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AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

RD19136 / RD14154

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals Act 2019* (the IC Act) and *Industrial Chemicals (General) Rules 2019* (the IC Rules) by following the *Industrial Chemicals (Consequential Amendments and Transitional Provisions) Act 2019* (the Transitional Act) and *Industrial Chemicals (Consequential Amendments and Transitional Provisions) Rules 2019* (the Transitional Rules). The legislations are Acts of the Commonwealth of Australia. The Australian Industrial Chemicals Introduction Scheme (AICIS) is administered by the Department of Health, and conducts the risk assessment for human health. The assessment of environmental risk is conducted by the Department of Agriculture, Water and the Environment.

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TABLE OF CONTENTS

SUMMARY 3
CONCLUSIONS AND REGULATORY OBLIGATIONS 3
ASSESSMENT DETAILS 5
1. APPLICANT AND APPLICATION DETAILS 5
2. IDENTITY OF CHEMICAL 5
3. COMPOSITION 5
4. PHYSICAL AND CHEMICAL PROPERTIES 5
5. INTRODUCTION AND USE INFORMATION 6
6. HUMAN HEALTH IMPLICATIONS 7
6.1. Exposure Assessment 7
6.1.1. Occupational Exposure 7
6.1.2. Public Exposure 7
6.2. Human Health Effects Assessment 8
6.3. Human Health Risk Characterisation 9
6.3.1. Occupational Health and Safety 9
6.3.2. Public Health 9
7. ENVIRONMENTAL IMPLICATIONS 9
7.1. Environmental Exposure & Fate Assessment 9
7.1.1. Environmental Exposure 9
7.1.2. Environmental Fate 10
7.1.3. Predicted Environmental Concentration (PEC) 10
7.2. Environmental Effects Assessment 10
7.2.1. Predicted No-Effect Concentration 11
7.3. Environmental Risk Assessment 11
APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES 12
APPENDIX B: TOXICOLOGICAL INVESTIGATIONS 14
B.1. Acute Dermal Toxicity – Rat 14
B.2. Skin Irritation – Rabbit 14
B.3. Eye Irritation – Rabbit 14
B.4. Skin Sensitisation – LLNA (maximum test concentration 10%) 15
B.5. Skin Sensitisation – LLNA (maximum test concentration 25%) 16
B.6. Combined Repeat Dose Oral Toxicity and One Generation Reproduction Toxicity – Rats 16
B.7. Genotoxicity – Bacteria 18
B.8. Genotoxicity – *In Vitro* Mouse Lymphoma Assay 19
B.9. Genotoxicity – *In Vitro* Chromosome Aberration Test in Human Lymphocytes 20
APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS 22
C.1. Environmental Fate 22
C.1.1. Ready Biodegradability 22
C.2. Ecotoxicological Investigations 22
C.2.1. Acute Toxicity to Fish 22
C.2.2. Acute Toxicity to Aquatic Invertebrates 23
C.2.3. Chronic Toxicity to Aquatic Invertebrates 24
C.2.4. Algal Growth Inhibition Test 24
C.2.5. Toxicity to soil microorganisms – Carbon Transformation Test 25
C.2.6. Toxicity to soil microorganisms – Nitrogen Transformation Test 25
C.2.7. Earthworms Toxicity Study 26
C.2.8. Terrestrial Plants Toxicity Study 26
BIBLIOGRAPHY 28

SUMMARY

The following details will be published on the AICIS website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1729	Cintox Australia Pty Ltd	RD19136 / RD14154	ND*	≤ 20 tonnes per annum	A component of industrial coatings

* Not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification
Based on the available information, the assessed chemical cannot be classified as hazardous according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

The environmental hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard Classification	*Hazard Statement*
Chronic Category 2	H411- Toxic to aquatic life with long lasting effects

Human Health Risk Assessment
Under the conditions of the occupational settings described, the assessed chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental Risk Assessment
On the basis of the low environmental exposure from the reported use pattern, the assessed chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

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 assessed chemical during reformulation and end-use:
  - Local exhaust ventilation when handling the assessed chemical in powder form
  - Spray booth for end-use application by spraying

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

- 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 assessed chemical during reformulation and end-use:
  - Respiratory protection if inhalation exposure to dust and aerosols may occur

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

- Spray applications should be carried out in accordance with the Safe Work Australia Code of Practice for *Spray Painting and Powder Coating* (SWA, 2015) or relevant State or Territory Code of Practice.

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

- If products and mixtures containing the assessed chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of 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.

Emergency procedures

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

Disposal

- Where reuse or recycling are not appropriate, dispose of the assessed chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Regulatory Obligations

Specific Requirements to Provide Information
This risk assessment is based on the information available at the time of the application. The Executive Director may initiate an evaluation of the chemical based on changes in certain circumstances. Under section 101 of the IC Act the introducer of the assessed chemical has post-assessment regulatory obligations to provide information to AICIS when any of these circumstances change. These obligations apply even when the assessed chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory).

Therefore, the Executive Director of AICIS must be advised in writing within 20 working days by the applicant or other introducers if:

- the function or use of the chemical has changed from a component in industrial coatings, 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 human health, or the environment.

The Executive Director will then decide whether an evaluation of the introduction is required.

Safety Data Sheet
The SDS of the assessed chemical provided by the applicant was reviewed by AICIS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND APPLICATION DETAILS

APPLICANT(S)
Cintox Australia Pty Ltd (ABN: 63 122 874 613)
26 Male Street
BRIGHTON VIC 3186

APPLICATION CATEGORY
Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Similar to a chemical previously assessed by AICIS (formerly NICNAS).

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
Data items and details taken to be protected information include: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, use details, import volume and identities of analogues.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for water solubility, hydrolysis as a function of pH, partition coefficient, flash point, flammability and genotoxic damage *in vivo*.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
RD19136 / RD14154

MOLECULAR WEIGHT
< 700 g/mol

ANALYTICAL DATA
Reference NMR and IR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 85 %

The assessed chemical is an unknown variable composition or biological (UVCB) substance with three major components comprising > 85% of the total concentration of the chemical.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: off-white powder

Property	*Value*	*Data Source/Justification*
Melting Point/Freezing Point	120 – 130 °C	SDS
Boiling Point	> 352 °C	SDS (Decomposition temperature)
Relative Density	1,020 kg/m^3	SDS
Vapour Pressure	< 1.1 × 10^{-6} kPa at 25 °C	Measured
Water Solubility*	0.1 – 147 mg/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Not expected to significantly hydrolyse in the environmental pH range (4-9)

