File No.: STD/1698

August 2020

AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

2-Pyrrolidinone, 1-butyl-

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.

This Public Report is available for viewing and downloading from the AICIS website. For enquiries please contact AICIS at:

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Executive Director
AICIS

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SUMMARY

The following details will be published on our website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1698	Eastman Chemical Australia Pty Ltd	2-Pyrrolidinone, 1-butyl-	Yes	≤ 500 tonnes per annum	Solvent in coatings, inks, fertiliser, industrial cleaning products and chemical synthesis

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Acute toxicity, oral (Category 4)	H302 – Harmful if swallowed
Skin irritant (Category 2)	H315 – Causes skin irritation
Eye irritant (Category 2A)	H319 – Causes serious eye irritation
Specific target organ toxicity (single exposure; narcotic effects) (Category 3)	H336 – May cause drowsiness or dizziness

Human Health Risk Assessment
Provided that the recommended controls are being adhered to, 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 following safe use instructions, the assessed chemical is not considered to pose an unreasonable risk to public health.

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemical should be classified as follows:
  - Acute toxicity, oral Category 4: H302 – Harmful if swallowed
  - Skin irritation Category 2: H315 – Causes skin irritation
  - Eye irritation Category 2A: H319 – Causes serious eye irritation
  - Specific target organ toxicity Category 3: H336 – May cause drowsiness or dizziness

  The above should be used for products/mixtures containing the assessed chemical, if applicable, based on the concentration of the assessed chemical present.

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 as introduced, during formulation of products, during chemical synthesis and in commercial/industrial printing products:
  - Automated processes where possible
  - Adequate local exhaust ventilation

- 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 assessed chemical as introduced, during formulation of products and end-use:
  - Avoid contact with skin and eyes
  - Avoid inhalation
  - Use in well-ventilated areas and clean up any spills promptly

- 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 as introduced, during formulation of products and end-use:
  - Gloves
  - Goggles
  - Protective clothing
  - Respiratory protection if inhalation exposure 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.

Public Health and Safety

- The paint and coating products containing the assessed chemical available for consumers should provide safe use instructions such as to use in well ventilated areas.

Storage

- The handling and storage of the assessed chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

Emergency procedures

- Spills or accidental release of the assessed chemical should be handled by physical containment, collection and subsequent safe disposal. Prevent spillage from entering drains or water courses.

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 notified in writing within 20 working days by the applicant or other introducers if:

- additional information on the repeated dose toxicity or reproductive/developmental toxicity of the chemical has become available;
- the function or use of the chemical has changed from a solvent in paints/coatings, inks, industrial cleaning products and chemical synthesis;
- the concentration of the assessed chemical exceeds 10% in paint products available for DIY use;
- the assessed chemical is likely to be used in consumer products other than in paints and inkjet inks;
- the fertiliser use of the assessed chemical has changed from a soil fertiliser for agricultural settings or farms;
- 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)
Eastman Chemical Australia Pty Ltd (ABN: 60 077 977 649)
832 High Street
KEW EAST VIC 3102

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

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
Data items and details exempt from publication include: other name, analytical data, degree of purity, import volume, and site of manufacture/reformulation.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for dissociation constant, particle size, explosive properties, oxidising properties, reactivity, and bioaccumulation.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
EU (REACH)
Canada (2019)
USA

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Tamisolve NxG

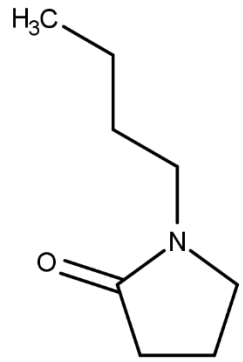
CAS NUMBER
3470-98-2

CHEMICAL NAME
2-Pyrrolidinone, 1-butyl-

OTHER NAME(S)
1-Butyl-2-pyrrolidone
1-Butyl-2-pyrrolidinone
N-butyl-2-pyrrolidone
N-butyl-2-pyrrolidinone
N-butylbutyrolactam
N-butylpyrrolidone
N-butylpyrrolidinone

MOLECULAR FORMULA
$C_8H_{15}NO$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
141.21 g/mol

ANALYTICAL DATA
Reference NMR and IR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 98%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None identified

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None identified

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless liquid

Property	*Value*	*Data Source/Justification*
Freezing Point	< -78.5 °C	Measured
Boiling Point	240.6 °C at 101.3 kPa	Measured
Density	959 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.013 kPa at 25 °C	Measured
Water Solubility	Fully soluble	Measured
Hydrolysis as a Function of pH	<10% after 5 days at pH 4, 7 and 9	Measured
Partition Coefficient (n-octanol/water)	log Pow = 1.265 at 25 °C (HPLC method)	Measured
	log Pow = 0.73 at 22°C (Shake flask method)	Measured
Adsorption/Desorption	log K_{oc} = 1.142 at 25 °C	Measured
Dissociation Constant	Not determined	Assessed chemical contains no dissociable functional groups
Surface tension	67.31 mN/m for 1% aqueous solution	Measured*
Flash Point	108 °C at 101 kPa	Measured, closed cup
Flammability Limits	Upper: 8.7% Lower: 0.9%	Measured*

Property	*Value*	*Data Source/Justification*
Autoignition Temperature	210 – 217 °C	Measured
	234 °C	Measured* (ASTM E659 method)
Explosive Properties	Not determined	Not expected to be explosive
Oxidising Properties	Not determined	Not expected to be oxidising

* Only results were provided

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 depicted in 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.

The assessed chemical has a flash point of 108 ºC which is greater than 93 °C. Based on *Australian Standard AS1940* definitions for combustible liquid, the assessed chemical may be considered as a Class C2 combustible liquid if the chemical has a fire point below the boiling point.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemical will not be manufactured in Australia. It will be imported into Australia in neat form and as a variety of products containing the assessed chemical as a solvent.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	10 – 500	10 – 500	10 – 500	10 – 500	10 – 500

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The assessed chemical will be imported in neat form in 205 L drums, or as a component of formulated finished products containing the assessed chemical at various concentrations. The finished products will be in a variety of different containers based on their specific use pattern.

USE
The assessed chemical will be used as an industrial solvent and replacement for *N*-methyl pyrrolidone (NMP). The main uses for the assessed chemical include coatings, fertiliser formulations, chemical synthesis, printing inks, and industrial cleaning agents. The assessed chemical will not be used in cosmetics or cleaning products available for consumer use.

The total introduced volume of the assessed chemical is expected to be divided into the following products: paints and coatings (15 – 40% of introduction volume), agricultural fertilisers (40 – 60%), solvent for chemical synthesis (20%), printing inks (5%), and industrial cleaning agents (5%).

OPERATION DESCRIPTION

Reformulation
At the reformulation site, the drums containing the assessed chemical will be pumped into a sealed blending vessel with other ingredients. The blending vessel will be fitted with a high-speed mixer and a local exhaust ventilation system. Each batch will be quality checked and adjustments made if necessary. The blended product is filtered and then dispensed into a variety of packaging sizes, based on the type of product that will be used. The end-use products will be warehoused and distributed to various end-users.

End Use
The assessed chemical will be available in a variety of products at different concentrations.

Paint and coating products containing the assessed chemical at 30 – 80% concentration will be mostly applied by spray in a spray booth in an industrial setting, with some possible application by brush or roller. Some paint and coating products containing the assessed chemical at ≤ 8% will be available for consumer use. The method of application would be by brush, roller or spray.

Fertiliser products containing the assessed chemical at ≤ 45% concentration will be used by farmers. The farmers will add concentrated products into a dilution tank (approximately 1 in 500 or 1 in 1000 dilution) to a final concentration of < 0.2%, and apply the mixture by spray boom on a tractor onto the soil. No fertiliser products will be available for use by the general public.

For chemical synthesis, enclosed systems including hoses and pumping equipment will be used to transfer the assessed chemical in neat form to a holding tank. The assessed chemical will then be dosed into the reactor vessel.

Ink products containing the assessed chemical at ≤ 10% concentration will be imported as finished products. Sealed cartridges containing these inks will be used for office and home inkjet printing, and larger containers will be used to supply the inks to large scale commercial or industrial printing.

Cleaning agents containing the assessed chemical at ≤ 30% concentration will be applied by professional cleaners, who will use these products to clean public areas such as bathrooms.

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 warehousing	2	12 – 24
Coating application	8	210
Fertiliser application	2	3 – 6
Plant operators (chemical synthesis)	8	24 – 48
Printer operators	0.5 – 8	5 – 210

EXPOSURE DETAILS

Transport and Storage
Transport and storage workers may come into contact with the assessed chemical in neat form or at lower concentrations in various end-use products only in the unlikely event of accidental breaching of containers. As the likelihood of such an event is expected to be low, the probability of repeated exposure to these workers is expected to be low.

Reformulation
Dermal, ocular and inhalation exposure to the assessed chemical in neat form may occur at reformulation sites during weighing and transferring the assessed chemical to the blending vessel or during equipment cleaning and maintenance. Exposure to the assessed chemical during reformulation is expected to be minimised through the use of enclosed and automated systems, local exhaust ventilation and workers wearing personal protective equipment (PPE), including gloves, safety goggles, coveralls and respiratory protection if exposure to mist or aerosol is likely to occur.

End use – paints and coatings
At industrial end use sites, dermal, ocular and inhalation exposure to coatings containing the assessed chemical at 30 – 80% concentration may occur during the transfer, application and cleaning processes. As stated by the applicant, during use in industrial sites, the potential for exposure is expected to be minimised through the use of engineering controls such as spray booths and PPE, including overalls, goggles and respiratory protection, such as

masks with ABEK-P3-filters, where ventilation is inadequate. Once the coating has been cured, any remaining assessed chemical will be bound within a coating matrix and is not expected to be available for exposure.

End use – agricultural
Dermal and ocular exposure to the assessed chemical concentrates (solutions containing ≤ 45% assessed chemical) is possible when the farmer is preparing the solutions for application. As stated by the applicant, exposure is expected to be minimised by the use of safety glasses, protective clothing and gloves. Dermal and ocular exposure to the assessed chemical at dilute concentrations of < 0.2% is also possible during the application of fertilisers containing the assessed chemical. However, the exposure is expected to be limited due to the method of application (boom spray) to the soil and the dilute concentrations of the assessed chemical used. Exposure could be further minimised with the use of PPE. Inhalation exposure to the assessed chemical in fertiliser solutions is also expected to be limited due to the method of spraying (boom spray), the diluted concentrations of the assessed chemical used, and non-enclosed (open spaces) spraying areas.

End use – chemical synthesis
Chemical synthesis using the assessed chemical is expected to be carried out within an enclosed, automated and remote operated system. Dermal, ocular and inhalation exposure to the assessed chemical in neat form will be possible during the connection and disconnection of hoses and pumping equipment to the reaction vessel, and during cleaning/maintenance processes. As stated by the applicant, dermal and ocular exposure is expected to be limited by the use of (PPE including gloves, overalls, safety boots and eye protection. General and local ventilation would be available in the facilities during chemical synthesis. A respirator will also be used during the connection and disconnection of the equipment. Any residual amounts of the assessed chemical will be washed and collected in solvent before any maintenance work is required.

