File No.: STD/1721

June 2020

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Diurea2

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 Agriculture, Water and 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 on our website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1721	Cintox Australia Pty Ltd	Diurea2	ND*	≤ 20 tonnes per annum	Component of industrial 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.

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

Hazard Classification	*Hazard Statement*
Chronic toxicity (Category 4)	H413 - May cause long lasting harmful effects to aquatic life

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
Based on the low hazard and reported use pattern, 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 safe work practices to minimise occupational exposure to the notified chemical during application 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 during application of products containing the notified chemical:
  - Impervious gloves
  - Protective clothing

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

- A copy of the 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.

Emergency procedures

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

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.

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 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.

Safety Data Sheet
The SDSs of products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the SDSs remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details exempt from publication include: chemical name, other name(s), CAS number, molecular and structural formulae, molecular weight, analytical data, use concentration, import volume and analogue identity.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for vapour pressure, water solubility, hydrolysis as a function of pH, partition coefficient, absorption/desorption, dissociation constant, particle size, explosive properties, oxidising properties, all toxicological endpoints (except eye irritation) and all ecotoxicological endpoints.

NOTIFICATION IN OTHER COUNTRIES
Europe and Taiwan

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Diurea2

MOLECULAR WEIGHT
> 500 g/mol

ANALYTICAL DATA
Reference NMR, IR, MS, HPLC-MS and UPC-MS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
100%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Light brown solid

Property	*Value*	*Data Source/Justification*
Melting Point	> 250 °C	Measured
Density	1,060 kg/m^3 at 20 °C	Measured
Vapour Pressure	Not measured	Expected to be low based on the relatively high molecular weight. Calculated vapour pressure (using US EPA, 2012) = 4.77 x 10^{-17} – 5.47 x 10^{-14} Pa
Water Solubility	Not measured	Expected to be highly insoluble. Calculated water solubility (using US EPA, 2012) = 6.516 x 10^{-10} – 7.935 x 10^{-6} mg/L
Hydrolysis as a Function of pH	Not measured	Contains hydrolysable functional groups but hydrolysis is not expected under environmental conditions (pH 4-9).

Property	*Value*	*Data Source/Justification*
Partition Coefficient (n-octanol/water)	Not measured	Insoluble in water. Calculated Log Kow (US EPA, 2012) = 9.25 – 13.18
Adsorption/Desorption	Not measured	Insoluble in water. Calculated Log Koc (US EPA, 2012) = 6.6 – 8.1
Dissociation Constant	Not measured	No dissociable functionality
Particle Size	Not determined	Will be imported and used in grease products
Flammability	Not highly flammable	Measured
Autoignition Temperature	395 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidative properties

DISCUSSION OF PROPERTIES
For 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 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 not be manufactured or reformulated in Australia. It will be imported into Australia as a component of finished lubricants at ≤ 15% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	12	12	12	15	20

PORT OF ENTRY
Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
Cintox Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia in finished lubricants in sealed aluminium cartridges (400 g), plastic tubs (0.25 L), plastic pails (18 kg) and metal drums (180 kg). The products containing the notified chemical will be transported by road to various warehouses, commercial outlets and service centres.

USE
The notified chemical will be used as a thickening agent at ≤ 15% concentration in lubricants for moving parts of equipment in industrial settings.

OPERATION DESCRIPTION
Manufacture, reformulation and repacking of the notified chemical or products containing the notified chemical will not occur in Australia.

Lubricants containing the notified chemical will be applied to equipment by workers using pre-filled cartridges via a grease gun or from larger containers by pneumatic or pump dispensers. Typically, the equipment will have a grease insertion port feeding into a grease reservoir which will then deliver the lubricant to internal moving parts of the equipment. When the lubricant is spent, it will be manually drained from the unit and collected for 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)*
Transport and warehousing	2 – 4	12 - 24
Service personnel	2 – 4	12 - 50

EXPOSURE DETAILS

Transport and Storage
Transport and storage workers are not expected to be exposed to the notified chemical except in the unlikely event of an accident, as the product containing the notified chemical will be sealed in original containers during transport and storage.

Service personnel
Dermal and ocular exposure of workers to the notified chemical at ≤ 15% concentration may occur during preparing and loading cartridges into grease guns, disconnecting and disposing of cartridges, connecting and disconnecting pneumatic and pump dispensers to large containers, and during clean-up of spills and maintenance. Inhalation exposure to the notified chemical is not expected given the estimated low vapour pressure of the notified chemical and the proposed application method (aerosols and mists are not expected to occur). Exposure will be minimised by the use of engineering controls (dedicated dispensing equipment without the need of cleaning between uses) and personal protection equipment (PPE) (including protective clothing, impervious gloves and safety glasses), as stated by the notifier.