Property	*Value*	*Data Source/Justification*
Partition Coefficient (n-octanol/water)*	log Kow > 6.2 at 20 °C	Measured
Adsorption/Desorption*	log Koc = 2.28 to > 5.6	Measured
Dissociation Constant	pKa < 0 and >14	Predicted values using ACD/Labs I-Lab 2.0
Surface Tension	60.5 mN/m at 20.5 °C	Measured
Particle Size		
Inhalable fraction (< 100 µm)	18.8%	Measured
Respirable fraction (< 10 µm)	2.25%	
Flash Point	Not determined	Not expected to form flammable vapour
Flammability	Not determined	Not expected to be highly flammable based on analogue information
Autoignition Temperature	> 140 °C	Measured. Not autoignite below melting temperature
Explosive Properties	Predicted negative	Based on chemical structure
Oxidising Properties	Predicted negative	Based on chemical structure

* Values were obtained for three main components of the assessed chemical

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemical will be introduced into Australia in neat form for reformulation or as a component of finished industrial coatings at ≤ 7% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The assessed chemical in neat powder form will be imported in 15 kg multi-ply paper bags. The finished industrial coating products containing the assessed chemical at ≤ 7% concentration will be imported in various size containers, including 1, 4 and 10 L cans and 210 kg lined steel drums.

USE
The assessed chemical will be used as an organic rheological additive in solvent-based industrial coatings at ≤ 7% concentration.

OPERATION DESCRIPTION
The assessed chemical will not be manufactured in Australia. It will be imported into Australia in neat powder form for reformulation into coatings for use in industrial settings. The assessed chemical will also be introduced as a component of finished industrial coatings at ≤ 7% concentration.

Reformulation
The assessed chemical in powder form will be manually weighed under fume hood into a dispensing container and transferred to a mixing vessel where it will be blended with additional additives in a mixture of solvents and resins

to form coatings. Following blending, samples will be taken for quality control testing. The finished coatings containing the assessed chemical at ≤ 7% concentration will be filled into containers by gravity feed or low-pressure pump and then distributed to industrial end-users.

End Use
The finished coatings containing the assessed chemical at ≤ 7% concentration will only be used by trained industrial users and will be applied by brush, roller or spray at various industrial locations. Spray applications will be conducted in purpose-built spray facilities.

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 and storage	1	4
Warehouse	1	4
Process operator	2.5	40
Quality control	0.5	40
Packaging	2	40
End Use	1	60

EXPOSURE DETAILS

Transport and storage
Transport, storage and warehouse workers may come into contact with the assessed chemical at ≤ 100% concentration only in the unlikely event of accidental rupture of containers.

Reformulation
Reformulation will be largely enclosed and automated; however dermal and ocular exposure of workers to the assessed chemical at ≤ 100% concentration may occur during weighing and transfer stages, quality control analysis, and cleaning and maintenance of equipment. Given the use of automated and enclosed processes and its low vapour pressure (< 1.1×10^{-6} kPa at 25 °C), inhalation exposure to the assessed chemical is not expected unless dust is generated, particularly during weighing and transfer of the neat powder form. According to the applicant's information, exposure of workers will be minimised through the use of engineering controls (such as a fume hood and closed weighing/dispensing containers) and personal protective equipment (PPE), such as protective clothing, eye protection and impervious gloves.

End-use
Finished coatings containing the assessed chemical will be applied by spray, brush or roller. Dermal and ocular exposure to the coatings containing the assessed chemical at ≤ 7% concentration may occur during transfer, application and cleaning of application equipment. Inhalation exposure may also occur during spray application. The potential for exposure will be minimised through the use of PPE (goggles, impervious gloves, protective clothing) by workers, including the use of appropriate respiratory protection, ventilation and spray booths (e.g. engineered facilities) during spray application.

Once the coatings are dried, the assessed chemical will be bound into an inert solid matrix and is not expected to be available for exposure.

6.1.2. Public Exposure
Finished coatings containing the assessed chemical at ≤ 7% concentration will be for industrial use only and will not be made available to the public. The public may come into contact with surfaces that have been coated with products containing the assessed chemical. However, once the coatings are dried, the assessed chemical will be bound into an inert solid matrix and is not expected to be available for exposure.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the assessed chemical are summarised in the following table. For details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Acute dermal toxicity – rat	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation – rabbit	non-irritating
Eye irritation – rabbit	slightly irritating
Skin sensitisation – mouse local lymph node assay conducted at up to 10% prepared in Propylene Glycol	no evidence of sensitisation
Skin sensitisation – mouse local lymph node assay conducted at up to 25% prepared in 1% Pluronic L92 in distilled water	no evidence of sensitisation
Repeat dose oral toxicity – rat, > 90 days*	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mouse lymphoma assay	non mutagenic
Genotoxicity – *in vitro* chromosome aberration test in human lymphocytes	non clastogenic
Reproductive toxicity (screening) – rat*	NOAEL = 1000 mg/kg bw/day**

* A combined study was conducted to evaluate repeated dose and reproductive toxicity

** Established by the study author, noting that some effects were observed in males at lower doses (see below).

Toxicokinetics, Metabolism and Distribution

Given the low molecular weight (< 700 g/mol), the assessed chemical may be absorbed across the respiratory and gastrointestinal tract, if exposed. Based on the measured partition coefficient (log Kow > 6.2) and water solubility (0.1 – 147 mg/L) values, percutaneous absorption could be limited.

Acute Toxicity

The assessed chemical was of low toxicity based on a rat dermal toxicity study. No information on acute toxicity via oral and inhalation routes were provided.

Irritation and Sensitisation

The assessed chemical was non-irritating to the skin based on a rabbit skin irritation study. The assessed chemical was slightly irritating to the eyes of rabbits. Slight to moderate conjunctival redness and chemosis were observed at 1 hour which resolved at 24 hour post administration.

The assessed chemical was determined not to be a skin sensitiser in mouse local lymph node assays at up to 25% concentration prepared in 1% Pluronic L92, and up to 10% concentration prepared in propylene glycol.

Repeated Dose Toxicity

A combined repeated dose 90-day oral toxicity (OECD TG 408) and one generation reproduction toxicity (OECD TG 415) test with the assessed chemical at 50, 250 and 1000 mg/kg bw/day was conducted in rats. The No Observed Adverse Effect Level (NOAEL) for systemic effects was established as 1000 mg/kg bw/day, the highest dose tested in this study.

Mutagenicity/Genotoxicity

The assessed chemical was not mutagenic in a bacterial reverse mutation assay and in an *in vitro* mouse lymphoma assay. The assessed chemical was not clastogenic in an *in vitro* chromosome aberration test in human lymphocytes.

