azo-Dyes In Cosmetic Products: Opinion Of The Scientific Committee On Cosmetic Products And Non-Food Products Intended For Consumers. SCCNFP/0495/01 (prepared in the context of Directive 76/768/EEC).

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/managing-risks-of-hazardous-chemicals-in-the-workplace.

TKL Research Inc. (1990) [Notified chemical]: Repeated Insult Patch Test (TKL Study Number 901001, March, 1990). Maywood, NJ 07601, TKL Research Inc. (Unpublished report provided by the notifier).

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.