End use – inkjet printing
Printer technicians and office workers may come into contact with the ink containing the assessed chemical at 10% concentration. Dermal exposure to the assessed chemical at ≤ 10% concentration may occur during operations including replacing spent ink cartridges and cleaning or maintaining printers. However, the exposure is expected to be infrequent or incidental, given the containment of the assessed chemical within purposely designed ink cartridges, the provision of safe use instructions and likely siting of printers in separate rooms.

Occasional and limited dermal exposure during printing may also occur if the printed pages are handled when wet, or if the ink-stained parts of the printer are touched. Inhalation exposure to the assessed chemical is not expected, given the low likelihood of aerosols being released from the printers. Once the ink dries, the assessed chemical will be bound to the matrix of the substrates and is not expected to be available for exposure.

Where larger scale commercial or industrial inkjet printing is carried out, use of separate rooms, engineering controls and a high rate of air replacement are expected to reduce worker exposure.

End use – cleaning agents
Exposure to the assessed chemical in end-use cleaning products (at ≤ 30% concentration) may occur in professions where the services provided involve the use of cleaning products. The principal route of exposure is expected to be dermal, while ocular and inhalation exposures are also possible. Such professionals may use PPE such as gloves to minimise repeated or prolonged exposure and ensure that good hygiene practices are in place.

6.1.2. Public Exposure
There could be repeated exposure of the public to the assessed chemical through the use of it in some consumer products such as paints. The principal route of exposure will be dermal, while ocular and inhalation exposures are also possible, particularly if paints are applied by spray.

Inkjet printer inks containing the assessed chemical at ≤ 10% may also be available to the general public for home use. However, inks containing the assessed chemical will be kept in sealed cartridges of low volume capacity until use, and will be released only slowly after intermittent use. Therefore, public exposure to the assessed chemical is expected to be low.

Public exposure to the paints and coating products containing the assessed chemical at up to 8% concentration is possible during the mixing and application of these products by do-it-yourself (DIY) users. Although the exposure will be mainly dermal, ocular and inhalation exposures are also possible with spray applications. Inhalation exposure to vapours is also possible if used in confined spaces. Considering the infrequent use of the paints/coatings (may be for a short duration), exposure is expected to be limited.

Members of the public may come into contact with surfaces coated or cleaned with products containing the assessed chemical or with printed materials containing the assessed chemical. However, any residual assessed chemical is expected to be bound into the coating matrix or within the substrate matrix or removed during post-application and not be available for exposure.

Fertilisers containing the assessed chemical will not be sold to the general public. Therefore, the general public will not be exposed to the assessed chemical through fertiliser use in home gardens. Furthermore, as the assessed chemical will be used in agricultural settings or farms, the general public is unlikely to come into contact with the assessed chemical through fertiliser applications. As the product will be applied by boom spray, bystander exposure is not expected. Therefore, based on the above information, exposure to the general public is expected to be low.

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 oral toxicity – rat	LD50 = 300 – 2,000 mg/kg bw; harmful
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute inhalation toxicity – rat	LC50 > 5.1 mg/L/4 hour; transient neurological effects observed immediately after exposure
Skin corrosion – *in vitro* Human Skin Model Test	Corrosive
Skin corrosion – *in vitro* Membrane Barrier Test	Non-corrosive
Skin irritation – rabbit	Irritating
Eye irritation – rabbit	Irritating
Skin sensitisation – mouse local lymph node assay	No evidence of sensitisation up to 50% concentration
Repeat dose oral toxicity – rat, 90 days	NOAEL = 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	Non mutagenic
Genotoxicity – *in vitro* mammalian cell micronucleus test	Non genotoxic
Developmental toxicity – rat (oral; gestation day (GD) 6 to 19)	NOAEL (maternal) = 50 mg/kg bw/day NOAEL (developmental) > 500 mg/kg bw/day
Developmental toxicity – rat (oral, GD 6 to 19)	NOAEL (maternal and developmental) = 400 mg/kg bw/day
Developmental toxicity – rabbit (oral, GD 7 to 28)	NOAEL (maternal) = 150 mg/kg bw/day NOAEL (developmental) = 300 mg/kg bw/day
Developmental toxicity – rat (dermal, GD 6 to 19)	NOAEL = 500 mg/kg bw/day
Developmental toxicity – rat (inhalation, GD 6 to 19)	NOAEC = 0.58 mg/L/day*

*Calculated as equivalent to NOAEL = 152.6 mg/kg bw/day

Toxicokinetics, Metabolism and Distribution

Based on the low molecular weight (< 500 g/mol), high water solubility and partition coefficient (log Pow = 0.73 – 1.265) of the assessed chemical, there is potential for the assessed chemical to cross biological membranes.

A toxicokinetic study conducted on a radio-labelled isotope of the assessed chemical indicated that the assessed chemical was fully absorbed to systemic circulation following oral dosing, and was widely distributed to all tissues. Tissue concentrations were correlated to the degree of perfusion by circulating blood. Almost all metabolic equivalents (99.5%) of the test substance were eventually excreted from the body through urine, faeces, and expired air within the duration of the study period of 7 days. Only a minor percentage (0.3%) was identified as the original test substance, suggesting that the assessed chemical was extensively metabolised. The major metabolites that were identified include *N*-hydroxybutyl succinimide, *N*-butyl-5-hydroxypyrrolidone, and a glucuronide conjugate of OH-*N*-butylpyrrolidone.

Acute Toxicity

The assessed chemical was found to be harmful to rats via the oral route, with LD50 determined to be between 300 and 2,000 mg/kg bw in rats. The assessed chemical was found to be of low acute toxicity to rats via the dermal route (LD50 > 2000 mg/kg bw).

Although the assessed chemical does not warrant hazard classification for acute inhalation toxicity (LC50 > 5.1 mg/L, no mortalities observed in exposed rats), there were transient neurological effects observed in rats immediately following exposure to the aerosol of the assessed chemical. These effects included clonic convulsions in 2/5 females and ataxia (impaired balance or coordination) in 5/5 males and 2/5 females. These effects were reduced at the next observation (1 – 2 hours) and no longer present afterwards. All animals were reported to be normal by day 4. Based on the effects observed in this study, the assessed chemical warrants classification for specific target organ toxicity after single exposure (may cause drowsiness or dizziness).

Irritation and Sensitisation
The assessed chemical was not corrosive in a membrane barrier test for skin corrosion using the Corrositex® model, but was found to be corrosive in an *in vitro* human skin model test using the EpiDerm model.

Based on *in vivo* irritation studies conducted in rabbits (according to the OECD TG 404 and 405), the assessed chemical was irritating to the skin and eyes of rabbits. Very slight to well-defined erythema was observed on all tested animals. The dermal irritation recovered in one animal on Day 7, but recurred in the same animal on Day 10. Dermal irritation did not completely recover in all animals at the end of the observation period. In the eye irritation study, conjunctivitis was observed in in all animals after application of the assessed chemical. Iritis was observed in all animals, and corneal opacity was observed in one animal. An additional ocular finding of neovascularisation was noted in two animals during the study. All observed effects eventually recovered by Day 10 in all animals.

The assessed chemical up to 50% concentration showed no evidence of skin sensitisation in a mouse local lymph node assay.

Repeated Dose Toxicity
A 90 day repeated dose oral toxicity study was conducted on the assessed chemical with dose levels of 10, 50 and 500 mg/kg bw/day. No changes were observed in the low dose group, but treatment-related effects observed in mid and high dose groups included an increase in the liver weights, liver hypertrophy, changes in blood chemistry, thymic atrophy and adrenal cortical vacuolation. These changes were considered non-adverse and adaptive effects to the treatment by the study authors. Renal changes, such as increased kidney weights, hyaline droplet accumulation, multifocal basophilic tubules, and proteinaceous casts were also observed in male rats, but these are specific to male rats and not relevant for humans. Thymic atrophy present in males in the mid and high dose groups and females in the high dose group was reported to be a secondary reaction to stress. Vacuolation in the adrenal cortex was observed in all males in the mid and high dose group at an increased incidence and severity and in one male in the low dose group, and considered to be related to lipid metabolism and liver changes.

There were no treatment-related effects on body weight gain and food consumption. Some dose groups had a statistically significant reduction in mean body weight gain in particular weeks, but it was recovered in subsequent weeks.

No adverse effects were detected during the oestrous cycle assessments or in sperm concentration or motility. A statistically significant increase in the number of sperm with abnormal morphology in high dose males was reported as likely due to artifactual abnormalities associated with the slide smearing procedure, based on the types of abnormalities.

Under the conditions of this study, the NOAEL for systemic toxicity is considered to be 500 mg/kg bw/day for both sexes.

Mutagenicity/Genotoxicity
The assessed chemical was not mutagenic in a bacterial reverse mutation test and an *in vitro* mammalian cell gene mutation test using the Thymidine Kinase Gene. The assessed chemical was not genotoxic in an *in vitro* mammalian cell micronucleus test

Toxicity for Reproduction and development
Five studies were provided for developmental toxicity of the assessed chemical, all performed according to OECD TG 414. Four of the studies were carried out in rats, two by the oral route, and one each by the dermal and inhalation routes. The study in rabbits used oral exposure. The maximum concentration tested varied from 300 to 500 mg/kg bw/day in oral studies and up to 750 mg/kg bw/day in the dermal study. The doses for each study were chosen on

the basis of tolerability, range-finding or preliminary studies, but details (e.g. effects observed at higher doses) of these studies were not provided.

The following NOAELs were obtained for developmental toxicity in these studies:

Species and route of exposure	Study Facility and Year	Developmental NOAEL (mg/kg bw/day)	Effects observed
Rat, oral	LPT (2013)	500	Reduced body weight and body weight gain, reduced food consumption, reduced mean foetal weight and placental weight at 500 mg/kg bw/day
Rat, oral	Charles River (2016)	400	Incidences of rales, reduced body weight gain, reduced food consumption, and reduced mean foetal body weights at 500 mg/kg bw/day
Rabbit, oral	Charles River (2020)	300	Reduced body weight gain, reduced food consumption and faecal output at 300 mg/kg bw/day
Rat, dermal	Charles River (2016)	500	Reduced body weights, body weight gain, gravid uterine weight, and foetal body weights at 750 mg/kg bw/day
Rat, inhalation	Charles River (2016)	152.6	Reduced body weights, body weight gain, gravid uterine weight, and foetal body weights; incidences of skeletal developmental variations at 315.8 mg/kg bw/day

Under the OECD TG 414 protocol, pregnant animals are exposed to the assessed chemical during gestation days 6-19 for rats and gestation days 7-28 for rabbits, covering the period from implantation until just before parturition. In all studies the main effects seen in the dams were dose-related reduced food consumption and reduced body weight gain. However, similar reductions in body weight gain were not seen in the 90-day rat study on the assessed chemical, suggesting that pregnant rats may be more sensitive to the chemical. The main effect in pups was dose-related reduced foetal weight. The extent of the changes in dams and pups, whether or not the effects were statistically significant compared to the controls, and the criteria for setting NOAELs varied between the studies. Gravid uterine weight was also reduced in a dose-dependent manner in all rat studies, and in one study where placental weights were measured, these also were reduced. In general, the effects on dams and foetuses occurred at similar dose levels.