Toxicity for Reproduction

A NOAEL for reproduction effects was established by the study authors at 1000 mg/kg bw/day, the highest dose tested in the combined repeated dose 90-day oral toxicity and one generation reproduction toxicity study in rats. Statistically significant increases in left epididymis and left cauda epididymis weights were observed in the males in mid and high dose groups. Blood haemoglobin in urine was also detected in males treated with the test substance. However, there were no related histopathological changes and given no changes in male reproduction capabilities from the same doses these effects were not considered to be adverse by the study authors.

Health Hazard Classification

Based on the available information, the assessed chemical cannot be classified as hazardous according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the toxicological information provided, the assessed chemical is slightly irritating to the eyes. When introduced in powder form for reformulation into end-use paint products, there is potential for inhalation and dermal exposure. However, there is no inhalation toxicity data available for the assessed chemical.

Reformulation

During reformulation, there is a concern for the health of workers related to slight eye irritation and potential lung overloading effects from inhalation of the dust particles generated during weighing and transfer of the chemical in powder form if control measures are inadequate. The risk should be minimised through the use of engineering controls (fume hood and closed weighing/dispensing containers). The use of PPE such as goggles, gloves and overalls and respiratory protection could also reduce exposure, where engineering controls may be inadequate.

Overall, provided control measures are in place to minimise ocular, dermal and inhalation exposure to the assessed chemical as introduced in powder form, including engineering controls and PPE, the risk to the health of workers during reformulation is not considered to be unreasonable.

End-use

Workers may experience dermal and possibly ocular exposure to the notified chemicals at ≤ 7% concentration during transfer, application (through brush or roller) and cleaning processes. The use of PPE by workers will minimise exposure to the assessed chemical. Inhalation exposure is also possible during spray application. The applicant has stated that workers will wear air-fed respirators and spray applications will be conducted under ventilation in purpose built spray facilities.

Once the coatings are dried, the assessed chemical will be bound into an inert solid matrix and will not be available for exposure.

Overall, based on the toxicity data provided for the assessed chemical and occupational settings described, the risk to workers during end use is not considered to be unreasonable.

6.3.2. Public Health

Finished coatings containing the assessed chemical at ≤ 7% concentration will be for industrial use only and will not be made available to the public. The public may come into contact with surfaces that have been coated with products containing the assessed chemical. However, once the coatings are dried, the assessed chemical will be bound into an inert solid matrix and will not be available for exposure. Therefore, when used in the proposed manner, the risk to public health 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 assessed chemical will be introduced into Australia as a component of finished coatings and in its neat form for reformulation into industrial coatings. The assessed chemical will be mixed with solvents and resins and reformulated into coating formulations in a closed system. Solvent used for equipment washing (which contains residues of the assessed chemical) is expected to be recycled for reuse on site or disposed of via accredited waste disposal contractors. Wastes and spills during reformulation activities (1% of annual import volume) are expected to be contained on site and disposed of in accordance with local regulations. Residues in import containers are expected to be disposed of via the trade waste stream in accordance with local regulations. Any spills of the assessed chemical during transportation and storage are expected to be contained with absorbent material and be disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

Coatings containing the assessed chemical may be applied by brush, roller or spray at industrial locations. The applicant has indicated that the coatings containing the assessed chemical will be mostly applied in spray facilities and that the main release of the assessed chemical during industrial spray painting operations will come from

overspray, accounting for up to 30% of the annual import volume. Overspray, accidental spills, application equipment washings (up to 5% of the annual import volume) and residues in empty paint containers (up to 2.5% of the annual import volume) are expected to be collected and disposed of to landfill in accordance with local, State and Federal regulations.

RELEASE OF CHEMICAL FROM DISPOSAL

The assessed chemical in coatings is expected to share the fate of articles to which it has been applied. The assessed chemical is likely to be either thermally decomposed during metal reclamation processes or disposed of to landfill at the end of the useful life of the article to which it has been applied.

7.1.2. Environmental Fate

As the assessed chemical is a UVCB, the individual components are characterised by different physical and chemical properties and will exhibit different behaviour in the environment. Three main components had measured water solubility ranging from 0.1 to 147 mg/L at 20 °C. The applicant's data indicate that the assessed chemical is not readily biodegradable (OECD TG 301B; 20% biodegradability over 28 days). For the details of the environmental fate study refer to Appendix C.

Three major components of the assessed chemical had measured octanol-water partition coefficients (log Kow) > 6.2 suggesting that they may potentially bioaccumulate in the environment. The chemical components were also determined to have an adsorption coefficient (log Koc) ranging from 2.28 to > 5.6.

Most of the assessed chemical is expected to be incorporated into an inert matrix of cured coatings as part of its use pattern. The assessed chemical is not expected to be bioavailable in this form. The assessed chemical will eventually degrade in landfill, or by thermal decomposition during metal reclamation processes, to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated as release of the assessed chemical to the aquatic environment will be limited based on its reported use pattern.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Aquatic Toxicity		
Acute Toxicity		
Fish	96 h LL50 > 100 mg/L	Not harmful to fish up to its water solubility limit
Daphnia	48 h EL50 > 1000 mg/L	Not harmful to aquatic invertebrates
Algae	72 h EL50 = 370 mg/L	Not harmful to algal growth
Chronic Toxicity		
Daphnia	21 d NOEC = 0.9 mg/L	Toxic to aquatic invertebrate reproduction
Algae	72 h NOEL = 125 mg/L	Not harmful to algal growth
Terrestrial Toxicity		
Nitrogen transformation activity of soil microorganisms	28 d EC50 > 1000 mg/kg	Does not inhibit the nitrogen transformation activity of soil microorganisms
Carbon transformation activity of soil microorganisms	28 d EC50 > 1000 mg/kg	Does not inhibit the carbon transformation activity of soil microorganisms
Terrestrial plants	21 d EC50 > 1000 mg/kg dry weight	Not harmful to terrestrial plants (seedling emergence and growth)
Earthworms	14 d EC50 > 1000 mg/kg soil	Very slightly toxic to earthworms

Based on the above acute ecotoxicological endpoints for the assessed chemical, it is not expected to be acutely harmful to aquatic life and not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) for short-term hazard (United Nations, 2009).

