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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

EXP1313100

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1584	IMCD Australia Limited	EXP1313100	ND*	< 50 tonnes per annum	Component of industrial engine lubricants

* ND = Not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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

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

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

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure during handling of products containing the notified chemical:
  - Enclosed and automated systems, where possible

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

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

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

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

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

Disposal

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

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of industrial engine lubricants, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

(Material) Safety Data Sheet
The (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
IMCD Australia Limited (ABN: 44 000 005 578)
1st Floor, 372 Wellington Road
MULGRAVE VIC 3170

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for dissociation constant, particle size, flash point, explosive properties, oxidising properties and skin sensitisation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
US, EU and Canada

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
EXP1313100

MOLECULAR WEIGHT
> 500 Da and < 2,000 Da

ANALYTICAL DATA
Reference ICP, NMR, IR, HPLC, LC-MS, GC, UV-Vis spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 80%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: amber crystalline solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Decomposes at 150 °C prior to melting	Measured
Boiling Point	Decomposes at 150 °C prior to boiling	Measured
Density	1,010 kg/m^3 at 20 °C	Measured
Vapour Pressure	< 1.5 × 10^{-6} kPa at 20 °C < 9.6 × 10^{-6} kPa at 25 °C	Measured
Water Solubility	2 × 10^{-5} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains no hydrolysable functionalities.
Partition Coefficient (n-octanol/water)	log Pow > 7.7 at 20 °C	Measured; expected to partition to phase boundaries based on surface activity

Property	Value	Data Source/Justification
Adsorption/Desorption	log K_{oc} = 4.45	Measured; expected to adsorb to soil and sediment based on low water solubility and surface activity
Dissociation Constant	Not determined	Expected to be ionised under environmental conditions (pH 4-9)
Particle Size	Not determined	The notified chemical is a non-granular crystalline solid and will be introduced as solutions in base oil.
Flammability	Not highly flammable	Measured
Autoignition Temperature	Not self-ignitable	Measured
Explosive Properties	Predicted negative	Based on the chemical structure
Oxidising Properties	Predicted negative	Based on the chemical structure and oxygen balance

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia as a component of additive packages of engine lubricants at < 1% concentration for reformulation or as a component of finished engine lubricants at < 0.5% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 50	< 50	< 50	< 50	< 50

PORT OF ENTRY
Australian ports

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of lubricant additive packages (at < 1% concentration) or as a component of finished lubricants (at < 0.5% concentration) in 205 L drums or bulk vessels including isotainers. The products containing the notified chemical will then be transported by road to distributor warehouses or customers' blending sites. Finished lubricants in drums or road tankers will be transported by road to end-users.

USE
The notified chemical will be used as a component of engine lubricants at < 0.5% concentration in industrial applications.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. The imported additive packages containing the notified chemical (at < 1% concentration) will be reformulated after importation.

Reformulation
At the customers' facilities, it is expected that the additive packages containing the notified chemical at < 1% concentration will be transferred into blending tanks using automated, enclosed and well-ventilated processes. After blending, it is expected that the end-use product containing the notified chemical at < 0.5% concentration

will be packaged using automated processes. The resulting engine lubricant products will be supplied to industrial end users.

End use
Engine lubricant products containing < 0.5% of the notified chemical will be used by industrial sites such as truck stops and fleet maintenance garages where the engine lubricants are expected to be pumped from the drums. The engine lubricants will remain in the engines until next oil change. Used oil will be captured for recycling or disposal.

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)*
Goods receipt	only during spillage	30
Transportation	only during spillage	30
Blending facilities operator	~ 10 mins	12
Quality assurance workers, maintenance workers	< 10 mins	12
End users operators	< 10 mins	200

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical at < 1% concentration in either the imported or the end use products only in the unlikely event of accidental rupture of containers.

Reformulation
Dermal and ocular exposure of workers to the notified chemical (at < 1% concentration) may occur when connecting and disconnecting hoses and during sample testing. The exposure should be limited as the blending and packaging processes are expected to be automated and within a closed system.

Dermal and ocular exposure to workers should be further mitigated through the use of personal protective equipment (PPE) including protective clothing, impervious gloves, goggles and safety shoes, as anticipated by the notifier. Inhalation of vapour is unlikely given the chemical's low vapour pressure. Although oil mists may be generated, exposure is expected to be limited due to the enclosed nature of the blending operation.

End-use
At automotive service sites, professional users such as mechanics may experience dermal or ocular exposure to the engine lubricant products containing the notified chemical at < 0.5% concentration when transferring engine lubricants to vehicles. The potential for dermal and ocular exposure may be mitigated through the use of PPE. Inhalation exposure is not expected given that aerosols are not likely to be generated and the notified chemical has a low vapour pressure.

6.1.2. Public Exposure
The notified chemical will be used in industrial settings only and will not be made available to the public. Public exposure to the notified chemical is not expected.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation (*in vitro*)	non-irritating
Eye irritation (*in vitro*)	no prediction on eye irritancy classification

Endpoint	*Result and Assessment Conclusion*
Eye irritation (*in vitro*) (10%)	no prediction on eye irritancy classification
Rabbit, eye irritation (50%)	slightly irritating
Guinea pig, skin sensitisation – non-adjuvant test*	no evidence of sensitisation
Rat, repeat dose oral/dermal toxicity – 14 days (dose range finding)	oral: no toxicologically relevant findings dermal: generally mild local effects
Rat, repeat dose oral toxicity – 28 days	NOAEL = 300 mg/kg bw/day
Rat, repeat dose dermal toxicity – 28 days	local NOEL = 50 mg/kg bw/day (males) local NOAEL = 150 mg/kg bw/day (males) = 50 mg/kg bw/day (females) systemic NOAEL = 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non genotoxic
Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test	non genotoxic

* Tested on the analogue

Toxicokinetics
No information on toxicokinetics of the notified chemical was provided. Based on the water solubility (2×10^{-5} g/L at 20 °C), partition coefficient (log Pow > 7.7) and relatively high molecular weight (> 500 Da) of the notified chemical, passive diffusion of the notified chemical across the skin is expected to be limited.