In the inhalation study in rats, test substance-related skeletal developmental variations were noted in the high dose group (1.2 mg/L, stated to be equivalent to 315.8 mg/kg bw/day). A significantly ($p < 0.01$) lower mean litter proportion of cervical centrum (no. 1) was ossified, compared to the control group (3.4% per litter versus 27.3% per control litter). A higher (not statistically significant) mean litter proportion of sternebrae (no. 5 and 6) were unossified in the 1.2 mg/L group (23.1% per litter) compared to the control group (9.4% per litter). These effects were considered to indicate developmental delay. The dermal rat study showed a slight but not statistically significant increase in the percentage of post-implantation loss (control group 7.0% versus high dose group 9.4%). Similar effects were not observed in the other studies. In one of the oral studies in rats, the death of a dam from the high dose group (500 mg/kg bw/day) on Day 10 was not considered by the study authors to be treatment related, based on clinical signs and macroscopic examination at necropsy.

Chernoff et al (2008) analysed a dataset of developmental studies carried out by the US National Toxicology Program (NTP). They concluded that the degree of foetal weight reduction was correlated with the extent of maternal weight loss in the dataset, raising the question of whether, in a particular study, the reduced foetal weight at term is due to maternal undernutrition caused by general toxicity or whether it is caused by direct developmental insult.

Section 3.7.2.4.2 of the GHS guidance for classification for Reproductive Toxicity (UN, 2009) states that maternal toxicity, depending on severity, may influence foetal development through secondary non-specific mechanisms. This guidance also lists several factors to take into account when assessing maternal toxicity. In the studies supplied for the assessed chemical, there was no test item related effect on maternal mortality, reproductive parameters such as mating or fertility indices and gestation length, post-mortem data or serious clinical signs.

Severe exhaustion or prostration of dams was not seen. However, there were substantial reductions in food consumption (some for only part of the study duration) and in maternal body weight gain.

The adjusted mean maternal body weight was calculated for all four developmental toxicity studies in rats, in order to explore the nature of the reduced body weight gain. This parameter is not considered useful for rabbit studies because of weight fluctuations in pregnancy. The adjusted mean maternal body weight, calculated by subtracting the gravid uterine weight from the net body weight gain during the duration of pregnancy, was reduced in a dose related manner in each study, with reductions in the body weight gain at the high dose of up to 40%, compared to the control mean. Gravid uterus weight was also reduced by up to 13%, compared to the control mean. These calculations confirm that base body weight was an important component of the weight changes, but does not confirm whether the effects are specific to the foetus or secondary to maternal toxicity.

Weight loss or food reduction later in the gestation period is thought to be more likely to decrease foetal growth (Chernoff et al, 2008, Faber, 2020). There is some correlation with this factor in the studies on the assessed chemical, where the highest reduction in foetal weight (13%) occurred in the rat inhalation study, where reduced food consumption occurred throughout the study. However, reductions in mean foetal weights of 5-10% were seen in other studies, where reduced food consumption occurred primarily at the beginning of dosing.

Although uncertainty remains about the relationship between reductions in foetal weight and reduced maternal body weight gain in the studies available for the assessed chemical, the GHS guidance (3.7.2.4.2) suggests that "developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case by case basis that the developmental effects are secondary to maternal toxicity". Mechanisms demonstrating that the effects are secondary have not been demonstrated for the assessed chemical, as the maximum doses tested were below the recommended maximum dose for the TG, due to the effects identified in the preliminary studies (details not available on some studies).

A second factor relevant to classification is whether the severity of developmental effects, in this case foetal weight reduction, warrants classification. The reversibility of effects is discussed in the GHS guidance (3.7.2.4.2) which states that "classification should be considered where there is significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies." It is not known whether the extent of reduced foetal weight seen in the available studies on the assessed chemical (5-13%) would have permanent effects on growth of the pups. One reference (Faber 2020) suggests that small decreases in foetal weight are considered reversible and typically disappear in study protocols where the dams are allowed to litter and nurse the offspring. However, on the basis of the results in animals it might be expected that reduced birth weight in humans would also occur, with potentially adverse effects on later development. The GHS guidance (3.7.2.3.3) also states that classification may not necessarily be needed if the effects seen are of low or minimal toxicological significance, such as small changes in foetal weights. However this is not quantified and there may be different approaches to the assessment of "small". Faber (2020) suggests that changes of 5-15% in foetal body weight should be considered "small", although often these levels will be statistically significant. The authors of some of the studies on the assessed chemical ignored reduced foetal weights unless statistically significant (e.g. one rat oral study by Charles River), despite signs of a dose relationship.

A third relevant factor for classification, apart from judging the severity of the foetal effects seen and their possible relationship to maternal toxicity, is the close structural relationship of the assessed chemical to an analogue, NMP, which is classified under the GHS for developmental toxicity (Cat 1B). A Tier III IMAP assessment is available on a NMP (NICNAS, 2018). NMP has a similar toxicology profile to the assessed chemical, as it is a skin (GHS Cat 2) and eye irritant (GHS Cat 2A) (HCIS, SWA), and has effects on body weight, liver, kidney, spleen, thymus and testes of rats/mice, with a classification for specific target organ toxicity with single exposure (GHS Cat 3).

For the analogue NMP, reduced foetal weight was observed at a lower dose than the maternal toxicity in the key oral developmental toxicity study used to derive the NOAEL for risk assessment (see Key studies for hazard assessment), which is not the case in the studies available for the assessed chemical, where foetal and maternal effects are seen at similar doses. In a two-generation rat inhalation study (Solomon et al, 1995) and another oral developmental toxicity study with doses up to mg/kg bw/day (Exxon, 1992) on NMP, decreased pup body weights were detected without effects on the maternal body weight. These studies indicate that the effects on the pups are direct rather than a secondary unspecific effect of maternal toxicity.

Based on the available information and the nature and severity of the effects seen in the studies provided, the assessed chemical does not warrant classification for the developmental toxicity. However, it is noted that the

assessed chemical has a close structural relationship to an analogue, *N*-methyl-2-pyrrolidone (NMP), which is classified under the GHS for developmental toxicity (Cat 1B) (NINCAS, 2018). Therefore, a strong suspicion exists that further effects warranting classification could still be possible under certain circumstances, such as different protocols or studies conducted at higher dosages.

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

Hazard Classification	*Hazard Statement*
Acute toxicity, oral (Category 4)	H302 – Harmful if swallowed
Skin irritant (Category 2)	H315 – Causes skin irritation
Eye irritant (Category 2A)	H319 – Causes serious eye irritation
Specific Target Organ Toxicity – Single exposure (Category 3)	H336 – May cause drowsiness or dizziness

6.3. Human Health Risk Characterisation
Based on available toxicological data, the assessed chemical could be harmful via the oral exposure and irritating to the skin and eyes. Single exposure to high aerosol concentrations of the assessed chemical could cause immediate transient neurological effects.

The assessed chemical has a close structural relationship to *N*-methyl-2-pyrrolidone (NMP), which is classified under the GHS criteria for developmental toxicity (Cat 1B). While the available information does not warrant a developmental toxicity classification for the assessed chemical, a relationship between the assessed chemical and NMP in this regards cannot be ruled out, due to the short duration of exposure used in the developmental toxicity studies provided for the assessed chemical and maximum dose levels tested in the studies of the assessed chemical compared to the NMP doses tested.

6.3.1. Occupational Health and Safety
Workers at reformulation and chemical synthesis sites may be exposed to the assessed chemical in neat form during weighing and transferring the assessed chemical to the blending vessel or during equipment cleaning and maintenance. However, exposure to the assessed chemical will be minimised with the proposed use of enclosed and automated systems, local exhaust ventilation and workers wearing personal protective equipment (PPE), including gloves, safety goggles, coveralls and respiratory protection if exposure to mist or aerosol is likely to occur.

During end-uses, professional workers may be exposed to the assessed chemical at up to 80% concentration during various uses such as paints and coatings, and 30% in cleaning agents. The principal route of exposure is expected to be dermal; ocular and inhalation exposures are also possible during spray painting and if used in enclosed spaces. However, as stated by the applicant, exposure to paints and coatings is expected to be minimised through the use of engineering controls such as spray booths and PPE, including overalls, goggles and respiratory protection, such as masks with ABEK-P3-filters, where ventilation is inadequate. Once the coating has been cured, the assessed chemical will be bound within a coating matrix and is not expected to be available for exposure. The applicant advised that the main controls used by professional cleaners would be gloves.

Exposure to the farmers to solutions containing up to 45% assessed chemical (while preparing the solutions for application) is expected to be minimised by the use of protective clothing, gloves and goggles. The exposure to the assessed chemical is also expected to be limited during the application of fertilisers containing the assessed chemical due to the method of application (boom spray) to the soil, the diluted concentration of the assessed chemical in fertiliser spray solution (< 0.2% concentration), and could be further minimised with the use of PPE.

Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the assessed chemical is not considered to pose an unreasonable risk to the health of workers, including farmers.

6.3.2. Public Health

Paints containing up to 8% concentration of the assessed chemical will be available for DIY consumer use. Irritation risks are not expected at this concentration and the DIY user exposure is expected to be of infrequent nature and may be for a short duration of time. The exposure to DIY users will be further limited by the use of small volumes of products containing the assessed chemical, and washing off at the end of each day. Transient neurological effects are not expected from exposure to paints containing 8% concentration of the assessed chemical if used in well ventilated areas. Considering the situations where DIY paint use is possible (e.g. indoor wall painting), products available to consumers should include safe use instructions such as to use in well-ventilated areas.

Members of the public may come into contact with surfaces coated or cleaned with products containing the assessed chemical or with printed materials containing the assessed chemical. However, the assessed chemical is expected to be bound into the coating matrix or within the substrate matrix or removed during post-application and not be available for exposure.

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The assessed chemical is not manufactured in Australia. Reformulation and repackaging will occur through various processes depending on the final product. The reformulation processes typically include liquid blending operations where the assessed chemical is transferred into a sealed blending tank from a metering pump. Any release from these processes are expected to be via accidental spills or leakages which are to be collected and recycled or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

Chemical synthesis processes are not expected to result in the release of the assessed chemical as they are expected to occur in closed, automated systems where waste is collected and disposed of via licensed waste contractors.

During the use in agricultural products, the assessed chemical is sprayed onto soils from a tractor, it is expected that the assessed chemical will penetrate past the top 30 cm of soils and may leech into the groundwater.

When used in coatings, the assessed chemical will be primarily applied via spray techniques, approximately 10-20% of the assessed chemical used for coatings is expected to be lost via overspray. Overspray will be collected via scrubbers and filters and contained in steel drums and disposed of via licensed waste contractors. An additional 1% of the import volume may be disposed of to the sewers from equipment cleaning.

When used in printing inks, the assessed chemical is expected to volatilise during the drying process and is not expected to remain on the substrate in any significant amount and is unlikely to enter the sewers as a result of the paper recycling process.

When used in industrial cleaning agents, the assessed chemical is expected to be collected and disposed of using licensed waste contractors. Waste waters containing the assessed chemical are expected to be treated prior to any potential release into waterways. Therefore significant concentrations of the assessed chemical is not expected to be released to surface waters from this use pattern. The assessed chemical is not used in any DIY or commercial cleaning agents.