The reproductive ecotoxicity study conducted on *Daphnia magna* was used to determine the GHS long-term classification. Based on the biodegradation data the assessed chemical is not rapidly biodegradable for the purposes of GHS classification. Therefore, based on the available data the assessed chemical is formally classified under the GHS as Chronic Category 2: H411 – Toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration (PNEC) was calculated based on the most sensitive chronic endpoint for daphnia (21-d NOEC = 0.9 mg/L) using an assessment factor of 50 as three acute endpoints and two chronic endpoints are available for aquatic environment.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
21-d NOEC (invertebrates)	0.9	mg/L
Assessment Factor	50	
Mitigation Factor	1	
PNEC:	18.0	µg/L

7.3. Environmental Risk Assessment

Risk quotients (Q = PEC/PNEC) for the assessed chemical have not been calculated as release to the aquatic environment in ecotoxicologically significant concentrations is not expected based on the reported use patterns as components of industrial coatings. Moreover, after curing, most of the imported quantity of the assessed chemical will be irreversibly incorporated into an inert matrix and the chemical is not expected to be mobile or bioavailable. On the basis of the low environmental exposure from the reported use pattern, the assessed chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Vapour Pressure $< 1.1 \times 10^{-6}$ kPa at 25 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Determined using vapour pressure balance. Measurements taken at several temperatures and linear regression analysis used to derive the value.
Test Facility	Harlan (2012a)

Water Solubility 0.1 to 147 mg/L at 20 °C

Method	OECD TG 105 Water Solubility
Remarks	Flask Method The assessed chemical is comprised of at least three main chemical components each of which has a different water solubility. The solubilities of each individual component were determined.
Test Facility	SPL (2001a)

Partition Coefficient (n-octanol/water) log Kow > 6.2 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method The assessed chemical is comprised of at least three main chemical constituents which have individual n-octanol/water partition coefficients. The log Kow of each component was determined.
Test Facility	SPL (2001a)

Surface Tension 60.5 mN/m at 20.5 °C

Method	EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	Determined using a White Electrical Institute interfacial tension balance and a procedure based on the ISO 304 ring method. Although correction was not used for surface tension result as it is not applicable to the apparatus used, this deviation was not considered to affect the integrity of the study.
Test Facility	SPL (2001a)

Adsorption/Desorption – screening test log Koc = 2.28 to > 5.63 at 30 °C

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	HPLC screening method. The log Koc values of each component were determined.
Test Facility	Harlan (2012b)

Dissociation Constant pKa < 0 and > 14

Method	OECD TG 112 Dissociation Constants in Water
Remarks	HPLC method The assessed chemical is comprised of at least three main chemical constituents which have similar, overlapping pKa values. Due to low water solubility, the experimental determination of dissociation constants was not practically possible. The predicted pKa values for the three components were determined using ACD/Labs I-Labs 2.0.
Test Facility	SPL (2001a)

Particle Size Inhalable fraction (< 100 µm): 18.8%
Respirable fraction (< 10 µm): 2.25%

Method	Screening test was conducted using sieve method and definitive test was conducted using cascade impactor method.
REMARKS	The result for respirable fraction was based on the mean of three definitive test determinations. According to the study report the mass median aerodynamic diameter was not calculated due to a very low level of particles < 10 µm.
Test Facility	SPL (2001a)

Autoignition Temperature > 140 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Remarks	The test item was determined not to have a relative autoignition temperature below its melting point.
Test Facility	Harlan (2012c)

Explosive Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties
Remarks	Based on the chemical structures of the components of the test item.
Test Facility	Harlan (2012d)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids)
Remarks	Based on the chemical structures of the components of the test item.
Test Facility	Harlan (2012e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test (1987) EC Regulation No 440/2008 B.3 Acute Toxicity (Dermal)
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	Arachis oil BP (moistened)
Type of dressing	Semi-occlusive
Remarks – Method	No significant protocol deviation.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 F & 5 M	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Slight erythema (score 1), light brown discolouration of the epidermis, slight desquamation and small superficial scattered scabs were noted in the test site of 2/5 female rats. The effects were reversed by day 14 of the study.
Signs of Toxicity – Systemic	No test substance-mediated toxicity was observed.
Effects in Organs	A renal cavity in the right kidney was noted in one male rat during necropsy. The defect was considered as genetic abnormality and not related to the test substance.
Remarks – Results	One female rat showed no body weight gain during the first week but expected body weight gain was observed in the second week of the study.
CONCLUSION	The assessed chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Harlan (2012f)

B.2. Skin Irritation – Rabbit

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (2002) EC Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)
Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	3
Vehicle	Distilled water (moistened)
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks – Method	No significant protocol deviation.

RESULTS

Remarks – Results	No erythema or oedema was observed in any of the test sites.
CONCLUSION	The assessed chemical is non-irritating to the skin.
TEST FACILITY	Harlan (2012g)

B.3. Eye Irritation – Rabbit

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion (2002)

	EC Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation)
Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	3
Observation Period	72 hours
Remarks – Method	No significant protocol deviation.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva – Redness	0.33	0.33	0.33	2	1 h	0
Conjunctiva – Chemosis	0.33	0.33	0.33	2	1 h	0
Conjunctiva – Discharge	0.0	0.0	0.0	1	1 h	0
Corneal Opacity	0	0	0	0	-	0
Iridial Inflammation	0	0	0	1	1 h	0

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

Remarks – Results	Slight to moderate conjunctival redness and chemosis were observed at 1 hour which resolved at 24 hour post administration.
CONCLUSION	The assessed chemical is slightly irritating to the eye.
TEST FACILITY	Harlan (2012h)

B.4. Skin Sensitisation – LLNA (maximum test concentration 10%)

TEST SUBSTANCE	Assessed chemical (up to 10%)
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2002)
Species/Strain	Mouse/CBA/Ca (CBA/CaBkl)
Vehicle	Propylene glycol
Preliminary study	Not conducted
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using: • α-hexylcinnamaldehyde in acetone/olive oil (4:1) at 5%, 10%, 25% (SI ≥ 5.5) • 2-mercaptobenzothiazole in dimethyl formamide at 1%, 5%, 25% (SI = 5.4)
Remarks – Method	The positive controls were not conducted in parallel with the test substance.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (Dpm/node)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	4F	448.67	–
2.5	4F	566.83	1.26
5	4F	848.90	1.89
10	4F	694.36	1.55

Remarks – Results	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the assessed chemical at up to 10%. The EC3 value was not determined due to lack of test substance mediated increase in stimulation index over 3.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the assessed chemical at up to 10% concentration.

TEST FACILITY SPL (2003)

B.5. Skin Sensitisation – LLNA (maximum test concentration 25%)

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/CBA/Ca (CaOlaHsd)
Vehicle 1% Pluronic L92 in distilled water
Preliminary study Yes
Positive control Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-Hexylcinnamaldehyde as a solution in 1% Pluronic L92 in distilled water at 5%, 10% and 25% (SI = 8.17)
Remarks – Method The vehicle used was selected as the test substance did not achieve desirable solubility in any of the OECD recommended vehicles.