Acute toxicity
The notified chemical was found to be of low toxicity via the oral and dermal routes in studies conducted in rats.

Irritation
The notified chemical was found to be non-irritating to the skin in an *in vitro* study conducted using the reconstructed human epidermis model. However, in an acute dermal toxicity study, a dose range finding study for repeated dose dermal toxicity and subsequent repeated dose dermal toxicity study, the notified chemical showed skin irritation effects to test animals.

In two *in vitro* bovine cornea opacity and permeability studies for the notified chemical at 100% concentration and 10% concentration respectively, no prediction on the eye irritancy classification could be made. No formal interpretation was done in a study conducted in rabbits due to the notified chemical in the neat form (solid) fell out of eye immediately following releasing the eyelids after instillation resulting in insufficient eye exposure. In a further study conducted in rabbits for the notified chemical at 50% concentration, the test substance was found to be slightly irritating to eyes.

Sensitisation
No sensitisation data for the notified chemical was provided. A skin sensitisation study carried out in guinea pigs (Buehler Test) using an acceptable analogue (Analogue 1, identify considered as Exempt Information) at 100% induction concentration and 50% challenge concentration showed no evidence of skin sensitisation. This result is consistent with the conclusion of a NICNAS Inventory Multi-tiered Assessment and Prioritisation (IMAP) assessment report (reference in Exempt information) on a group of chemicals (including Analogue 1) having similar structure to the notified chemical.

Repeated dose toxicity
A 14-day oral/dermal toxicity range-finding study on the notified chemical was conducted in rats. The study authors concluded that the oral gavage did not result in toxicologically relevant findings at up to 1000 mg/kg bw/day and the dermal exposure resulted in generally mild local effects on the treated skin area due to irritation.

In a subsequent 28-day repeated dose oral toxicity study, rats were administered via gavage with the notified chemical. The doses selected were 100, 300 and 1000 mg/kg bw/day. No premature deaths occurred during the study and no toxicologically relevant clinical signs were noted. It was considered by the study authors that an adverse effect on liver integrity occurred at the dose level of 1000 mg/kg bw/day, given the observed changes in alanine aminotransferase activity, liver necrosis in one male animal and other supporting clinical biochemistry changes. The No Observed Adverse Effect Level (NOAEL) was thus established as 300 mg/kg bw/day.

A 28-day repeated dose dermal toxicity study was also conducted in rats. The doses applied were 50, 150 and 500 mg/kg bw/day. The NOAEL for systemic toxicity was established as 500 mg/kg bw/day (the highest dose tested) for both sexes, based on an absence of treatment-related adverse effects at all dose levels.

Effects on the treated skin area during treatment were generally moderate to severe at the high dose and primarily included scales, scabs, general erythema, swelling, fissuring and scar formation. Maculate erythema or focal erythema and wounds were also noted at lower incidence. At the mid dose, scales and/or scabs were noted during treatment up to moderate degree. Maculate erythema or focal erythema, scar formation and wounds were also noted for most female animals, with lower incidence in male animals. At the low dose, effects on the treated skin area were all minimal and primarily included scales for both sexes and also scabs for female animals. Maculate erythema or focal erythema was noted at lower incidence. Most of the local effects remained at similar degrees during the 14-day recovery period.

The NOAEL for local toxicity was established as 150 mg/kg bw/day for males, based on moderate to severe effects on the treated skin area noted at the higher dose (500 mg/kg bw/day) and no higher incidence/severity of adverse local effects noted at 150 mg/kg bw/day compared to the controls. For females, the local NOAEL was established as 50 mg/kg bw/day, based on higher incidence/severity of local effects noted at the higher doses (150 mg/kg bw/day and 500 mg/kg bw/day) and no higher incidence/severity of adverse local effects noted at 50 mg/kg bw/day compared to the controls.

The No Observed Effect Level (NOEL) for local toxicity was established as 50 mg/kg bw/day for males, based on that local effects noted at this dose were comparable to the controls. No NOEL for local toxicity was established for females, based on higher incidence/severity of local effects noted at all doses tested compared to the controls.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation study and was not clastogenic in an *in vitro* mammalian chromosome aberration test and an *in vivo* mammalian erythrocyte micronucleus test.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation
Based on available toxicological studies on the notified chemical and an analogue, the notified chemical is expected to be of low acute toxicity and may cause slight eye irritation. The notified chemical may also cause skin irritation effects. Repeated or prolonged exposure to high concentrations of the notified chemical may have the potential to result in adverse liver effects.

6.3.1. Occupational Health and Safety
Dermal and ocular exposure of workers to the notified chemical (at < 1% concentration) during reformulation should be limited by enclosed and automated/semi-automated processes, and by the stated use of PPE including protective clothing, impervious gloves and goggles.

Workers handling the engine lubricants containing the notified chemical may come into contact with the chemical at < 0.5% concentration during engine servicing. However, the exposure is expected to be limited by the use of PPE and the low use concentrations of the notified chemical in the products. Therefore, given the assessed use pattern, the risk to workers is not considered unreasonable.

6.3.2. Public Health
The notified chemical will be used in industrial settings only and will not be made available to the public. Public exposure to the notified chemical is not expected. Therefore, the risk to public health is not considered unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of lubricant additive packages for reformulation into engine lubricant oils, or as a component of finished engine lubricant oils. No significant release of the notified chemical is expected from transportation and storage, except in the unlikely event of accidental spill and leaks.