RELEASE OF CHEMICAL FROM DISPOSAL

During the use in coating materials and inks the assessed chemical is expected to either volatilise or share the fate of the substrate it is applied to. For the other use patterns the assessed chemical is expected to be primarily disposed of via landfill. Some of the assessed chemical will remain in the packaging materials as residues which are to be disposed of via landfill. Approximately 5% of the total import volume of the assessed chemical is expected to be disposed of via landfill.

7.1.2. Environmental Fate

As a result of the end use in the agricultural industry, a significant proportion of the assessed chemical is expected to be dispersed onto the top soil. As the assessed chemical is volatile, a major portion is expected to partition to air; however, if rainfall occurs shortly after application, due to its high water solubility and low adsorption coefficient (Log Koc = 1.142), the assessed chemical may become mobile and run-off and reach adjacent surface waters or leach and reach ground waters.

When used in coatings and inks, the assessed chemical is expected to share the fate of the substrate it is applied to, primarily disposed of to landfill. Use of the assessed chemical as an industrial synthesis solvent is to occur in a controlled environment where it is to be collected and eventually disposed of to landfill via a licensed waste contractor.

Some of the assessed chemical may reach the sewer system through improper disposal and equipment washings where it will likely volatilise. In the environment the assessed chemical is expected to be ultimately biodegradable after a lag phase of approximately 30-35 days based on several biodegradation studies (0% degradation after 28 days using OECD TG 301 B and C, 80% degradation after 112 days using OECD TG 302B and 100% degradation after 56 days using OECD TG 302C). The assessed chemical is not expected to bioaccumulate based on its low log Pow values (0.73 - 1.142). The assessed chemical is expected to eventually degrade via biotic and abiotic processes to form landfill gases (e.g. methane), water and oxides of carbon and nitrogen.

For further details of the environmental fate studies, refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A Predicted Environment Concentration (PEC) was not calculated as the amount of assessed chemical released into the environment cannot be accurately quantified. Limited quantities of the assessed chemical may reach surface water or groundwater during use in fertilisers or released into sewers from equipment washings from spray coating use.

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*
Fish Toxicity	EC50 >100 mg/L	Not harmful to fish
Fish early life-stage toxicity	NOEC = 82 mg/L	Not harmful to fish development
Daphnia Toxicity	EC50 >100 mg/L	Not harmful to invertebrates
Daphnia reproductive toxicity	NOEC = 100 mg/L	Not harmful to invertebrate reproduction
Algal Toxicity	EyC50 = 130 mg/L NOEC = 40 mg/L	Not harmful to algal growth
Inhibition of Bacterial Respiration	EC50 > 315 mg/L	Not harmful to bacterial respiration at tested levels.

Based on the above ecotoxicological endpoints for the assessed chemical, it is not expected to be harmful to aquatic organisms. Therefore, the assessed chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* for acute and chronic toxicities (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated, since the assessed chemical is not harmful to aquatic life based on the above studies.

7.3. Environmental Risk Assessment

A risk quotient was not calculated as the PEC and PNEC were not determined. Although the assessed chemical has the potential to reach surface water or groundwater, it is not considered acutely, or chronically harmful to aquatic species and is not expected to be persistent in the environment. The assessed chemical is not expected to bioaccumulate based on the low log Pow. Therefore, on the basis of the low hazard and the reported use pattern, the assessed chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -78.5 °C

Method OECD TG 102 Melting Point/Melting Range
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks Test substance was stored in dry ice for 1 hour, with no freezing or crystallisation observed in this time.
Test Facility Allessa (2012a)

Boiling Point 240.6 °C at 101.3 kPa

Method OECD TG 103 Boiling Point
EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks Differential scanning calorimetry (DSC) was used.
Test Facility Allessa (2012b)

Density 959 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids
Remarks Pycnometer method was used.
Test Facility Smithers Viscient (2017)

Vapour Pressure 0.013 kPa at 25 °C

Method OECD TG 104 Vapour Pressure
EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks A vapour pressure balance was used.
Test Facility Envigo (2016a)

Water Solubility Fully soluble

Method OECD TG 105 Water Solubility
Remarks Flask Method
Test Facility Smithers Viscient (2017b)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH

pH	*T (°C)*	*Amount hydrolysed after 5 days*
4	50 ± 0.5 °C	3.4%
7	50 ± 0.5 °C	1.9%
9	50 ± 0.5 °C	2.3%

Remarks Samples analysed by HPLC-UV
Test Facility Smithers Viscient (2017c)

Partition Coefficient (Study 1) (n-octanol/water) log Pow = 1.265 ± 0.003 at 25 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method
Test Facility LAUS GmbH (2013a)

Partition Coefficient (Study 2) (n-octanol/water) log Pow = 0.73 ± 0.05 at 22 °C

Method OECD TG 107 Partition Coefficient (n-octanol/water): Shake Flask Method
Remarks Flask Method
Test Facility Taminco (2012)

Adsorption/Desorption – main test log K_{oc} = 1.142 at 25 °C

Method Chemical Registration Center of MEP TG 106 Adsorption – Desorption Using a Batch Equilibrium Method. This method is equivalent to OECD TG 106.

Soil Type	*Organic Carbon Content (%)*	*Koc (mL/g)*
Jianxi red soil	0.407	1.26
Heilongjiang black soil	1.97	1.29
Xinjiang sierozem	1.68	1.01
Hubei paddy soil	1.80	1.02
Anhui fluvo-aquic soil	1.73	1.13

Remarks The desorption process was irreversible in all soil types.
Test Facility Guandong Detection Center of Microbiology (2017a)

Flash Point 108 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point
Remarks Closed cup method. A Gardco Rapid Flash Point Tester was used.
Test Facility Smithers Viscient (2017)

Autoignition Temperature 210 – 217 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks Blue M Electric Furnace was used. There was a lag time of 109 seconds before ignition. The protocol used by the test laboratory suggested that it is most relevant when the chemical has vapourised at the autoignition temperature, however the measured autoignition temperature is below the boiling point of 240.6 °C.
Test Facility Smithers Viscient (2017)

Appendix B: Toxicological Investigations

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001) EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/RccHan: WIST
Vehicle	Distilled water
Remarks – Method	GLP Certificate. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	2,000	3/3
2	3 F	300	0/3
3	3 F	300	0/3

LD50	> 300 and < 2,000 mg/kg bw
Signs of Toxicity	Two animals treated at 2,000 mg/kg bw died on the same day of dosing, and the remaining one was euthanized within 1 day of treatment. Signs of toxicity included moderate convulsions, tachypnea (rapid breathing), prostration, clear lacrimation in eyes and loss of consciousness. All animals in the 300 mg/kg bw dose group survived. The animals in group 2 showed slight to moderate decreased activity, hunched posture and ruffled fur on Day 1. The animals in group 3 showed no signs of toxicity.
Effects in Organs	Excess fluid was found in the stomachs of all prematurely deceased animals. No abnormalities were noted in any surviving animals.
Remarks – Results	All surviving animals showed expected gains in bodyweight over the observation period.
CONCLUSION	The assessed chemical is harmful via the oral route.
TEST FACILITY	Harlan (2013)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (1987) –Limit Test EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal)
Species/Strain	Rat/RccHan: WIST
Vehicle	None. The assessed chemical was applied undiluted.
Type of dressing	Semi-occlusive
Remarks – Method	GLP Certificate No significant protocol deviations. After the 24 h contact period the test substance was wiped from the skin with cotton wool moistened with distilled water.

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	Very slight erythema was noted in two females, with scattered scabs and glossy skin also observed in one of the two.
Signs of Toxicity – Systemic	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	All animals showed expected body weight gain over the observation period of 14 days, except for two females that only showed expected body weight gain in the second week

CONCLUSION The assessed chemical is of low acute toxicity via the dermal route.

TEST FACILITY Harlan (2014a)

B.3. Acute Inhalation Toxicity – Rat

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 403 Acute Inhalation Toxicity
US EPA OPPTS Guideline 870.1300 Acute Inhalation Toxicity

Species/Strain	Rat/Crl:CD (SD)
Vehicle	
Method of Exposure	Nose-only exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol, particle size = 2.4 µm
Remarks – Method	GLP Certificate No significant protocol deviations. The humidity was lower than the protocol-specified minimum of 30% at one stage of the study, but was considered acceptable and did not affect the validity of this study. Clinical observations were made once during exposure, immediately after exposure, 1-2 h after exposure and daily for 14 days

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 F, 5 M	6.0	5.1	0/10

LC50	> 5.1 mg/L/4 hours
Signs of Toxicity	Laboured respiration was observed in one female during exposure. Signs immediately after exposure included clonic convulsions in 2 females, decreased or laboured respiration in 3 males and 5 females, and ataxia in 5 males and 2 females. These observations were reduced at the next observation and no longer present afterwards. The incidence of clinical signs from Day 1 onwards was low, and included unkempt appearance and dried material on the body. All animals were considered normal by Day 4.
Effects in Organs	Clear fluid was observed in the uterus of 1 female at necropsy.
Remarks – Results	All animals showed expected gains in bodyweight over the observation period.

CONCLUSION The assessed chemical is of low acute toxicity via inhalation.

TEST FACILITY Charles River (2017)

B.4. Skin Corrosion – *In Vitro* Human Skin Model Test

TEST SUBSTANCE Assessed chemical

METHOD	OECD TG 431 *In vitro* Skin Corrosion – Human Skin Model Test 2004) EC Council Regulation No 440/2008 B.40 bis. *In vitro* Skin Corrosion – Human Skin Model Test
Vehicle	None. The assessed chemical was directly applied.
Remarks – Method	GLP Certificate No significant protocol deviations EpiDerm Model. Negative control (de-ionised water) and positive control (potassium hydroxide, 8 mol/L) were run concurrently with the assessed chemical.

RESULTS

3 Minute Exposure

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability* (%)*	*SD of mean viability*
Negative control	1.940	100	0.271
Test substance	1.887	97.3	0.072
Positive control	0.573	29.5	0.110

1 Hour Exposure

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability* (%)*	*SD of mean viability*
Negative control	2.019	100	0.029
Test substance	0.177	8.8	0.018
Positive control	0.257	12.7	0.015

OD = optical density; SD = standard deviation
*Relative to the negative control, which is assigned a value of 100%.

Remarks – Results	Because the relative mean tissue viability was < 15% after the 1 hour treatment with the assessed chemical, it is categorised as corrosive according to the criteria of the test. The mean tissue viability at 3 minutes was 97.3%, which is well above the cut-off of <50% and would not lead to a corrosive classification. The mean OD_{570} from the negative control and positive control were within the historical control values. It was demonstrated in a pre-test that the assessed chemical does not react directly with formazin. Therefore, it was concluded by the study authors that the test conditions of this study were adequate and functioned properly.
CONCLUSION	The assessed chemical was corrosive to the skin under the conditions of the test.
TEST FACILITY	LAUS GmbH (2013b)

B.5. Skin Corrosion – *In Vitro* Corrositex®

TEST SUBSTANCE	Assessed chemical
METHOD	Similar to OECD TG 435 *in vitro* Membrane Barrier Test Method for Skin Corrosion
Vehicle	None
Remarks – Method	No GLP Certificate No significant protocol deviations. Only a summary was provided. The test substance was considered a Category 2 irritant based on results from indicator solutions, showing weak acidic/basic properties.