The positive controls were not conducted in parallel with the test substance.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response Mean ±S.D.*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5F	1450.40 ± 932.59	-
5	5F	1359.72 ± 327.69	0.94
10	5F	1316.12 ± 648.60	0.91
25	5F	1194.38 ± 374.90	0.82

Remarks – Results The positive control was not conducted in parallel with the test substance. Only the positive control concentration tested and stimulation index values were reported. For concentrations of 5%, 10% and 25% the stimulation index values were 1.3, 2.37 and 8.14 respectively.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the assessed chemical at up to 25% concentration. The EC3 value was not determined due to lack of test substance mediated increase in stimulation index over 3.

TEST FACILITY Harlan (2010a)

B.6. Combined Repeat Dose Oral Toxicity and One Generation Reproduction Toxicity – Rats

TEST SUBSTANCE Assessed chemical

METHOD Combined OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (1998) and OECD TG 415 One Generation Reproduction Toxicity in Rodents (1983)
Species/Strain Rat/Wistar (HsdRccHan^{TM}:WIST)
Route of Administration Oral – gavage
Exposure Information Total exposure days: 90–108 days (at least 28 days prior to mating and throughout the mating, gestation and lactation for group B animals)
Dose regimen: 7 days per week
Vehicle Arachis oil BP
Remarks – Method No significant protocol deviation. In a 21-day dose-range finding study, the test substance in arachis oil BP was administered by oral gavage in Wistar Han:RccHan rats (3/sex/dose) at 0, 500 and 1,000 mg/kg bw/day. No clinical signs of toxicity or adverse body weight changes or

macroscopic abnormalities was observed in treated animals. Based on the results, doses of 50, 250 and 1000 mg/kg bw/day were chosen for the main study.

For reproduction toxicity evaluation, group B female rats were introduced from week 8 of the study and were paired with group A male rats after week 11 on a one male to one female basis within each dose group for maximum of 21 days.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Group A			
Control	10 M / 10 F	0	0 / 20
Low Dose	10 M / 10 F	50	0 / 20
Mid Dose	10 M / 10 F	250	0 / 20
High Dose	10 M / 10 F	1000	0 / 20
Group B			
Control	22 F	0	0 / 22
Low Dose	22 F	50	0 / 22
Mid Dose	22 F	250	0 / 22
High Dose	22 F	1000	0 / 22

Mortality and Time to Death

No unscheduled mortalities were observed during the study.

Clinical Observations

No clinical signs of toxicity were detected. Increased salivation was detected up to 1 hour post dosing in group A at ≥ 50 mg/kg bw/day and in Group B at ≥ 250 mg/kg bw/day. The effects were more pronounced in the male group. Salivation was considered to be due to the unpleasant taste or irritant effect of the test substance by the study author.

No treatment related changes in body weight and weight gain were observed, apart from weight gain reductions in male rats from all dose groups during week 10, with 32% reduction in low dose, 10% reduction in mid dose and 39% reduction in high dose group animals when compared to controls. Given the lack of dose-related response and no significant changes in weight and weight gain observed for other weeks, these reductions were not considered due to adverse effect of the test substance by the study author.

A slightly increased food consumption was noted in male (mid and high dose group A) and female rats (high dose group B during gestation and lactation, with a statistically significant increase of 19% only during the first week of gestation). The food consumption increases did not correlate with weight gains, and thus were not considered adverse. No differences were seen in water consumption between treated and control animals.

No treatment related changes in functional performance, sensory reactivity or behavioural tests were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Compared to controls, the following statistically significant changes were observed in group A animals:

- Reduction (9%) in prothrombin clotting time in male rats at mid dose.
- Increase (32%) in lymphocyte counts in male rats at mid and high dose.
- No eosinophils detected in female rats at high dose.
- Increase (18%) in activated partial thromboplastin times in female rats at high dose group.
- Dose-dependent increase (13%, 15% and 18%) in blood cholesterol levels in male rats (at low, mid and high dose respectively).
- Blood haemoglobin detected in urine of male rats at low (2/10 animals), mid (1/10 animal) and high (2/10 animals) dose.

These changes were not considered by study authors to represent toxic effects of the test substance due to lack of dose-related response or histopathological correlation.

Reproductive and developmental screening parameters

In the mid dose group only, a 40% reduction in progressive velocity of sperm was observed although overall there were no changes in sperm concentration, motility or morphology, compared with the controls. Increased cauda epididymis weights and reduced cauda epididymis counts were statistically significant at high dose. Such higher epididymis weights and lower epididymis spermatid counts were considered by the study authors to be attributable to unexpected lower or higher control values, rather than an effect of treatment, given semen quality and fertility were unaffected.

No treatment related changes were observed in the oestrus cycle, mating performance, fertility, gestation length, litter size or live births between the treated group B female rats and the controls. Also, there were no differences in implantation loss, survival indices or pup sex ratio.

No treatment related changes in pup body weight or weight gain were observed. Reflex action responses and macroscopic observations were also not affected. Patchy fur growth was noted in 11 pups from one particular litter in the mid dose group. However no dose response was observed.

Effects in Organs

Left epididymis weights and left cauda epididymis weights were statistically increased at mid and high doses. The increases in left epididymis were 15 – 16% and 20 – 21% at mid and high doses respectively, while the increases in left cauda epididymis were 14% and 12% at the mid and high doses respectively, when compared to controls. In the absence of dose-dependent effects and treatment related histopathological changes the findings were considered to be not toxicologically significance by the study authors.

Necropsy and Histopathology finding

No treatment–related macroscopic or histopathological findings were detected in the organs examined in either the adult animals or their offspring.

Remarks – Results

The observed effects either failed to show a dose response relationship, were not statistically significant or were spontaneous changes often observed in laboratory animals; hence they were not considered to be toxicologically important by the study authors.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for both systemic and reproductive effects was established as 1000 mg/kg bw/day, the highest dose tested in this study, by the study authors. Given that effects were noted in the males treated with the test substance, including blood haemoglobin in urine and weight increase in epididymis, the NOAEL might be lower.