Local blending and repackaging of the additive containing the notified chemical into engine oils is expected to occur within enclosed automated systems. Blending tanks and equipment are expected to be cleaned with mineral oil, which is expected to be recycled during subsequent blending, or collected for disposal by licensed waste management services. Accidental spills and leaks during transport, normal blending and packaging procedures are expected to be contained and collected for recycling where appropriate, or disposed of in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The finished products containing the notified chemical will be used as a component of automotive engine oils in industrial settings. Release during use may arise from spills when pouring lubricants into engines or from engine leaks, and is expected to be very low. The notifier has indicated that there will be no do-it-yourself (DIY) application of the engine oils containing the notified chemical.

RELEASE OF CHEMICAL FROM DISPOSAL

After reformulation, empty import drums containing residues of the notified chemical are expected to be sent to a container recycling facility for reconditioning. Empty import drums will be washed with mineral oil, and the wastes containing the notified chemical collected for disposal in accordance with local government regulations, most likely to landfill or by licensed waste management services. Therefore, the release of the notified chemical to surface waters from the cleaning of empty drums is expected to be limited.

Products containing the notified chemical including waste oils are expected to be collected for disposal by licensed waste management facilities. Consequently, the notified chemical in engine oils is expected to be recycled or incinerated. Release of the notified chemical from professional activities is expected to be limited by the requirement for appropriate disposal of waste oils in accordance with local government regulations.

7.1.2. Environmental Fate

Based on the results of a biodegradability study, the notified chemical is not expected to be readily biodegradable (13% in 28 days). For details of the environmental fate study, please refer to Appendix C. The notified chemical, however, is not expected to be bioaccumulative based on its high molecular weight and surfactant properties. The majority of the notified chemical in engine oils will be either thermally decomposed during use or recycling. Notified chemical disposed of to landfill is not expected to be mobile nor bioavailable based on its high molecular weight, low water solubility and surfactant properties. In landfill, the notified chemical is expected to eventually degrade by biotic and abiotic processes to form water and oxides of carbon and sulphur.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated, as significant release of the notified chemical to the aquatic environment is not expected based on its reported use pattern as a component of engine oils.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LL50 > 0.0038 mg/L (WAF*)	Not harmful to fish up to water solubility limit (acute)
	32 d NOELR = 0.14 mg/L (WAF*)	Not harmful to fish up to water solubility limit (chronic)
Daphnia Toxicity	48 h EL50 > .0.21 mg/L (WAF*)	Not harmful to aquatic invertebrates up to water solubility limit
Algal Toxicity	72 h EL50 > 7.7 mg/L (WAF*)	Not harmful to algae up to water solubility limit
Inhibition of Bacterial Respiration	3 h IC50 > 1,000 mg/L	Not inhibitory to microbial respiration

* Water Accommodated Fraction

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

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has not been calculated, as the notified chemical is not expected to be harmful to aquatic life up to the limit of its solubility in water, and no significant aquatic release is expected from the reported use pattern.

7.3. Environmental Risk Assessment

A Risk Quotient (RQ = PEC/PNEC) has not been calculated, as no significant release of the notified chemical to the environment is expected from the proposed use pattern. Although the notified chemical is not considered readily biodegradable, it is not expected to be bioaccumulative. On the basis of the assessed use pattern as a component of engine lubricant oils and the expected limited aquatic release, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point No melting temperature

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Differential scanning calorimetry method was used. Slight decomposition of the test substance was noted starting at 150 °C.
Test Facility	WIL (2014a)

Boiling Point No boiling temperature

Method	OECD TG 103 Boiling Point.
Remarks	Differential scanning calorimetry method was used. Slight decomposition of the test substance was noted starting at 150 °C.
Test Facility	WIL (2014a)

Density 1,010 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	A gas comparison stereopycnometer was used.
Test Facility	WIL (2014a)

Vapour Pressure < 1.5× 10^{-6} kPa at 20 °C
< 9.6× 10^{-6} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Isothermal thermogravimetric effusion method was used.
Test Facility	WIL (2014a)

Water Solubility 2 × 10^{-5} g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Column Elution Method
Test Facility	WIL (2014a)

Partition Coefficient (n-octanol/water) log Pow > 7.7 at 20 °C

Method	OECD TG 107 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Estimated from the solubility of the notified chemical in n-octanol and the solubility in water.
Test Facility	WIL (2014a)

Adsorption/Desorption log K_{oc} = 4.45

Method	OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC). EC Council Regulation No 440/2008 C.19 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	HPLC Screening Method. The notified chemical eluted after the reference chemical phenanthrene.
Test Facility	WIL (2014a)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks	Screening test. No propagation of combustion of the test substance along 200 mm of the

pile within 4 minutes was noted.
Test Facility WIL (2014a)

Autoignition Temperature Not self-ignitable

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks No endothermic or exothermic effect of the test substance was noted between 29 °C and 400 °C. The test substance was decomposed and/or reacted during the experiment.
Test Facility WIL (2014a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations were noted. The test substance contains > 10% impurity and no correction was made for the purity. The substance was mixed with the vehicle, heated in a water bath at a maximum temperature of 96 °C for 1 h to obtain homogeneity and dosed at ≤ 40 °C.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	Clinical signs including lethargy, hunched posture and/or piloerection were noted for all animals on Days 1 and/or 2.
Effects in Organs	No abnormalities were noted at macroscopic post mortem examination.
Remarks - Results	All animals showed expected body weight development.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	WIL (2015a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Wistar
Vehicle	Propylene glycol
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations were noted. The test substance contains > 10% impurities and no correction was made for the purity. The substance was mixed with the vehicle, heated to a maximum of 93 °C for 1 h to obtain visual homogeneity and dosed at < 40 °C.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Erythema, fissuring, scales and/or scabs were noted on the treated skin area and/or left flank of all animals. In addition, necrosis of the treated skin area and/or left flank was noted on Days 4-8 for all female animals, with one of them having a wound on the left flank on Days 7 and 8 and scar formation on Days 14 and 15.
Signs of Toxicity - Systemic	None reported
Effects in Organs	No abnormalities were noted at macroscopic post mortem examination.