RESULTS

Test Material	*pH*	*Corrositex Time (minutes)*
Test substance	9.1	> 60

Remarks – Results: Positive and negative controls were run concurrently with the test substance.
Four replicate samples were run to confirm the reproducibility of the test conditions.

CONCLUSION: The assessed chemical was considered non-corrosive under the conditions of the test.

TEST FACILITY: InVitro (2013)

B.6. Skin Irritation – Rabbit

TEST SUBSTANCE: Assessed chemical

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion
US EPA OPPTS Guideline 870.2500 Acute Dermal Irritation

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3
Vehicle: None. The assessed chemical was directly applied.
Observation Period: 21 days (1 animal), 14 days (2 animals)
Type of Dressing: Semi-occlusive
Remarks – Method: GLP Certificate
No significant protocol deviations.
One animal was initially dosed for 3-minutes, 1-hour, and 4-hours using different exposure sites. The remaining 2 animals were dosed for a single exposure period of 4 hours.

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	2	1	1	2	> 14 or 21 days	1
Oedema	0	0	0	1	< 24 hours	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal after 4 h exposure.

Remarks – Results: After 3 minutes exposure to the test substance in one animal, no effects were seen except very slight erythema in one animal at 14 days, which had resolved by Day 21.

After 1 h exposure, very slight erythema was seen intermittently from patch removal to Day 14, but had resolved by Day 21. Desquamation was seen from Days 7-14.

After 4 h exposure, very slight erythema was seen on all tested animals and very slight oedema on one animal at the 1-hour scoring interval. Well-defined erythema was seen in one animal and very slight erythema in the other two animals at 24- , 48-, and 72-hours after treatment.

Dermal irritation was not observed in one animal on Day 7, but was again observed in the same animal on Day 10. Very slight erythema was seen in all animals at the end of the observation period (14 days for 2 animals and 21 days for 1 animal).

Additional observations included blanching (focal or pinpoint areas up to 10% of the treated site) in one animal at 24-72 h after treatment and desquamation in all animals from Day 10 onwards.

CONCLUSION The assessed chemical is irritating to the skin.

TEST FACILITY Charles River (2014a)

B.7. Eye Irritation – Rabbit

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion (date not specified)
US EPA OPPTS Guideline 870.2400 Acute Eye Irritation

Species/Strain Rabbit/New Zealand White
Number of Animals 3
Observation Period 10 days
Remarks – Method GLP Certificate
No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva – Redness	3	2.7	2	3	< 10 days	0
Conjunctiva – Chemosis	2	2	0.3	3	< 7 days	0
Conjunctiva – Discharge	2	1	1	2	< 7 days	0
Corneal Opacity	2	0	0	3	< 7 days	0
Iridial Inflammation	1	1	1	1	< 10 days	0

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

Remarks – Results Exposure to the test substance produced corneal opacity in one animal from 24 h after treatment. Iritis was observed in one animal after 1 hour of treatment and in the other two by the 24-hour scoring interval. Conjunctivitis and discharge were observed in in all animals from 1 hour after treatment. These effects had recovered by Day 7 to Day 10 in all treated animals.

An additional ocular finding of neovascularisation was noted in two animals during the study, persisting to the end of the observation period of 7 or 10 days respectively, when it was considered level 1 (<10% coverage of the cornea). At this time the extent of neovascularisation had significantly reduced since the previous observation, when it was level 3 (25-50% coverage of the cornea or level 4 (>50% coverage of the cornea). Therefore it might be expected that the neovascularisation would resolve by 21 days after treatment.

Decreased faecal output was observed in all animals. This observation was likely due to the buprenorphine (an opioid) treatment that the animals received from Days 0 to 5.

CONCLUSION The assessed chemical is irritating to the eye.

TEST FACILITY Charles River (2014b)

B.8. Skin Sensitisation – LLNA

TEST SUBSTANCE Assessed chemical

METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2010) EC Council Regulation No 440/2008 B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/Ca
Vehicle	Acetone/olive oil (4:1)
Preliminary study	Yes, at 50% and 75%
Positive control	Conducted in parallel with the test substance using α-hexyl cinnamaldehyde.
Remarks – Method	A pre-test was carried out to determine concentrations for the main test. GLP Certificate No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5 F	1407.09	0
10	5 F	978.85	0.7
25	5 F	1278.1	0.91
50	5 F	1720.98	1.22
Positive Control			
25	5 F	7577.97	5.39

Remarks – Results	In the preliminary study, the animal tested at 75% was found dead on Day 3. There were no signs of systemic toxicity, local irritation or increase in ear thickness > 25% in the animal treated at 25%, and this was chosen as the highest concentration in the main study. No deaths or signs of systemic toxicity were observed in the main study. There were also no signs of local irritation or increases in ear thickness (≥ 25%) in any of the tested animals. All animals showed expected gains in bodyweight over the study period.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the assessed chemical up to 50% concentration.
TEST FACILITY	Harlan (2015)

B.9. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (1998)
Species/Strain	Rat/RccHan: WIST
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Distilled water
Remarks – Method	GLP Certificate. Doses for the main study were determined as a result of an earlier study using dose levels of 0, 50, 200, 500, 750 and 1000 mg/kg bw/day. Adverse effects such as ataxia and respiratory difficulties were seen at the highest two dose levels after 2-3 days, so these dose levels were terminated.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10 F, 10 M	0	0/20
Low Dose	10 F, 10 M	10	0/20
Mid Dose	10 F, 10 M	100	0/20
High Dose	10 F, 10 M	500	0/20

Mortality and Time to Death

There were no unscheduled deaths for the duration of this study.

Clinical Observations

Animals of either sex from the high dose group had statistically significantly lower overall activity. Clinical signs in both sexes included post-dose salivation and isolated episodes of noisy respiration. All animals showed expected body weight gain over the total duration of the study.

There was no adverse effect of treatment on body weight development and food consumption at all doses. Males in the high dose group showed a statistically significant reduction of body weight gain during Week 8, Week 10 and Week 11, female in the high dose group showed a statistically significant reduction of body weight gain during Week 11, and males in the mid dose group showed a statistically significant reduction of body weight gain during Week 8. However, recovery was evident thereafter with body weight gains superior to controls, leading to an overall body weight gain comparable to the control. Some variations in food efficiency was also observed on certain weeks, consistent with the intergroup differences in body weight gain observed, but no obvious overall effect on food consumption was observed. This was considered to represent normal biological variations rather than an adverse effect of treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Males in the high dose group showed a statistically significant increase in total protein, calcium, creatinine and bile acid and a statistically significant reduction in albumin/globulin ratio. Females in the high dose group showed a statistically significant reduction in albumin/globulin ratio, chloride and alkaline phosphates and a statistically significant increase in cholesterol, bilirubin and bile acid. Males in the mid dose group showed a statistically significant increase in chloride levels. The study authors attributed the clinical chemistry effects to mixed function oxidase induction in the liver.

Males in the high dose group had a statistically significant increase in neutrophil and platelet counts and a statistically significant reduction in clotting time. Females in the high dose group showed a statistically significant reduction in haemoglobin and haematocrit and a statistically significant increase in neutrophil count.

Effects in Organs

Compared to the controls, mean liver weights were statistically significantly increased in high dose males (+48%) and females (+33%) and mid dose males (+9%). Enlarged livers were seen in 8/10 high dose males. Males in the high and mid dose groups had increased mean kidney weights (+23% and +6% increase respectively, compared to the control mean). A statistically significant increase in high dose female mean kidney weight (+8%) compared to controls was still within the historical control range. Hypertrophy or centrilobular hypertrophy of the liver was observed in 7/10 and 3/10 males in the mid and high dose groups respectively and in all high dose females. An increased incidence and severity of hyaline droplet accumulation, multifocal basophilic tubules and the presence of proteinaceous casts in the tubules of kidneys were observed in males at the mid and high dose groups. Thymic atrophy present in males in the mid and high dose group and females in the high dose group was considered by the study authors as likely to be a secondary reaction to stress. Vacuolation in the adrenal cortex was observed in all males in the mid and high dose group at an increased incidence and severity and in one male in the low dose group, and considered to be related to lipid metabolism and liver changes.

No adverse effects were detected during the oestrous cycle assessments or in sperm concentration or motility. A statistically significant increase in the number of sperm with abnormal morphology in high dose males was considered by the study authors as likely due to artifactual abnormalities associated with the slide smearing procedure, based on the types of abnormalities. There was a non-statistically significant reduction in mean homogenisation resistant spermatid counts in the high dose compared to the control group (the only groups tested).

Remarks – Results

The study authors established that the No Observed Effect Level (NOEL) for the assessed chemical was 10 mg/kg bw/day for males and 100 mg/kg bw/day for females. They concluded that the effects observed in the liver, blood chemistry, thymus and adrenals to be treatment-related adaptive changes, and not considered to be adverse. The renal changes observed are specific to male rats and it does not correlate to humans.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 500 mg/kg bw/day in this study.

TEST FACILITY Harlan (2014b)

B.10. Genotoxicity – Bacteria

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1997)
EC Council Regulation No 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria
Plate incorporation procedure (test 1)/Pre incubation procedure (test 2)

Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	S9 mix from Aroclor-1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 31.6 – 5,000 µg/plate b) Without metabolic activation: 31.6 – 5,000 µg/plate
Vehicle	Acetone
Remarks – Method	GLP Certificate. Vehicle and positive controls were run concurrently with the test substance.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	Not reported	Negative
Test 2		> 5,000	Not reported	Negative
Present				
Test 1		> 5,000	Not reported	Negative
Test 2		> 5,000	Not reported	Negative

Remarks – Results

No significant increases in the frequency of revertant colonies were observed for any of the bacterial strains, at any test concentration, either with or without metabolic activation.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

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

TEST FACILITY LPT (2013a)

B.11. Mutagenicity – *In Vitro* mammalian cell gene mutation test

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test
EC Directive 2008/440/EC B.17 Mutagenicity – *In vitro* Mammalian Cell Gene Mutation Test (2008)

	US EPA OPPTS Guideline 870.5300 Detection of Gene Mutations in Somatic Cells in Culture
Species/Strain	Mouse
Cell Type/Cell Line	L5178Y Mouse lymphoma cells ($TK^{+/-}$-3.7.2C)
Metabolic Activation System	S9-Mix from phenobarbital (PB)/β-naphthoflavone (NF) induced rat liver
Vehicle	RPMI medium
Remarks – Method	GLP certificate. A dose range-finding study was carried out at 5.51 – 1411.2 µg/mL. The dose selection for the main experiments was based on toxicity observed in the range-finding study and the solubility test. Based on this, the maximum dose in the main study was 10 mM (1411.2 µg/mL), which is the maximum recommended dose for relatively non-cytotoxic compounds. A vehicle control and two positive controls (ethyl methanesulfonate in the absence of metabolic activation and cyclophosphamide in the presence of metabolic activation) were run concurrently with the assessed chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0, 44.1*, 88.2*, 176.4*, 352.8*, 705.6*, 1411.2*	4 hours	2 days
Test 2	0, 44.1*, 88.2*, 176.4*, 352.8*, 705.6*, 1411.2*	24 hours	2 days
Present			
Test 1	0, 44.1*, 88.2*, 176.4*, 352.8*, 705.6*, 1411.2*	4 hours	2 days
Test 2	0, 44.1*, 88.2*, 176.4*, 352.8*, 705.6*, 1411.2*	4 hours	2 days