TEST FACILITY	Harlan (2010b)

B.7. Genotoxicity – Bacteria

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA$^-$
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	With metabolic activation: 50–5000 µg/plate Without metabolic activation: 50–5000 µg/plate
Vehicle	Polyethylene glycol 400 (PEG 400)
Remarks – Method	Preliminary toxicity study was conducted only with the bacterial strains TA100 and WP2uvrA$^-$. Positive controls: • With S9-mix: 2-aminoanthracene (TA100, TA1535, TA1537 and WP2uvrA$^-$) and benzo(a)pyrene (TA98)

- Without S9-mix: *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (TA100, TA1535 and WPuvrA$^-$), 9-aminoacridine (TA1537) and 4-nitroquinoline-1-oxide (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	≥ 1500	Negative
Test 2	-	> 5000	≥ 1500	Negative
Present				
Test 1	> 5000	> 5000	≥ 1500	Negative
Test 2	-	> 5000	≥ 1500	Negative

Remarks – Results: No increase in the number of revertant colonies of any tested strains were observed following two experiments with the test substance at any dose level, with or without metabolic activation.

The positive controls induced a distinct increase of revertant colonies during the study indicating the validity of the test system.

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

TEST FACILITY: SPL (2000)

B.8. Genotoxicity – *In Vitro* Mouse Lymphoma Assay

TEST SUBSTANCE: Assessed chemical

METHOD: OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test Using the Thymidine Kinase Gene

Species/Strain: Mouse

Cell Type/Cell Line: L5178Y Mouse lymphoma cells (TK +/- 3.7.2c)

Metabolic Activation System: S9 mix from phenobarbital/β-naphthoflavone induced rat liver

Vehicle: RPMI 1640 medium without serum (R0 medium)

Remarks – Method: No significant protocol deviation. A preliminary test was conducted with the test substance at concentrations from 9.77 to 2500 µg/mL for 4h with S9 mix and for 4h and 24h without S9 mix. For test 1, 2% S9 mix and for test 2, 1% S9 mix were used.
Positive controls were ethylmethanesulphonate (without S9-mix) and cyclophosphamide (with S9-mix).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 39.06, 78.13, 156.25, 312.5, 625, 1250, 1875, 2500	4 h	2 days	10–14 days
Test 2	0, 50, 100, 200, 400, 600, 800, 1000, 1200	24 h	2 days	10–14 days
Present				
Test 1	0, 39.06, 78.13, 156.25, 312.5, 625, 1250, 1875, 2500	4 h	2 days	10–14 days
Test 2	0, 50, 100, 200, 400, 600, 800, 1000, 1200	4 h	2 days	10–14 days

RESULTS

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

Test 1	≥ 1250	≥1250	≥ 39.06	Negative
Test 2	≥ 1250	≥ 600	≥ 200	Negative
Present				
Test 1	≥ 1250	≥ 2500	≥ 78.13	Negative
Test 2	-	≥ 2500	≥ 312.5	Negative

Remarks – Results

There was no evidence of any dose-related increase in the mutant frequency at any test concentration with or without S9-mix.

The authors considered that the integrity of the study was unaffected by precipitation.

The positive controls induced marked increases in the mutant frequency validating the sensitivity of the assay and the efficacy of the S9-mix.

CONCLUSION

The assessed chemical was not mutagenic to mouse lymphoma cells under the conditions of the test.

TEST FACILITY

Harlan (2009a)

B.9. Genotoxicity – *In Vitro* Chromosome Aberration Test in Human Lymphocytes

TEST SUBSTANCE

Assessed chemical

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: S9 mix from phenobarbitone/β-naphthoflavone induced rat liver

Vehicle: Eagle's minimal essential medium (MEM)

Remarks – Method: No significant protocol deviation. A preliminary test was conducted with the test substance at concentrations from 19.53 to 5000 µg/mL for 4h with S9 mix and for 4h and 24h without S9 mix. For test 1 2% S9 mix was used and for test 2 1% S9 mix was used.

The positive controls were:

- With S9 mix: Cyclophosphamide 5 µg/mL
- Without S9 mix: Mitomycin C 0.4 µg/mL for 4 hour exposure and 0.2 µg/mL for 24 hour exposure.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 156.25, 312.5, 625*, 1250*, 2500*, 5000	4 h	24 h
Test 2	0*, 312.5*, 625*, 1250*, 2500, 3750, 5000	24 h	24 h
Present			
Test 1	0*, 156.25, 312.5, 625, 1250*, 2500*, 5000*	4 h	24 h
Test 2	0*, 312.5, 625, 1250, 2500*, 3750*, 5000*	4 h	24 h

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 2500	≥ 156.25	Negative
Test 2	≥ 1250	> 1250	≥ 312.5	Negative
Present				
Test 1	≥ 5000	> 5000	≥ 156.25	Negative
Test 2	-	≥ 5000	≥ 312.5	Negative

Remarks – Results	The test substance did not induce any statistically significant increase in the number of cells with chromosome aberrations at any test concentration with or without S9 mix. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The assessed chemical was not clastogenic to human lymphocytes under the conditions of the test.
TEST FACILITY	Harlan (2014)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Activated sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total organic carbon analyser
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with good laboratory practice (GLP) standards and principles. No major deviations from the test guidelines were reported. A toxicity control was also conducted.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
8	12	8	83
14	10	14	90
28	20	28	95

Remarks – Results	All validity criteria of the test guideline were satisfied. The degradation of sodium benzoate was 83% by day 8 and 95% after 28 days. These values confirm the suitability of the inoculum and test conditions. The difference in extremes were less than 20% at the end of the test, the inoculum blank reached a maximum of 30.95 mg CO_2/L and the inorganic carbon in the test suspension was < 5% of total carbon. The test substance is not considered toxic to the inoculum as the toxicity control reached > 25% by day 14. The assessed chemical showed 20% biodegradation in 28 days.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	SPL (2006a)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static test
Species	*Danio rerio* (zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	LC-MS
Remarks – Method	A limit test was performed using a Water Accommodated Fraction (WAF) of the test substance based on the results of a range finding test. The WAF (loading rate of 100 mg/L) was used directly, without dilution, as the test medium. WAFs were prepared by stirring solid test item in water for 48 hours followed by two hour settling period. The supernatant liquid was filtrated. The filtrated solution was aerated for 30 minutes before use for exposure and analysis. Test media were replaced daily.

RESULTS

Concentration (mg/L)		Number of	Mortality				
Nominal	*Actual*	*Fish*	*0 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	< LOD*	10	0	0	0	0	0
100	16.25-20.75 (component 1) 0.008-.016 (component 2) < LOD* or < LOQ** (component 3)	10	0	0	0	0	0

*LOD - limit of detection (0.0002 mg/L)
** LOQ - limit of quantification (0.005 mg/L)

LL50	> 100 mg/L at 96 hours
Remarks – Results	All the validity criteria were met. The dissolved oxygen concentrations ranged from 80.7 to 104.8%, pH values ranged from 7.29 to 8.36 and temperature ranged from 22.1 to 23.2 during the 96 h exposure period in both control and test solutions.
CONCLUSION	The test substance is not harmful to fish up to its water solubility limit.
TEST FACILITY	SYRICI (2014)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Assessed chemical
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	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	No major deviations from the test guidelines were reported. Water Accommodated Fraction (WAF) at 1000 mg/L of the test substance in test medium was prepared and used as the highest test concentration in a limit test. The WAFs were removed by syphon and used in exposure experiments. Although undissolved test material was observed throughout the water column at the end of the mixing and settling period, filtration was considered unnecessary as the range finding studies indicated no physical effect of undissolved test material on the test organisms. The test solutions were not renewed during the exposure period.