Remarks - Results	All animals showed expected body weight development.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	WIL (2015b)

B.3. Irritation – skin (*in vitro*)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method - EPISKIN-SM™ Reconstructed 3D Human Epidermis Model
Vehicle	None
Remarks - Method	The test substance contains > 10% impurity and no correction was made for the purity/composition. The solid test substance (10.2-10.9 mg) was ground and spread to the tissues in triplicate (tissues were moistened with Milli-Q water to ensure close contact with the test substance). Following exposure periods of 15 minutes (room temperature), the tissues were rinsed, incubated at 37 °C for 42 hours, treated with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide] and then incubated at 37 °C for 3 hours. In a preliminary test the test substance was shown not to directly reduce MTT. Positive and negative controls were run in parallel with the test substance: - Negative control (NC): phosphate buffered saline - Positive control (PC): 5% sodium dodecyl sulphate in distilled water

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.073	100	0.093
Test substance	1.287	120	0.074
Positive control	0.109	10	0.025

OD = optical density; SD = standard deviation

Remarks - Results	The test substance showed no irritation effects. The positive and negative controls gave satisfactory results, confirming the validities of the test systems.
CONCLUSION	The notified chemical was non-irritating to the skin under the conditions of the *in vitro* test.
TEST FACILITY	WIL (2014b)

B.4. Irritation – eye (*in vitro*)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants
Vehicle	None
Remarks - Method	No significant protocol deviations were noted. The test substance contains > 10% impurity and no correction was made for the purity/composition.

Negative control was physiological saline and positive control was 10% benzalkonium chloride in physiological saline.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Negative control	0	0.000	0.0
*Test substance**	4	0.022	4.3
*Positive control**	85	2.762	126

IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results: The controls gave satisfactory results confirming the validity of the test system. The IVIS of the test substance was > 3 but < 55.

CONCLUSION: No prediction on the eye irritancy classification could be made.

TEST FACILITY: WIL (2014c)

B.5. Irritation – eye (*in vitro*, 10%)

TEST SUBSTANCE: Notified chemical (tested at 10%)

METHOD: OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants

Vehicle: Propylene glycol

Remarks - Method: No significant protocol deviations were noted.

The test substance contains > 10% impurities and no correction was made for the purity/composition. The test substance was tested as a 10% suspension in propylene glycol homogenised at a maximum temperature of 69 °C for 1 h with ultrasonic waves.

Negative control was physiological saline, solvent control was propylene glycol and positive control was 10% benzalkonium chloride in physiological saline.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0	0.063	1.0
Negative control	-1	0.000	-1.0
*Test substance**	4	0.010	4.1
*Positive control**	74	2.476	110.8

IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results: The controls gave satisfactory results confirming the validity of the test system. The IVIS of the test substance at 10% (w/v) was > 3 but < 55.

CONCLUSION: No prediction on the eye irritancy classification could be made.

TEST FACILITY: WIL (2015c)

B.6. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	1
Observation Period	72 hours
Remarks - Method	The study was performed in a stepwise manner and stared by treatment of a single rabbit. The test substance was the notified chemical in the neat form (a solid) with > 10% impurities.
RESULTS	Redness and discharge were noted following the application and the irritation was completely resolved within 24 hours. No iridial irritation or corneal opacity were noted.
Remarks - Results	The test substance fell out of eye immediately after releasing the eyelid after instillation. Therefore, exposure period was considered by the study authors to be not long enough to allow a meaningful interpretation of the eye irritancy. No further rabbits were therefore treated.
CONCLUSION	No formal interpretation of the study results was done.
TEST FACILITY	WIL (2015d)

B.7. Irritation – eye (50%)

TEST SUBSTANCE	Notified chemical (tested at 50%)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	72 hours
Remarks - Method	The study was performed in a stepwise manner and stated by treatment of a single rabbit and 2 other rabbits were treated in a similar manner 1 week later. The test substance contains > 10% impurities and no creection was made for the purity. The test substance was tested as a 50% formulation in propylene glycol. Visual homogeneity of the formulation was obtained using a water bath at a maximum temperature of 96.4 °C for 45 minutes. The formulation was cooled down to 26.4 °C before dosing.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.3	0.3	0	1	< 48 hours	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	0	-	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	Redness, chemosis and discharge were noted following application and the irritation effects were completely resolved within 24 hours in 1 animal and within 48 hours in the other 2 animals. No iridial irritation or corneal opacity were noted.
CONCLUSION	The test substance is slightly irritating to the eye.
TEST FACILITY	WIL (2015e)

B.8. Skin sensitisation

TEST SUBSTANCE	Analogue 1 (identity in Exempt Information)
METHOD	OECD TG 406 Skin Sensitisation – Buehler Test.
Species/Strain	Guinea pig/ albino Hartley
PRELIMINARY STUDY	Concentrations chosen for the main study: Induction – 100% Challenge – 50% in Kaydol white mineral oil
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
Vehicle	Kaydol white mineral oil
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using octanal, 2-(phenylmethylene)- (CAS No. 101-86-0).
INDUCTION PHASE	Induction Concentration: topical: 100%
Signs of Irritation	Grade 1-2 erythema was noted in all treated animals and scaliness was noted in 5 treated animals.
CHALLENGE PHASE	
1st challenge	topical: 50% in Kaydol white mineral oil
2nd challenge	topical: 50% in Kaydol white mineral oil
Remarks - Method	No significant protocol deviations. Very slight skin reactions were noted at 75% and 100% exposure concentrations in the preliminary study and therefore, 100% concentration was selected for the induction phase.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50%	0	3	0	0
Control Group	0%	0	1	0	0

Remarks - Results	Grade 1 skin reactions were noted in 1 treated animal and scaliness was noted in 2 treated animals in the 1st challenge phase. No skin reactions were noted in the 2nd challenge phase.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	WIL (2012)

B.9. Repeat dose toxicity – dose range finding

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD TG 410 Repeated Dose Dermal Toxicity: 21/28-day Study.
Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage Dermal – occluded
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week Duration of dermal exposure: 6 hours/day Post-exposure observation period: none
Vehicle	Propylene glycol
Remarks - Method	The study was a 14-day range-finding test. The test substance contains > 10% impurity and no correction was made for the purity. Evaluated

parameters were clinical signs, body weight, food consumption, macroscopy at termination and organ weights on a selection of tissues.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control (oral)	3M	0	0/3
low dose (oral)	3M	500	0/3
high dose (oral)	3M	1000	0/3
control (dermal)	3M	0	0/3
low dose (dermal)	3M	500	0/3
high dose (dermal)	3M	1000	0/3

Mortality and Time to Death

No test substance related deaths occurred during the study.