*Cultures selected for mutation frequency (MF) 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	> 1411.2	> 1411.2	Negative	Negative
Test 2	≥ 1411.2	≥ 1411.2	Negative	Negative
Present				
Test 1	> 1411.2	> 1411.2	Negative	Negative
Test 2		> 1411.2	Negative	Negative

Remarks – Results	The assessed chemical did not lead to a statistically significant increase in the number of mutation frequencies at the TK-locus, either in the presence or absence of metabolic activation. The number of small and large colonies in treated cultures was within the range of the concurrent vehicle control and the historical vehicle control data. The increase in the frequencies of mutant colonies induced by the positive control demonstrated the sensitivity of the test method and the metabolic activity of the S9 mix.
CONCLUSION	The assessed chemical was not mutagenic to mouse lymphoma cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2014c)

B.12. Genotoxicity – *In Vitro* mammalian cell micronucleus test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 487 *In vitro* Mammalian Cell Micronucleus Test

Species/Strain	Human peripheral blood cells
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9-Mix from phenobarbital (PB)/β-naphthoflavone (NF) induced rat liver
Vehicle	Culture media
Remarks - Method	GLP Certificate A dose range-finding study was carried out at 5.51 – 1411.2 µg/mL. The dose selection for the main experiments was based on toxicity observed in the range-finding study and the solubility test. The highest concentration chosen for the main study was the maximum recommended concentration of 10 mM (1411.2 µg/mL) A vehicle control and three positive controls (mitomycin C, cyclophosphamide and demecolcin) were run concurrently with the assessed chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0, 44.1, 88.2, 176.4, 352.8*, 705.6*, 1411.2*	4 hours	28 hours
Test 2	0, 44.1, 88.2, 176.4, 352.8*, 705.6*, 1411.2*	24 hours	28 hours
Present			
Test 1	0, 44.1, 88.2, 176.4, 352.8*, 705.6*, 1411.2*	4 hours	28 hours

*Cultures selected for micronucleus analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µL/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation in Main Test*	*Genotoxic Effect*
Absent				
Test 1	> 1411.2	> 1411.2	Negative	Negative
Test 2	> 1411.2	> 1411.2	Negative	Negative
Present				
Test 1	> 1411.2	> 1411.2	Negative	Negative

Remarks - Results	The assessed chemical did not cause any dose related or statistically significant increase in the number of cells carrying micronuclei in either the absence or presence of metabolic activation when tested up to the highest concentration. The micronucleus rate of the treated cells was within the range of historical control data, exceeding this slightly only for the highest dose tested at 24 h treatment without metabolic activation. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The assessed chemical was not genotoxic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2014d)

B.13. Developmental Toxicity – Rat (oral)

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 414 Prenatal Developmental Toxicity Study (2001)
Species/Strain	Rat/Crl:CD (SD)
Route of Administration	Oral – gavage
Exposure Information	Exposure days: 14 days (gestation days 6 – 19)
Vehicle	Tap water
Remarks – Method	GLP Certificate

The dose levels were selected based on the results of a previous range-finding study by LPT (Study No. 29177, not included) using the same doses and 3 animals/dose (gestation days 6 – 19), and reported briefly in this study.

In the range-finding study, the following effects were seen at the high dose only (500 mg/kg bw/day). The commencement of treatment led to a distinct reduction in food consumption and a decline in body weight. Piloerection was seen in 2/3 dams and laboured breathing in 1/3 dams. There was total loss of implantation sites in 1/3 dams but no effect in reproduction parameters in the remaining 2 dams. Foetuses from the remaining 2 dams showed slightly reduced body weight, but no malformations or variations were found during the external examination.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1 (vehicle control)	20 F	0	0/20
2	20 F	5	0/20
3	20 F	50	0/20
4	20 F	500	0/20

Mortality and Time to Death

There were no unscheduled deaths for the duration of this study.

Effects on Dams

At the high dose, the animals showed a statistically significant reduction in terminal body weight and mean body weight gain at the end of exposure (-5.8% and -12.9% respectively). A reduction of food consumption was noted on gestation day 7 and 8, but normal levels of consumption were observed after this. A reduced mean gravid uterine weight and carcass weight in the high dose group was observed during laparotomy (-11.4% and -4.3% respectively). No behavioural effects were observed. Breathing sounds reported for one high dose dam on days 8 and 9 only were not considered test item related. Changes to body weight, body weight gain or clinical signs were not seen in low or mid dose animals.

No treatment-related findings in reproductive parameters were seen in any group. An increase in pre-implantation loss in all test groups was considered not relevant as dosing did not start until day 6. An increase in early resorptions per dam in the low dose group was not considered relevant as it was not dose related and was well within the historical control range. .

Effects on Foetus

No deaths, foetal malformations or other developmental variations were observed at all dose levels. A reduction in mean foetal weight overall (5.9% reduction) and mean weight of female foetuses (8.8% reduction and statistically significant) and reduction in mean placental weight (11.3% reduction and statistically significant) in the high dose group compared to the control group were discounted by the study authors because the values were within historical controls. However, the foetal weights were at the bottom end of the historical control range.

CONCLUSION

The study authors established a NOAEL of 50 mg/kg bw/day for maternal toxicity, based on lower body weight and lower body weight gain observed in dams of the high dose group. A NOAEL of > 500 mg/kg bw/day was established for developmental toxicity.

TEST FACILITY LPT (2013b)

B.14. Developmental Toxicity – Rat (oral)

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 414 Prenatal Developmental Toxicity Study (2001)

	US EPA OPPTS Guideline 870.3700 Prenatal Development Toxicity Study
Species/Strain	Rat/Crl:CD (SD)
Route of Administration	Oral – gavage
Exposure Information	Exposure days: 14 days (gestation days 6 – 19)
Vehicle	Deionised water
Remarks – Method	GLP Certificate

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1 (vehicle control)	25 F	0	0/25
2	25 F	200	0/25
3	25 F	300	0/25
4	25 F	400	0/25
5	25 F	500	1/25

Mortality and Time to Death

One animal in the 500 mg/kg bw/day group was found dead on gestation day 10. No remarkable clinical observations, effects on body weight or food consumption prior to death, or macroscopic findings during necropsy were found. The study authors concluded that this death was not caused by the test substance.

Effects on Dams

Clear and red material around the mouth was noted in animals at 500 mg/kg bw/day after the dose administration.

Rales was observed in some animals during daily observations and/or 1 h after dose administration in the 300, 400 and 500 mg/kg bw/day dose groups, but on the basis of the incidence, the effects were only considered adverse at 500 mg/kg bw/day by the study authors.

The animals in the 500 mg/kg bw/day dose group showed a lower mean body weight gain, and reduced overall food consumption (all statistically significant compared to the control mean), leading to a lower mean body weight (-4.9%, but not statistically significant) than the control group at the end of dosing. However mean net body weight and gravid uterine weight in the 500 mg/kg bw/day dose group was comparable to the control group. Mean net body weight gains at 300 and 400 mg/kg bw/day were also slightly lower than the control group (statistically significant).

No treatment-related macroscopic findings were noted at any dose level. No effects were seen on numbers of viable foetuses, sex ratio, number of dead foetuses or on early or late resorptions.

Effects on Foetus

A statistically significantly reduced mean foetal body weight (-5.0%, -5.0% and -10.0%) was observed at the 300, 400 and 500 mg/kg bw/day dose levels compared to the controls. This was partially attributed by the study authors to high foetal weights of the concurrent control group (at the high end of the historical controls), which was in turn assumed to be due to lower numbers of foetuses in the control group. Based on these factors and the smaller reduction in foetal body weights at 300 and 400 mg/kg bw/day, the study authors determined that only the effects at 500 mg/kg bw/day were test substance related and indicated adverse developmental toxicity. Foetal survival at all dose levels were considered to be unaffected by treatment.

No foetal deformations or developmental variations related to the test substance were observed at all dose levels.

CONCLUSION

The NOAEL was established as 400 mg/kg bw/day for both maternal toxicity and developmental toxicity in this study, based on incidences of rales, lower body weight gain, and lower mean foetal body weights observed at 500 mg/kg bw/day.

TEST FACILITY Charles River (2016a)

B.15. Developmental Toxicity – Rabbit (oral)

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 414 Prenatal Developmental Toxicity Study (2001) US EPA OPPTS Guideline 870.3700 Prenatal Development Toxicity Study
Species/Strain	Rabbit/New Zealand White
Route of Administration	Oral – gavage
Exposure Information	Exposure days: 21 days (gestation days 7 – 28)
Vehicle	Deionised water
Remarks – Method	GLP Certificate The dose levels were selected based on the results of a previous 7-day tolerability test in non-pregnant animals at 50, 125, 250 and 500 mg/kg bw/day and a subsequent range-finding study similar to TG414 at 100, 200 and 300 mg/kg bw/day, both conducted by Charles River (Study No. 00387126 and 00387127, not included). In the tolerability test, using 2 animals/dose, clinical observations such as decreased faecal output, body weight losses of 8% and minimal food consumption were seen in animals in the 500 mg/kg bw/day dose group. Based on this result, the highest dose used in the range finding and main studies was 300 mg/kg bw/day.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1 (vehicle control)	24 F	0	0/24
2	24 F	50	0/24
3	24 F	150	0/24
4	24 F	300	0/24

Mortality and Time to Death

There were no unscheduled deaths for the duration of this study.

Effects on Dams

The females in the 300 mg/kg bw/day dose group showed mean body weight loss during Days 7-10, a lower but not statistically significant mean body weight gain during Days 10-13, and a statistically significantly higher body weight gain during Days 20-29. Overall, the mean body weight gain over Days 7-29 at 300 mg/kg bw/day was lower than the control group (-28%) but not statistically significant. The mean total food consumption in the 300 mg/kg/day group was statistically significantly lower than the control group, was associated with a lower fecal output, and was considered by the study authors to be related to the body weight effects. Overall, the lower mean maternal body weight gains and reduced food consumption at 300 mg/kg bw/day were considered by the study authors to be test substance-related and adverse.

The mean corrected body weight and gravid uterine weight in the 300 mg/kg bw/day group were generally comparable to the control group (-2.2% and -4.6% respectively); no statistically significant differences were noted.

Lower (not statistically significant) mean body weight gains were noted in the 50 and 150 mg/kg/day groups following the initiation of dosing (Gestation Days 7–10), which were considered test substance-related but not adverse. Mean absolute body weights, corrected body weights, corrected body weight gains, and gravid uterine weights in the 50 and 150 mg/kg/day groups were generally comparable to the control group; no statistically significant differences were noted. Food consumption in the 50 and 150 mg/kg/day groups were unaffected by the treatment and was generally comparable to the control group throughout the study.