RESULTS

Concentration (mg/L)		Number of D.	Number Immobilised	
Nominal	*Actual*	*magna*	*24 h*	*48 h*
Control	< LOQ*	20	0	0
1000	115-117 (filtered) 234-274 (unfiltered)	40	0	0

*LOQ-limit of quantification (0.3 mg/L)

EL50	1000 mg/L at 48 hours
Remarks – Results	No significant differences were shown between the measured values of test substance at 0 and 48 h, indicating that the test material was stable over the study duration. All the validity criteria were met. Dissolved oxygen was maintained at ≥ 8.1 mg/L, pH was maintained between 7.5 and 8.0 and temperature was maintained at 21 °C.
CONCLUSION	The test substance is not harmful to aquatic invertebrates.

TEST FACILITY SPL (2000b)

C.2.3. Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 211 *Daphnia magna*, Reproduction test
Species *Daphnia magna*
Exposure Period 21 d
Auxiliary Solvent None
Water Hardness 140 mg $CaCO_3$/L
Analytical Monitoring HPLC-MS
Remarks – Method After a range finding test, a definitive test was conducted at five nominal concentrations: 0.007, 0.022, 0.07, 0.22 and 0.7 mg/L. A saturated solution was prepared by stirring 50 mg/L of test chemical in dechlorinated tap water at 1500 rpm for 24 h and by removing any undissolved test material by filtration. The test solutions were renewed three times per week. Measured concentrations were variable and exceeded the nominal concentrations. The time-weighted mean measured concentrations were determined to be 0.025, 0.071, 0.24, 0.9 and 2.5 mg/L assessed chemical.

RESULTS

Test Day 21

Time-weighted mean measured concentrations (mg/L)	*Mean Percent Survival*	*Mean Number of Offspring Released Per Female (SD)*	*Mean Total Body Length (mm) (SD)*	*Mean Dry Weight (mg) (SD)*
Control	90	72 (15)	4.0 (0.2)	ND
0.025	100	67 (13)	4.0 (0.1)	ND
0.071	90	65 (21)	3.8 (0.1)	ND
0.24	100	68 (12)	3.9(0.1)	ND
0.90	70	52 (17)	3.8 (0.2)	ND
2.5	30	9* (11)	3.3* (0.3)	ND

*significant differences from control

EC50 (21 d Immobilisation) 1.6 mg/L (95% CI:0.21-20) mg/L
EC50 (21 d Reproduction) 1.2 mg/L (95% CI:0.51-9.3) mg/L
NOEC (21 d) 0.9 mg/L

Remarks – Results Analysis Analysis of the freshly prepared media showed measured concentrations of 0.0146 to 4.22 mg/L and analysis of the old media showed concentrations of 0.0153 to 4.40 mg/L. All validity criteria were met. Mortality of the parent population in the control group was < 20% and the amount of living offspring in the control group was > 60 after 21 days and coefficient of variation for control group was < 25%.

CONCLUSION The test substance is toxic to aquatic invertebrate reproduction.

TEST FACILITY Harlan (2009b)

C.2.4. Algal Growth Inhibition Test

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
Species *Scenedesmus subspicatus*
Exposure Period 72 hours
Concentration Range Nominal: 62.4, 125, 500 and 1000 mg/L
Auxiliary Solvent None

Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks – Method	The test was conducted according to GLP principles. No significant deviations from the test guidelines were reported. After a range finding study, a definitive test was conducted. Water Accommodated Fractions (WAF) of the test substance were prepared at various loading rates in test medium (see above). The WAFs were removed by syphon and used in exposure tests. Although undissolved test material was observed throughout the water column at the end of the mixing and settling period, filtration was considered unnecessary as the range finding studies indicated no physical effect of undissolved test material on the test organisms.

RESULTS

Biomass		*Growth*	
E_bL50 (mg/L at 72 h)	*NOE_bL (mg/L)*	*E_rL50 (mg/L at 72 h)*	*NOE_rL (mg/L)*
250	125	370	125

Remarks – Results	The pH values of the control cultures increased from 7.6 at 0 h to 9.9 at 72 h but this increase was not considered to have an adverse effect on the results. The cell density in the control increased by a factor of 172 within 72 h. Analysis of the 72 h test samples showed a decline in measured concentrations ranging from 6.15 to 222 mg/L for the unfiltered samples and from 0.234 to 0.682 mg/L in the filtered samples due to possible adsorption to algal cells.

CONCLUSION The test substance is not harmful to algal growth.

TEST FACILITY SPL (2001c)

C.2.5. Toxicity to soil microorganisms – Carbon Transformation Test

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 217 Soil Microorganisms: Carbon Transformation Test

Test system	Natural soil: A field loamy sand soil
Exposure Period	28 d
Concentration Range	Nominal: 1,000 mg/kg
Analytical Monitoring	Nitrate
Remarks – Method	After a range finding test, soil microorganisms were exposed to the test material at 1000 mg/L. Inhibitory effect on soil microorganisms was assessed by the determination of glucose induced respiration rates in soils.

RESULTS

Remarks - Results	No significant long-term effect on soil carbon transformation was recorded at the maximum test concentration.

CONCLUSION The test substance does not inhibit carbon transformation activity of soil microorganisms.

TEST FACILITY SPL (2007a)

C.2.6. Toxicity to soil microorganisms – Nitrogen Transformation Test

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 216 Soil Microorganisms: Nitrogen Transformation Test

Test system	Natural soil: A field Loamy sand soil
Exposure Period	28 d

Concentration Range	Nominal: 1,000 mg/kg
Analytical Monitoring	Nitrate
Remarks – Method	After a range finding test, soil microorganisms were exposed to the test material at 1000 mg/kg. Inhibitory effect on soil microorganisms was assessed by the determination of nitrate concentration in soils.

RESULTS

Remarks - Results	No significant long-term effect on soil nitrogen transformation was recorded at the maximum test concentration.