Clinical Observations

Oral exposure

No toxicologically relevant clinical signs were noted. Food consumption and organ weights were considered by the study authors to have been unaffected by the treatment. A slight decrease in body weights and body weight gains was considered by the study authors to be of no toxicological relevance.

Dermal exposure

Increased incidence of erythema, scales and scabs was noted on the treated skin area for all treated animals from Day 3 onwards, compared to the control group. A reversible fissuring was noted in 1 animal at 1000 mg/kg bw/day between Days 4 and 8. Hunched posture was shown by 1 animal at 500 mg/kg bw/day and all animals at 1000 mg/kg bw/day. Body weights and food consumption were considered by the study authors to have been unaffected by the treatment.

Effects in Organs

Oral or dermal exposure

No toxicologically relevant macroscopic lesions were noted. Organ weights were considered by the study authors to have been unaffected by the treatment.

CONCLUSION

Oral exposure did not result in toxicologically relevant findings at up to 1000 mg/kg bw/day and dermal exposure resulted in generally mild local effects.

TEST FACILITY WIL (2015f)

B.10. Repeat dose toxicity - oral

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations were noted. The test substance contains > 10% impurities and no correction was made for the purity. Visual homogeneity of the formulation was obtained at a maximum temperature of 95.5 °C for 75 minutes. The formulation was cooled down to < 40 °C before dosing.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0/10
low dose	5 per sex	100	0/10
mid dose	5 per sex	300	0/10
high dose	5 per sex	1000	0/10
control recovery	5 per sex	0	0/10
high dose recovery	5 per sex	1000	0/10

Mortality and Time to Death

No premature deaths occurred during the study.

Clinical Observations

No toxicologically relevant clinical signs were noted.

Food consumption was unaffected. There were no treatment-related changes in functional observation. Body weight gain was lower in male animals of the high dose group during the treatment and at initiation of the recovery phase in both sexes of the high dose group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

In the high dose group, increased alanine aminotransferase activity (with a factor of approximately 12 and 17 for male and female animals respectively) was noted for both sexes and higher alkaline phosphatase activity was noted for male animals. In combination with an observation of multifocal necrosis of liver in a male of the group, these laboratory findings were considered to support hepatocellular damage in the test animals. Other treatment-related changes noted included a lower total protein, albumin, cholesterol and calcium levels and higher total bilirubin and bile acid levels in both sexes, and lower creatinine level in male animals. At the end of the recovery period, only total protein level remained lower in the males of the group.

Clinical biochemistry changes, including a higher alanine and aspartate aminotransferase activity, lower total protein, albumin and calcium levels, higher total bilirubin and bile acid levels for the mid dose group and lower total protein, calcium and albumin levels and higher total bilirubin level for the low dose group were not supported histopathologically and absent at the end of recovery period. Therefore these changes were not considered by the study authors to be adverse in nature.

Higher white cell counts in both sexes of the high dose group were noted at the end of treatment. For males, the higher white blood cell counts were due to high values of two test animals including the one with multifocal liver necrosis. Hence, this change was considered to be toxicologically significant. For females, the change of white blood cell counts was not considered by the study authors to be toxicologically relevant as the individual values remained within the range considered normal for rats of this age and strain.

Effects in Organs

One male animal of the high dose group showed a sight multifocal necrosis of the liver, which was considered by the study authors to be treatment-related. Multifocal necrosis of liver was absent at the end of the recovery period.

Higher relative kidney weights and lower absolute/relative prostate and seminal vesicle weights were noted in male animals of the high dose group. Lower absolute/relative heart and thymus weights and higher relative spleen weight were noted in female animals of the group at the end of the treatment. All these organ weight changes were similar to the control group at the end of the recovery period, except for relative kidney weights in male animals that remained slightly higher.

Remarks – Results

It was considered by the study authors that an adverse effect on liver integrity occurred at the high dose, based on the presence of multifocal necrosis of liver in one male and the magnitude of change of primarily alanine aminotransferase activity in combination with other clinical biochemistry changes that point to a disturbance in liver function.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 300 mg/kg bw/day in this study, based on the treatment-related effects on liver function at the dose level of 1000 mg/kg bw/day.

TEST FACILITY WIL (2015g)

B.11. Repeat dose toxicity - dermal

TEST SUBSTANCE Notified chemical

METHOD OECD TG 410 Repeated Dose Dermal Toxicity: 21/28-day Study.

Species/Strain	Rat/Wistar
Route of Administration	Dermal – occluded
Exposure Information	Total exposure days: 28 days Dose regimen: 6 hours per day, 7 days per week Post-exposure observation period: 14 days
Vehicle	Propylene glycol
Remarks - Method	No significant protocol deviations were noted. The test substance contains > 10% impurities and no correction was made for the purity/composition. Visual homogeneity of the formulation was obtained at a maximum temperature of 96 °C for 1 hour. The formulation was cooled down to < 58.3 °C before use.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	1/10
low dose	5 per sex	50	1/10
mid dose	5 per sex	150	0/10
high dose	5 per sex	500	0/10
control recovery	5 per sex	0	1/10
high dose recovery	5 per sex	500	0/10

Mortality and Time to Death
Two control female animals and 1 female animal of the low dose group were found dead during or immediately after the 6-hour application period, after 14, 20 and 8 days of treatment respectively. The deaths were considered by the study authors to be not treatment-related, based on that the mortality showed no relationship to the dose and occurred also in the control groups. No cause of death could be established histopathologically.