Effects on Foetus

No other treatment-related clinical signs or macroscopic findings were noted at any dose level. Mean foetal weights were slightly reduced (-4.4%) at 300 mg/kg bw/day without statistical significance. No effects were seen on litter proportions of post-implantation loss, mean number of viable foetuses or foetal sex ratio.

Although malformations and developmental variations were observed in some foetuses in the 150 and 300 mg/kg bw/day dose groups, the total incidences occurred infrequently or at a frequency similar to that in the control group, were not seen to have any dose-dependent patterns, and were within the historical control data ranges.

CONCLUSION
The NOAEL was established as 150 mg/kg bw/day for maternal toxicity, based on the effects observed at 300 mg/kg bw/day including reduced body weight gain, reduced food consumption, and corresponding excreta-related clinical observations. The NOAEL for developmental toxicity was established as 300 mg/kg bw/day (the highest dose tested).

TEST FACILITY Charles River (2020)

B.16. Developmental Toxicity – Rat (Dermal)

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 414 Prenatal Developmental Toxicity Study (2001) US EPA OPPTS Guideline 870.3700 Prenatal Development Toxicity Study
Species/Strain	Rat/Crl:CD (SD)
Route of Administration	Dermal – non-occluded
Exposure Information	Exposure days: 14 days (gestation days 6 – 19) Duration of exposure (dermal): 23 hours/day
Vehicle	Deionised water
Remarks – Method	GLP Certificate The dose levels were selected based on a previous dose range-finding dermal study in rats. Some animals were replaced, due to an error in feeding.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
1	24 F	0	0/24
2	23 F	375	0/24
3	24 F	500	0/24
4	24 F	750	0/24

Mortality and Time to Death
There were no unscheduled deaths for the duration of this study.

Effects on Dams
Incidences of red material around the eyes of animals were noted in all treatment groups throughout the treatment period, but this finding was not considered adverse.

Mean body weight of treated groups compared to the controls was reduced in a dose-dependent manner and the reduction was statistically significant at 750 mg/kg bw/day on most days and at 500 mg/kg bw/day on days 18, 19 and 20. Lower body weight gain was statistically significant in all treated groups on days 0-6 (prior to dosing) compared to the control mean (-17.6%, -17.6% and -23.6% for each dose group respectively). Reduction in body weight gain was statistically significant for days 17-19 and 6-20 (total treatment time) at 750 mg/kg bw/day and for days 17-18 at 500 mg/kg bw/day. Mean gravid uterine weight (-12.6%) and net body weight (-6.1%) were lower and statistically significant at 750 mg/kg bw/day and net body weight gain was statistically significantly lower than the control group for the 500 and 750 mg/kg bw/day groups (-13.4% and -27.8% respectively).

Food consumption was evaluated both as g/animal/day and as g/kg bw/day and identified reduced consumption primarily on days 6-9 and overall for days 6-20, for all treatment groups. The g/kg bw/day evaluation was only statistically significant for days 6-9, for the 500 and 750 mg/kg bw/day groups.

The body weight changes and gravid uterine weights in the 375 mg/kg bw/day dose group were not considered to be affected by the treatment (-2.8% and -0.7% respectively).

No treatment-related macroscopic findings were noted at any dose levels. No changes related to treatment were observed in the numbers of viable foetuses and dead foetuses, sex ratio and early or late resorptions.

Effects on Foetus
A statistically significantly reduced mean foetal body weight (-5.3% compared to control mean) was observed at the 750 mg/kg bw/day dose level. This was considered to be an effect of low food consumption and body weight gain in maternal animals during the treatment period.

Intrauterine growth and survival at dose levels of 500 mg/kg bw/day and below were considered to be unaffected (-3% foetal weight for 375 and 500 mg/kg bw/day).

No test substance related foetal malformations or developmental variations were observed at any dose level.

Remarks – Results
Mean body weight losses were noted in all groups, including the control, following the initiation days of administration (gestation day 6-7). These changes were believed by the study authors to be related to the placement of Elizabethan collars on the animals.

CONCLUSION
The NOAEL was established as 500 mg/kg bw/day for both maternal toxicity and developmental toxicity in this study, based on the lower body weights, body weight gain, gravid uterine weight, and lower mean foetal body weights observed at 750 mg/kg bw/day.

TEST FACILITY Charles River (2016b)

B.17. Developmental Toxicity – Rat (Inhalation)

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 414 Prenatal Developmental Toxicity Study (2001)
US EPA OPPTS Guideline 870.3700 Prenatal Development Toxicity Study

Species/Strain	Rat/Crl:CD (SD)
Route of Administration	Inhalation – whole body exposure
Exposure Information	Exposure days: 14 days (gestation days 6 – 19) Duration of exposure (inhalation): 6 hours/day
Vehicle	None
Physical Form	Vapour/liquid aerosol
Particle Size	2.4 – 2.7 µm
Remarks – Method	GLP Certificate The dose levels were selected based on a previous dose range-finding study in non-pregnant rats.

RESULTS

Group	*Number of Animals*	*Dose/Concentration (mg/L)*		*Mortality*
		Target	*Measured*	
1	24 F	0	0	0/24
2	24 F	0.3	0.29	0/24
3	24 F	0.6	0.58	0/24
4	24 F	1.2	1.2	0/24

The target concentrations 0.3, 0.6, and 1.2 mg/L were reported to correspond to mean estimated inhaled dosage levels of 76.2, 152.6, and 315.8 mg/kg bw/day, respectively.

Mortality and Time to Death
There were no unscheduled deaths for the duration of this study.

Effects on Dams

Red material around the nose, mouth and forelimbs were observed daily in the 0.58 and 1.2 mg/L dose groups after the dose administration. A clear material on the ventral neck area was also noted in animals in the 1.2 mg/L dose group during the latter half of the gestation period.

There was a dose-related reduction in body weight in all test groups compared to the controls, from the commencement of dosing, which was statistically significant at the end of the study only at 1.2 mg/L (-11.5% reduction compared to control mean). Reduced body weight gain compared to the control group occurred at some days of the dosing period, and was statistically significant over the whole period at 0.58 and 1.2 mg/L (- 12.5% and -37.5% reduction compared to control mean). Gravid uterine weight, net body weight and net body weight gain were also reduced in a dose dependent manner and were statistically significant at 1.2 mg/L, compared to the control group.

Food consumption was reduced to some extent in all test groups from the commencement of dosing onwards. The reduction was statistically significant in all test groups over the entire period (14 days), and at several other days. The greatest reduction in food consumption compared to the controls occurred in the 1.2 mg/L group (- 25% and -19.4% through GD 6-20, when calculated by g/animal/day or g/kg bw/day respectively).

No treatment-related macroscopic findings in dams were observed at any dose level. Lung weights were not affected. No effects were seen on numbers of viable foetuses and dead foetuses, sex ratio and early or late resorptions.

Effects on Foetus

A statistically significant reduced mean foetal body weight was observed in all treatment groups. The approximate reduction of 5% observed in the 0.29 and 0.58 mg/L does groups were not considered by the study authors to be related to treatment, based on the historical control data (75th quartile) and the magnitude of the change. The reduced mean foetal body weight observed in the 1.2 mg/L dose group (-12.5% in male pups and -13.2% in female pups) was attributed to the reductions in mean body weight gain and food consumption in dams during the gestation period, and considered an adverse effect.

Two skeletal developmental variations found in the 1.2 mg/L group were considered test substance related and indicative of developmental delay. These were a statistically significant lower mean litter proportion with cervical centrum no. 1 ossified and a higher mean litter proportion with sternebrae no 5 and 6 unossified (not statistically significant, but outside the historical controls).

No other test substance related foetal deformations or developmental variations were observed at any dose level.

Remarks – Results

The reduced foetal weights in the 0.29 and 0.58 mg/L groups were considered to be partially due to slightly higher concurrent control foetal weights.

CONCLUSION

The NOAEL was established as 0.58 mg/L in this study for both maternal toxicity and developmental toxicity, equivalent to approximately 152.6 mg/kg bw/day, based on the incidences of skeletal developmental variations observed in the highest dose.

TEST FACILITY	Charles River (2016c)

B.18. Toxicokinetics – Rat (Oral)

TEST SUBSTANCE	Assessed chemical, [carbonyl-^{14}C] (^{14}C-substituted in the 2' position)
METHOD	OECD TG 417:Toxicokinetics
Species/Strain	Rat/Crl:CD (SD)
Route of Administration	Oral – gavage Intravenous (i.v.) – bolus
Vehicle	0.9% saline

Remarks – Method	GLP Certificate. No significant protocol deviations.

STUDY DESIGN AND OBJECTIVE

The objectives of this study were to determine plasma pharmacokinetics of the test substance in male rats following a single oral (gavage) or i.v. (bolus) doses. The routes of elimination and excretion mass balance as well as the tissue distribution and tissue pharmacokinetics of test substance were determined by tracing test substance-derived radioactivity.

For the pharmokinetic phase, the animals received either a single oral (300 mg/kg bw) or i.v. (30 mg/kg bw) dose of the test substance. Following dosing, blood samples were collected from 4 animals/group/time point at approximately 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, and 72 hours.

For the excretion balance phase, one group of 4 animals received a single oral dose of the test substance at 300 mg/kg bw. After dosing, animals were placed into glass metabolism cages for separate collection of expired air through 48 hours and urine and faeces for 168 hours (7 days).

For the tissue distribution phase, the animals received a single oral dose of test substance. At approximately 0.25, 1, 4, 8, 24, 72, and 168 hours post-dose, a whole blood sample was collected from 1 animal/time point. Following blood collection, animals were euthanized and their carcasses were frozen for analysis by quantitative whole body autoradiography (QWBA).

Group	*Number and Sex of Animals*	*Dose (mg/kg)*	*Target Radioactivity (µCi/kg)*	*Sample Collected*
1 (Pharmcokinetic phase)	12 M	300	200	Blood
2 (Pharmcokinetic phase)	12 M	30*	200	Blood
4 (Excretion balance phase)	4 M	300	200	Excreta, expired air, carcass
5 (Tissue distribution phase)	7 M	300	200	Blood, carcass

* Applied by i.v.

RESULTS

Bioavailability and excretion data confirmed that the test substance was fully absorbed to systemic circulation following oral dosing in male rats. After administration of the test substance by oral route (first dose group), the plasma half-life was calculated to be 7.96 hours. [^{14}C]-labelled test substance was widely distributed to all tissues, and tissue concentrations were correlated to the degree of perfusion by circulating blood. The second dose group with i.v. administration gave a calculated half-life of 9.89 hours. Tissue exposure to the test substance was lowest in fat and highest in the adrenal glands, kidneys (all sections), and spleen. The study authors concluded that there is no difference in clearance following administration by either route.

The test substance was extensively metabolized by the rat following oral administration; the test substance was responsible for < 3% of the radioactivity circulating in plasma by 8 hours post-dose. The two major metabolites detected in plasma were *N*-butyl-5-hydroxypyrrolidone (70%) and one other hydroxyl metabolite (11.3%).