CONCLUSION	The test substance does not inhibit nitrogen transformation activity of soil microorganisms.
TEST FACILITY	SPL (2007b)

C.2.7. Earthworms Toxicity Study

TEST SUBSTANCE	Assessed chemical

METHOD	OECD TG 207 Earthworm, Acute Toxicity Test
Species	*Eisenia foetida*
Exposure	14 days
Concentration range	Nominal: 1000 mg/kg soil
Auxiliary solvent	None
Remarks – Method	The test was conducted according to GLP principles. No significant deviations from the test guidelines were reported. Following a range finding test, a definitive test was conducted in a defined artificial soil as a test substrate with 70, 20 and 10% of quartz sand, clay and peat, respectively. Chloroacetamide was used as a reference substance.

RESULTS

Nominal Concentration (mg/kg dry weight)	*Total number of test earthworms*	*Mean Bodyweight [g (SD)]*		*Mortality (%)*
		0 d	*14 d*	
Control	40	0.38 (0.07)	0.34 (0.06)	0
1000	60	0.42 (0.08)	0.36 (0.07)	0

14 d LC50	> 1000 mg/kg dry weight soil
NOEC	1000 mg/kg dry weight soil
Remarks – Results	All the validity criteria were met. No significant effect was observed in the treated soils. The 14 d LC50 = 42 mg/kg dw soil for the reference substance was within the normal range.

CONCLUSION	The test substance is very slightly toxic to earthworms.
TEST FACILITY	SPL (2006b)

C.2.8. Terrestrial Plants Toxicity Study

Test substance	Assessed chemical

METHOD	OECD TG 208 Seedling emergence and seedling growth test
Species	Three plant species: One monocotyledon (onion), and two dicotyledons (Cucumber and soybean).
Exposure	21 days
Concentration range	Nominal: 1,000 mg/kg dry matter
Auxiliary solvent	None

Remarks – Method	The test was conducted according to GLP principles. No significant deviations from the test guidelines were reported. The test substance was incorporated into the soil in which seeds were sown.

Results

Onion

Concentration	*Mortality**	*%Emergence (SD)*	*Mean shoot weight [g (SD)]*
Control	5	88 (15)	0.016 (0.007)
1000 (mg/kg)	6	85 (14)	0.016 (0.008)

Soyabean

Concentration	*Mortality**	*%Emergence (SD)*	*Mean shoot weight [g (SD]*
Control	3	93 (10)	0.221 (0.075)
1000 (mg/kg)	3	93 (10)	0.257 (0.060)

Cucumber

Concentration	*Mortality**	*%Emergence (SD)*	*Mean shoot weight [g (SD)]*
Control	1	98 (7)	0.169 (0.068)
1000 (mg/kg)	3	93 (10)	0.218 (0.059)

*No Emergence

21 d EC50 (shoot weight)	> 1000 mg/kg dry weight
Remarks – Results	The results of the control for all plant species met the required validity criteria.
CONCLUSION	The test substance is not harmful to terrestrial plants (seedling emergence and growth).
TEST FACILITY	SPL (2007c)

BIBLIOGRAPHY

Harlan (2009a) [Assessed chemical]: L5178Y TK +/- Mouse Lymphoma Assay (Project No. 1843/0005, August, 2009). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2009b) [Assessed chemical]: *Daphnia magna* Reproduction Test (Project No. 2219/0010, August, 2009). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2010a) [Assessed chemical]: Local Lymph Node Assay in the Mouse (Project No. 1843/0010, February, 2010). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2010b) [Assessed chemical]: Oral Gavage One Generation Reproduction Study in the Rat with Evaluation of Sub Chronic Toxicity (OECD 408 and OECD 415) (Project No. 1843-0004, March, 2010). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012a) [Assessed chemical]: Determination of Vapour Pressure (Study No.: 41201034, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012b) [Assessed chemical]: Determination of Adsorption Coefficient on Soil and Sludge (Study No.: 41201046, November, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012c) [Assessed chemical]: Determination of Relative Self-Ignition Temperature for Solids (Study No.: 41201036, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012d) [Assessed chemical]: Determination of Explosive Properties (Study No.: 41201035, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012e) [Assessed chemical]: Determination of Oxidising Properties (Study No.: 41201037, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012f) [Assessed chemical]: Acute Dermal Toxicity (Limit Test) in the Rat (Project No, 41201039, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012g) [Assessed chemical]: Acute Dermal Irritation in the Rabbit (Project No. 41201040, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2012h) [Assessed chemical]: Acute Eye irritation in the Rabbit (Project No. 41201041, June, 2012). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

Harlan (2014) [Assessed chemical]: Chromosome Aberration Test in Human Lymphocytes in vitro (Project No. 41201042, October, 2014). Shardlow, Derbyshire, UK, Harlan Laboratories Ltd. (Unpublished report submitted by the applicant).

SPL (2000) [Assessed chemical]: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* and *Escherichia coli* (Project No. 1343/006, July, 2000). Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2001a) [Assessed chemical]: Determination of General Physico-chemical Properties (Project No: 1343/004, Jan, 2001), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2001b) [Assessed chemical]: Acute Toxicity to *Daphnia magna* (Project No. 1343/010, Jan, 2001), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2001c) [Assessed chemical]: Algal Inhibition Test (Project No. 1343/011, Jan, 2001), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2003) [Assessed chemical]: Local Lymph Node Assay in the Mouse (Project No. 1776/004, August, 2003). Shardlow, Derbyshire, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2006a) [Assessed chemical]: Assessment of Ready biodegradability: Carbon dioxide evaluation test (Project No: 2219/0001, June, 2006), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2006b) [Assessed chemical]: Acute Toxicity to Earthworms (Project No: 2219/0002, June, 2006), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2007a) [Assessed chemical]: Carbon Transformation Test (Project No: 2219/0005, Jan, 2007), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2007b) [Assessed chemical]: Nitrogen Transformation Test (Project No. 1219/0004, Jan, 2007), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SPL (2007c) [Assessed chemical]: Terrestrial Plant Test. Seedling Emergence and Growth Test (Project No. 1219/0003, Jan, 2007), Derby, UK, Safepharm Laboratories Limited (Unpublished report submitted by the applicant).

SWA (2015) Code of Practice: Spray Painting and Powder Coating, Safe Work Australia, https://www.safeworkaustralia.gov.au/doc/model-code-practice-spray-painting-and-powder-coating.

SYRICI (2014) [Assessed chemical]: Fish Acute Toxicity Test (Study No.: G1223J0130, May, 2014). Shenyang, China, Safety Evaluation Center of Shenyang Research Institute of Chemical Industry Ltd. (Unpublished report submitted by the applicant).

United Nations (2017) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 7th revised edition. United Nations Economic Commission for Europe (UN/ECE), <https://unece.org/ghs-rev7-2017 >