Clinical Observations

Local findings
A range of findings in the treated skin area was noted across all dose groups during the treatment. The incidence, severity and number of these findings showed an apparent dose-related trend.

At the high dose, effects on the treated skin area during treatment were generally moderate to severe and primarily included scales, scabs, general erythema, swelling, fissuring and scar formation. Maculate or focal erythema and wounds were also noted.

At the mid dose, scales and/or scabs were noted during treatment up to moderate degree. Maculate or focal erythema, scar formation and wounds were also noted for most female animals, with lower incidence in male animals.

At the low dose, effects on the treated skin area were all minimal and primarily included scales for both sexes and scabs for female animals. Maculate or focal erythema was noted at lower incidence for females.

Most of the local effects remained at similar degrees during the recovery period.

Findings in the treated skin area of control animals included minimal degrees of scales and/or scabs in several

animals, supported by background levels of histopathological effects including minimal or slight degrees of hyperkeratosis, ulceration and epidermal hyperplasia. These findings were considered by the study authors to be related to the slightly irritating properties of the vehicle propylene glycol combined with the wrapping procedure.

Non-local findings
Incidental cases of piloerection, uncoordinated movements, abnormal or hunched posture, diarrhoea, restless behaviour and/or pallor noted in animals of mid and high dose groups were considered by the study authors to be related to the burden of dermal treatment in combination with local effects, rather than being representative of systemic toxicity.

A dose-related lower body weight and body weight gain was noted for male animals of all treatment groups, being adverse in nature for high dose group where weight gain was approximately 60% lower than controls. This weight loss was considered by the study authors to be due to the local effects noted on the treated skin. Body weights of female groups remained unaffected.

Female animals of the high dose group showed a lower motor activity at the end of the treatment period, which was considered by the study authors to be secondary to the local skin effects and had resolved at the end of the recovery period. Male animals did not show any apparent treatment-related changes in motor activity.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
At the high dose, higher alanine and aspartate aminotransferase activity and lower albumin and creatinine in both males and females, and lower glucose in males were recorded. At the low and mid doses, higher alanine aminotransferase activity and lower glucose in males and/or females were also noted. At the end of the recovery, in the high dose recovery group higher alanine aminotransferase activity and lower albumin remained present in males and females respectively. Alkaline phosphatase activity was increased in females in this group which was not seen at the end of the treatment. These statistically significant clinical biochemistry changes showed no apparent correlation to the histopathological lesions and were not considered by the study authors to be toxicologically relevant.

Changes in (differential) white blood cell counts in all dose groups were recorded and considered to have occurred secondary to the observed skin effects. These changes in (differential) leucocyte counts had largely recovered at the end of the recovery.

Effects in Organs

Local findings
Histopathological examination of the treated skin showed increased incidence and/or severity of hyperkeratosis, epidermal hyperplasia, exudative inflammation, ulceration, inflammatory infiltrate of the dermis and/or fibrosis in male animals of the mid and high dose groups and in female animals staring at the low dose, indicative of severely irritating properties of the test substance. Necropsy findings included scales, scabs, sores, and scars correlated to several of these histopathological changes. No recovery for these histopathological changes was noted for the high dose group after the recovery period. These histopathological findings were considered by the study authors to be adverse in both sexes of the high dose group and in female animals of the mid dose group. Local non-adverse treatment-related microscopic findings were present in the treated skin of female animals of the low dose group and in male animals of the mid dose group.

Systemic findings:
Adrenal glands: Vacuolar degeneration was recorded for the adrenal gland of a few females at the mid and high doses at the end of treatment at a minimal or slight degree. Based on the low incidence and severity of the change, the reversibility after the recovery and the absence of a clinically apparent effect on the well-being of the animals, the vacuolar degeneration was considered by the study authors to be non-adverse.

Inflammatory infiltrate in the adrenal cortex was reported in several females at the mid and high doses at a minimal severity and showed partial recovery for the high dose group. This lesion was considered to be a non-adverse microscopic finding.

Spleen: Increased haematopoiesis was seen in females at mid and high doses with complete recovery. This microscopic finding was considered by the study authors to be an adaptive change and reflected an increased erythropoiesis to compensate for a decrease in erythrocytes as recorded for the females at the high dose. Red

blood counts were marginally lower than the control group at the end of the treatment. Other haematological changes in females at the high dose consisted of higher reticulocyte counts, red cell distribution width and mean corpuscular volume. At the end of the recovery, lower red blood cell counts and haemoglobin remained for females, while the splenic haematopoiesis had fully recovered. This finding in the spleen with associated haematological changes was regarded by the study authors to be non-adverse.

At the end of the treatment, haematological changes in male dose groups included lower red blood cell counts, haemoglobin and haematocrit at high dose and lower haematocrit at mid dose. At the end of recovery, lower haematocrit and red blood cell count, and higher reticulocyte counts and red cell distribution width were noted. These changes were not supported by histopathological changes and were not considered toxicologically relevant by the study authors.

Lacrimal glands: Increased incidence and/or severity (up to slight degree) of glandular vacuolation and/or hypertrophy with karyomegaly was seen in males and females at mid and high doses and Harderian alteration was seen in males at high dose. At necropsy, this observation was supported by enlarged exorbital lacrimal glands in a few animals at mid dose and most animals at high dose (as well as a single male case at low dose without microscopic correlation). There was no recovery for these findings. Glandular vacuolation at a minimal degree could be seen as a background finding and the slight increase in incidence and severity as seen in the study was therefore not considered by the study authors to be adverse. For the Harderian alteration and the hypertrophy with karyomegaly, the exact pathogenesis and toxicological significance could not be determined and were not regarded by the study authors to be precursor findings.