Urine samples contained up to 38 metabolites, with 3 primary metabolites identified as *N*-hydroxybutyl succinimide (6%), *N*-butyl-5-hydroxypyrrolidone (11%), and a glucuronide conjugate of OH-*N*-butylpyrrolidone (11%). The parent test substance was only found as a minor component of urine (0.3%). Twelve metabolites were identified in faecal extractions and 8 were present at > 5% of the total radioactivity (ROI) in at least 1 sample, but no metabolites were present at > 5% of the administered dose. Similar to urine, only small quantities of the parent test substance (0.3%) was present in the faecal matter.

The test substance's metabolic equivalents were almost completely excreted by the end of the study period. In the excretion balance phase group, about 99.5% of the administered dose was recovered in expired air (1.17%), urine (94.1%) and faeces (4.28%). The carcasses were not analysed further because mass balance was achieved.

CONCLUSION
Bioavailability and excretion data confirmed that the test substance is fully absorbed to system circulation following oral dosing. The test substance is widely distributed to all tissues, and tissue concentrations were correlated to the degree of perfusion by circulating blood. The test substance was extensively metabolised and distributed around the entire body. Almost all metabolic equivalents of the test substance were completely excreted from the body in urine, faeces, and expired air within the duration of the study period (7 days), with only a minor percentage identified as the original test substance.

TEST FACILITY Charles River (2016d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	Sewage effluent
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	ThOD
Remarks – Method	As per OECD test guidelines. Sodium benzoate was used for the reference substance and a toxicity control was conducted.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	0*	3	49.5%
8	0*	8	66.7%
14	0*	14	67.7%
20	0*	20	68.1%
28	0*	28	73.9%

*The % degradation of the test substance were all negative values.

Remarks – Results	All validity criteria were met. The oxygen consumption levels in the inoculum blank were <1.5 mg/L, the residual oxygen concentration was maintained above 0.5 mg/L in all test vessels, the difference between replicates was maintained at <20%. The test substance is not considered inhibitory as the ThOD was 33.6% at day 14 in the toxicity control.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	LAUS GmbH (2013c)

C.1.2. Inherent Biodegradability (Study 1)

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 302 B Inherent Biodegradability: Zahn-Wellens/ EVPA Test
Inoculum	Activated sludge
Exposure Period	112 days
Auxiliary Solvent	None
Analytical Monitoring	TOC
Remarks – Method	The test guidelines only contains scope for 28 day study periods unless adaption occurs. Adaption was observed during the study, therefore an extension of the study duration is acceptable. The chemical is moderately volatile and OECD 302B is not suitable for volatile substances, so the results from this test should be treated with caution. Notable deviations from the OECD test guidelines include: A temperature range of 18.7°C – 26.0°C instead of 20°C - 25°C, however as the control sample behaved normally, this is not considered to have impacted the study.

RESULTS

Test Substance		*Aniline*	
Day	*Mean % Degradation*	*Day*	*Mean % Degradation*
2	5.5	2	16.4
8	-3.2	8	97.7
14	2.9	14	99.8
20	6.8	20	100.4
28	7.1	28	99.7
56	19.0	56	99.5
71	39.2	71	99.7
84	51.7	84	99.0
98	68.2	98	99.3
112	80.8	112	99.2

Remarks – Results: All validity criteria were met. The degradation of the reference substance was >70% at 14 days and the degradation in the toxicity control was >35%.

There is a large discrepancy between the replicate values of the test substance at day 112 (60% vs 101.6%), and therefore there is large uncertainty in the mean value. The values in both replicates, however is sufficient to conclude that the test substance is ultimately biodegradable.

CONCLUSION: The test substance is ultimately biodegradable, however the results should be treated with some caution.

TEST FACILITY: LAUS GmbH (2013d)

C.1.3. Inherent Biodegradability (Study 2)

TEST SUBSTANCE: Assessed chemical

METHOD: OECD TG 302 C Inherent Biodegradability: Modified MITI Test (II)

Inoculum:

Exposure Period: 56 Days

Auxiliary Solvent: None

Analytical Monitoring: BOD

Remarks – Method: As per OECD test guidelines. The following deviations were noted:
Activated sludge inoculum was only obtained from one site, this is not expected to have impacted the study as the reference substance degraded within the expected timeframe.
The recovery rate of the control sample was not conducted and instead was conducted on a test sample. This deviation is unlikely to have affected the study as the test substance was successfully detected.
The test was conducted over 58 days rather than the standard 28 days. This deviation is not expected to affect the validity of the study as the test substance appears to have a lag phase in the biodegradation process.

RESULTS

Test Substance			*Sodium benzoate*	
Day	*% Degradation ($ThOD_{NH4}$)*	*% Degradation ($ThOD_{NO3}$)*	*Day*	*% Degradation*
4	1.1	0.9	4	87.2
8	1.7	1.4	8	95.2
16	0.8	0.7	16	91.6
28	16.3	13.7	28	81.7
32	56.9	47.8	32	79.5
36	98.9	83.0	36	77.0
48	127.9	107.4	48	72.2
56	133.6	112.2	56	70.0

Remarks – Results: All validity criteria were met. The reference substance reached >40% degradation by day 7 and >65% after 14 days. And the recovery rate was determined to be 89% of the nominal value.

CONCLUSION: The test substance is ultimately biodegradable.

TEST FACILITY: IBACON GmbH (2015)

C.1.4. Inherent Biodegradability (Study 3)

TEST SUBSTANCE: Assessed chemical

METHOD: OECD TG 302 C Inherent Biodegradability: Modified MITI Test (II)
- Inoculum: Activated sludge
- Exposure Period: 28 Days
- Auxiliary Solvent: None
- Analytical Monitoring: BOD
- Remarks – Method: As per OECD test guidelines, no deviations were noted.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	1.2	2	39.0
8	3.7	8	79.7
14	4.4	14	85.5
22	4.8	22	86.2
28	3.0	28	85.7

Remarks – Results: All validity criteria were met. The degradation of the reference substance was >40% at day 7 and >65% at day 14, the residual concentration of the test substance in the abiotic control was >10% at the end of the test.

CONCLUSION: The test substance is not inherently biodegradable.

TEST FACILITY: Guangdong Detection Center of Microbiology (2017b)

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
Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	none
Water Hardness	104 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS
Remarks – Method	As per OECD test guidelines. A limit test only was conducted.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0
100	100	7	0	0	0	0	0

LC50	>100 mg/L at 96 hours
NOEC	>100 mg/L at 96 hours
Remarks – Results	All validity criteria were met, dissolved oxygen was maintained at >60% and the concentration of the test substance was maintained at >80% of the nominal concentration.
CONCLUSION	The test substance is not harmful to fish
TEST FACILITY	Harlan (2014e)

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	240 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	As per OECD test guidelines. A positive control using potassium dichromate was also conducted.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	20	0	0
100	100	20	0	0

LC50	>100 mg/L at 48 hours
NOEC	>100 mg/L at 48 hours

Remarks – Results	All validity criteria were met. The test pH was maintained within ±1.5 units, the dissolved oxygen was maintained at >3 mg/L in all vessels and the temperature was maintained at 20°C ± 1°C. The positive control test showed an EC50 of 1.21 mg/L which is within the expected range for potassium dichromate.
CONCLUSION	The test substance is not harmful to invertebrates.
TEST FACILITY	Guangdong Detection Center of Microbiology (2017c)

C.2.3. Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE

METHOD	OECD TG 211 *Daphnia magna* Reproduction test
Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	None
Analytical Monitoring	HPLC-MS
Remarks – Method	As per OECD test guidelines. No deviations were noted.

Test substance loading (mg/L)	Survival (% parental generation)	Mean no. offspring per female	Number of dead young	Number of unhatched eggs
Control	90	130	0	0
1	100	120	0	0
3.2	90	126	0	0
10	80	125	0	0
32	90	129	0	0
100	90	121	0	0

NOEC	>100 mg/L
EC50	>100 mg/L
Remarks – Results	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.
CONCLUSION	The test substance is not harmful to invertebrate reproduction.
TEST FACILITY	Harlan (2014f)

C.2.4. Algal Growth Inhibition Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation No 440/2008 C.3 Algal Inhibition Test
Species	
Exposure Period	72 hours
Concentration Range	Nominal: 10 - 160 mg/L
Auxiliary Solvent	None
Analytical Monitoring	HPLC-MS
Remarks – Method	As per OECD test guidelines. A positive control test was run using potassium dichromate ($K_2Cr_2O_7$).

RESULTS

Yield *EyC50*	*Growth rate* *ErC50*
130 mg/L	> 160 mg/L

Remarks – Results	All validity criteria were met. The growth factor in the control culture was > 16, the coefficient of variation was 9% for section specific growth rate and 1% for average growth rate. The positive control sample showed an ErC50 of 1.2 mg/L which is within the expected range for potassium dichromate. It is noted that there was some contamination of the control test (0.00019 mg/L) however due to the low concentration of contamination and validity criteria being met, this is not considered to have negatively impacted the study.
CONCLUSION	The test substance is not harmful to algal growth.
TEST FACILITY	Harlan (2014g)

C.2.5. Inhibition of Microbial Activity

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 3.0 - 315 mg/L Actual: 3.0 - 315 mg/L
Remarks – Method	As per OECD test guidelines. No significant deviations from the guidelines were noted. A reference test using 3,5-dichlorophenol was conducted.
RESULTS	
IC50	>315 mg/L
NOEC	>315 mg/L
Remarks – Results	All validity criteria were met. Oxygen uptake rate was >20 mg/L and the EC50 of 3,5-dichlorophenol was within the expected range (EC50 = 12.3).
CONCLUSION	Test substance was not harmful to microbial respiration at the levels tested.
TEST FACILITY	Smithers Vincent (2017d)

C.2.6. Fish Early Life-Stage Toxicity Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 210 Fish, Acute Toxicity Test –Semi-static
Species	*Pimephales promelas* (Fathead Minnow)
Exposure Period	33 Days
Auxiliary Solvent	None
Water Hardness	145 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	As per OECD test guidelines. No deviations were noted.

RESULTS

Nominal concentration	Measured concentration	Number exposed	Number hatched	Number surviving to termination	Mean total length (mm)	Mean wet weight (mg)	Mean dry weight (mg)
Control	0	80	70	61	24.6 ± 0.44	122 ± 7.1	23.9 ± 2.0
10	10	80	73	65	24.6 ± 0.21	126 ± 4.2	24.1 ± 1.2
20	20	80	73	66	24.5 ± 0.37	124 ± 4.1	23.8 ± 1.0
40	41	80	79	68	24.4 ± 0.42	118 ± 3.9	22.3 ± 1.1
80	82	80	63	57	24.7 ± 0.62	130 ± 8.6	25.6 ± 1.5
160	167	80	72	63	23.4 ± 0.68*	110 ± 6.5*	22.0 ± 0.94

* Indicates a statistically significant difference in mean total length and wet weight from the control

NOEC	82 mg/L at 33 Days
LOEC	167 mg/L at 33 Days
EC20	>167 mg/L at 33 days for all measurements.
Remarks – Results	All validity criteria were met, dissolved oxygen was maintained at >60%, water temperature was maintained at 25°C ± 1°C, the concentration of the test substance was maintained at ±20% of the nominal concentration. In the control group 87% of the embryos hatched and >75% of larval survival was achieved.
CONCLUSION	The test substance is not harmful to the early life-stage of fish.
TEST FACILITY	EAG Laboratories (2016)

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