The above mentioned systemic microscopic findings in adrenal gland, spleen and exorbital lacrimal gland were not recorded in the repeated dose oral 28-Day rat study (see Appendix B.10).

Other findings

Draining lymph nodes: lymphoid hyperplasia in both sexes starting at low dose was considered by the study authors to be secondary to the degenerative and inflammatory changes in the treated skin and was not adverse in nature.

Thymus: Minimal to moderate degree of increased lymphocytosis and minimal lymphoid depletion noted in treated females were considered by the study authors to be secondary to stress resulting from treatment with an irritating test substance combined with the wrapping procedure and not directly treatment-related.

Adrenal glands: An increase in adrenal gland weight and microscopic adrenocortical hypertrophy were considered by the study authors as a result of treatment stress and not directly treatment-related.

Liver: Coagulative/lobar necrosis, capsular fibrosis and/or yellow-brown pigmentation, supported at necropsy by a combination of reduces/increased size of lobes, discoloration, hardening and/or adhesions, were considered by the study authors to be procedure-related and not test-substance-related.

Remarks – Results

The microscopic findings in the adrenal cortex, combined with the organ weight change, suggested a stress response with possible superimposed test substance-related effects on the adrenal cortex. The reduced thymus weight and increased adrenal gland weight in females suggested that stress occurred in the study. The study authors suggested that when adrenal effects occur in a study in conjunction with other markers of stress response, it could not be excluded that stress may have contributed to the occurrence of these findings.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for local toxicity was established as 150 mg/kg bw/day for males in this study, based on moderate to severe effects on the treated skin area noted at higher dose (500 mg/kg bw/day) and no higher incidence/severity of adverse local effects noted at 150 mg/kg bw/day, compared to the controls.

The No Observed Effect Level (NOEL) for local toxicity was established as 50 mg/kg bw/day for males, based on that local effects noted at this dose were comparable to the controls.

The NOAEL for local toxicity was established as 50 mg/kg bw/day for females in this study, based on higher incidence/severity of local effects noted at higher doses (150 mg/kg bw/day and 500 mg/kg bw/day) and no higher incidence/severity of adverse local effects noted at 50 mg/kg bw/day, compared to the controls.

No NOEL for local toxicity was established for females, based on higher incidence/severity of local effects than controls were noted at all doses tested.

The NOAEL for systemic toxicity was established as 500 mg/kg bw/day, the highest dose tested, for both sexes, based on an absence of treatment-related adverse effects at all dose levels.

TEST FACILITY WIL (2015h)

B.12. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure

Species/Strain Preliminary test:
S. typhimurium TA100 and *E. coli* WP2uvrA

Main test 1:
S. typhimurium TA1535, TA1537 and TA98

Main test 2:
S. typhimurium TA1535, TA1537, TA98, TA100 and *E. coli* WP2uvrA

Metabolic Activation System S9 mix prepared using Aroclor 1254 induced rat liver

Concentration Range in Main Test a) With metabolic activation: 52-5000 µg/plate
b) Without metabolic activation: 52-5000 µg/plate

Vehicle Dimethyl sulfoxide (DMSO)

Remarks - Method The test substance contains > 10% impurities and no correction was made for the purity/composition.

Tests with vehicle control and positive controls were run concurrently. Positive controls were:
- With metabolic activation: 2-aminoanthracene
- Without metabolic activation: sodium azide (TA1535); ICR-191 (TA1537), 2-nitrofluorene (TA98), methylmethanesulfonate (TA100), and 4-nitroquinoline-1-oxide (WP2uvrA)

RESULTS

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

Remarks - Results In both tests, no significant increases in the frequency of revertant colonies were observed in the presence or absence of metabolic activation.

In strain TA1535, fluctuations in the number of revertant colonies above the historical control data range were observed in the absence of metabolic activation at the dose levels of 164 and 5000 µg/plate. Since the increases reached a maximum of 1.1 fold and were less than 3 folds, they were not considered by the study authors to be relevant.

The positive and negative controls gave a satisfactory response confirming the validity of the test system.

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

TEST FACILITY WIL (2014d)

B.13. Genotoxicity – *in vitro*

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test.

Species/Strain	Human peripheral lymphocytes
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 mix prepared from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks - Method	The test substance contains > 10% impurities and no correction was made for the purity/composition.

A dose range-finding study was carried out at 5.4-512 µg/mL (3 h exposure) and 1.7-1600 µg/mL (24 h or 48 h exposure). The dose selection for the main experiments was based on the solubility and results from the preliminary study.

Vehicle and positive controls (mitomycin C and cyclophosphamide) were run concurrently with the notified chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	52*, 164*, 512*	3 h	24 h
Test 2	50, 75*, 100*, 125*, 150, 175, 200	24 h	24 h
Test 3	50*, 75*, 100*, 125, 150, 175, 200	48 h	48 h
Present			
Test 1	52*, 164*, 512*	3 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 512	> 512	≥ 512	negative
Test 2	≥ 164	≥ 125	≥ 200	negative
Test 3	≥ 164	≥ 100	≥ 200	negative
Present				
Test 1	> 512	> 512	≥ 512	negative

Remarks - Results In the tests, no effect of the test substance on the number of polyploidy cells and cells with endoreduplicated chromosomes were noted in the presence or absence of metabolic activation.

CONCLUSION The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY WIL (2014e)

B.14. Genotoxicity – *in vivo*

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Vehicle	Propylene glycol
Remarks - Method	No significant deviations of protocol were noted. The test substance contains > 10% impurity and no correction was made for the purity/composition. The selection of the highest dose for the main test was based on a range-finding study. Cyclophosphamide (CP) was used as a positive control.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
vehicle control	5M	0	24
low dose	5M	500	24
mid dose	5M	1000	24
high dose 1	5M	2000	24
high dose 2	5M	2000	48
positive control (CP)	5M	20	48

CP=cyclophosphamide.

RESULTS	
Doses Producing Toxicity	No premature death occurred. No treatment-related clinical signs were noted. There was no evidence of cytotoxicity in any treatment groups based on the comparison of the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) ratios between the treatment groups and the negative control group.
Genotoxic Effects	There was no biologically relevant increase in the mean frequency of micronucleated PCEs.
Remarks - Results	The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* mammalian erythrocyte micronucleus test.
TEST FACILITY	WIL (2014f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	29 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Carbon Dioxide ($ThCO_2$)
Remarks - Method	The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Test substance		*Toxicity control*		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
6	1	6	10	6	29
14	11	14	28	14	74
22	13				
29	13				

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound surpassed the threshold level of 60% by 11 days (64%) and reached 74% degradation by 14 days. Therefore, the test indicates the suitability of the inoculums. The percentage degradation of the toxicity control surpassed the threshold level of 25% by 14 days (28%), indicating that toxicity was not a factor inhibiting the biodegradability of the test substance. The test substance attained 13% degradation by 28 days. Therefore, the test substance is not considered to be readily biodegradable according to the OECD (301 B) guideline.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	WIL (2014g)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static.
Species	*Cyprinus carpio* (carp)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	UPLC
Remarks – Method	The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 2 days. Any undissolved material was removed by membrane filtration. The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*¾ h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
100	0.0038	7	0	0	0	0	0

LL50 > 0.0038 mg/L (WAF) at 96 hours
NOEL Not determined
Remarks – Results All validity criteria for the test were satisfied. The test solutions were not renewed during the 96 h test period. The actual concentrations of the test substance were measured at the start and end of the 96 h test period. No abnormalities in behaviour or appearance were observed. The 96 h LL50 for fish was determined to be > 0.0038 mg/L (WAF), based on measured concentrations.

CONCLUSION The notified chemical is not considered to be harmful to fish up the limit of its water solubility.

TEST FACILITY WIL (2014h)

C.2.2. Chronic toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD TG 210 Fish, Early-life Stage Toxicity Test – Semi-static.
Species *Pimephales promelas* (Fathead minnow)
Exposure Period 32 days
Auxiliary Solvent None
Water Hardness 180 mg $CaCO_3$/L
Analytical Monitoring UPLC
Remarks – Method The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 10 mg/L was prepared by stirring the test substance in water for 2 days. Any undissolved material was removed by membrane filtration. The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Concentration mg/L		*Number of eggs*	*Mortality post-hatch (%)*				
Nominal	*Actual*		*0 d*	*8 d*	*16 d*	*24 d*	*32 d*
Control	Control	80	0	8.75	11.25	12.5	16.25
1	0.015	80	0	5	8.75	8.75	10
10	0.14	80	0	7.5	8.75	11.25	12.5

NOELR 0.14 mg/L (WAF) at 32 days.
Remarks – Results All validity criteria for the test were satisfied. The test solutions were renewed every 48 hours during the test period. The actual concentrations of the test substance were measured at the start of each test solution renewal and at the end of the test period. No significant abnormalities in behaviour or appearance were observed between the control and test groups. The 32 d post-hatch NOELR for fish was determined to be 0.14 mg/L, based on measured concentrations.

CONCLUSION The notified chemical is not considered to be harmful to fish on a chronic basis up to the limit of its water solubility.

TEST FACILITY WIL (2015i)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified 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	180 mg $CaCO_3$/L
Analytical Monitoring	UPLC
Remarks - Method	The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 2 days. Any undissolved material was removed by membrane filtration. A total of 20 daphnids were used. The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
100	0.21	20	0	0

EL50	> 0.21 mg/L (WAF) at 48 hours
NOEL	Not determined
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. The actual concentrations of the test substance were measured at the start and end of the 48 h test period. No immobilisation or abnormalities in behaviour or appearance were observed. The 48 h EL50 was determined to be > 0.21 mg/L (WAF), based on measured concentrations.
CONCLUSION	The notified chemical is not considered to be harmful to aquatic invertebrates up to the limit of its water solubility.
TEST FACILITY	WIL (2014i)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 1-100 mg/L Actual: 0.27-7.7 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	UPLC
Remarks - Method	The test substance was prepared as a Water Accommodated Fraction (WAF) due to its low water solubility. A stock solution with a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 2 days. Any undissolved material was removed by membrane filtration. The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported.

RESULTS

Biomass		*Growth*	
E_bL50	*NOEL*	*E_rL50*	*NOEL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 7.7	7.7	> 7.7	7.7

Remarks - Results: All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. The actual concentrations of the test substance were measured at 0, 24 and 72 hours of the 72 h test period. No effects were observed. The 72 h E_bL50 and E_rL50 were both determined to be > 7.7 mg/L (WAF), based on measured concentrations. The corresponding NOEL was determined to be 7.7 mg/L (WAF).

CONCLUSION: The notified chemical is not considered to be harmful to algae up to the limit of its water solubility.

TEST FACILITY: WIL (2014j)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test – Carbon and Ammonium Oxidation.

Inoculum: Activated sludge
Exposure Period: 3 hours
Concentration Range: Nominal: 10-1,000 mg/L (loading rate)
Actual: Not determined
Remarks – Method: The test was conducted in accordance with the test guideline above, with no significant deviation in protocol reported. 3,5-Dichlorophenol was used as the reference control. The respiration rate was determined by measurement of Biological Oxygen Demand during the test after 3 hours of exposure.

RESULTS

IC50: > 1,000 mg/L at 3 hours
NOEC: 1,000 mg/L at 3 hours
Remarks – Results: All validity criteria for the test were satisfied. The 3 h IC50 and NOEC were determined to be > 1,000 mg/L and 1,000 mg/L, respectively, based on nominal concentrations.

CONCLUSION: The notified chemical is not inhibitory to microbial activity.

TEST FACILITY: WIL (2014k)

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