6.1.2. Public Exposure
Lubricants containing the notified chemical will be for industrial use only and will not be available to the public.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemical and an analogue chemical (identity is Exempt Information) 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 > 2,000 mg/kg bw; low toxicity
Skin irritation – rabbit*	non-irritating
Eye irritation – rabbit	slightly irritating
Skin sensitisation – mouse local lymph node assay*	no evidence of sensitisation at up to 10% concentration
Combined repeat dose oral toxicity with reproductive / developmental toxicity screening test – rat*	NOAEL = 1,500 mg/kg bw for systemic and reproductive toxicity
Mutagenicity – bacterial reverse mutation*	non-mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration*	non-genotoxic
Genotoxicity – *in vitro* mammalian cell gene mutation test*	non-genotoxic

* Analogue chemical

Toxicokinetics
Based on the relatively high molecular weight (> 500 g/mol), estimated low water solubility and estimated high Log Kow, absorption of the notified chemical across biological membranes is expected to be limited.

Acute Toxicity
No acute toxicity data were submitted for the notified chemical. An analogue chemical is of low acute oral toxicity based on a study conducted in rats.

Irritation
No skin irritation data were submitted for the notified chemical. An analogue chemical was found to be non-irritating to the skin in a study conducted in rabbits.

The notified chemical was found to be slightly irritating to eyes in a study conducted in rabbits.

Sensitisation
No sensitisation data were submitted for the notified chemical. An analogue chemical was found to be a non-sensitiser in a mouse local lymph node assay when tested up to 10% concentration. Skin sensitisation potential of the notified chemical at higher concentrations cannot be ruled out.

Repeated Dose Toxicity
In a combined repeated dose oral toxicity study with reproduction/developmental screening test, an analogue chemical was administered (oral gavage) in rats at doses of 375, 750 and 1,500 mg/kg bw/day for up to 6 weeks for males and up to 8 weeks for females. Males from all treatment groups showed a statistically significant increase in thyroid weight, both absolute (37.9% for low dose group, 27.2% for mid dose group and 13.5% for high dose group) and relative to terminal body weight (25% for each dose group). Females treated at 375 mg/kg bw/day showed a statistically significant reduction in absolute (12.4%) and relative thyroid weight (28.5%). These changes were not considered by the study authors to be toxicologically relevant as there were no true dose related responses or histological correlation. Statistically significant increases in mean body weights on Day 1 *post partum* were observed in female offspring from litters of dams treated at 1,500 mg/kg bw/day (10.7%) and 750 mg/kg bw/day (10.9%) and in male offspring from litters of dams treated at 750 mg/kg bw/day (10.2%). This effect was not considered by the study authors to be an adverse effect of treatment. The No Observed Adverse Effect Level (NOAEL) for systemic and reproductive toxicity was established as 1,500 mg/kg bw/day by the study authors.

Mutagenicity/Genotoxicity
No mutagenicity/genotoxicity data were submitted for the notified chemical. An analogue chemical was negative in a bacterial reverse mutation assay, an *in vitro* mammalian chromosome aberration test using human lymphocytes and an *in vitro* mammalian cell gene mutation test using mouse lymphoma L5178Y cells.

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.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information, skin sensitisation potential of the notified chemical at the proposed end-use concentrations (≤ 15%) cannot be ruled out. The notified chemical is expected to be of low systemic toxicity and non-irritating at the concentrations of use. No inhalation toxicity data were provided, but due to the estimated low vapour pressure of the notified chemical and the proposed application manner, inhalation exposure to vapours, aerosols or mists of the notified chemical is not expected.

The use of engineering controls (dedicated dispensing equipment without the need of cleaning between uses) and personal protection equipment (PPE) (including protective clothing, impervious gloves and safety glasses), as anticipated by the notifier, are expected to minimise the potential for exposure.

Therefore, under the conditions of the occupational settings described, the risk to workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
The notified chemical is only intended for use in industrial settings. Therefore, when used in the proposed manner, the risk to public health is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a component of finished lubricant greases. It will not be reformulated or repackaged in Australia. No release is expected except from accidental spills where the packaging is breached. Accidental spills of the products containing the notified chemical during import, transport, or storage are expected to be collected for recycling or treating at on-site treatment where residual hydrocarbons are separated from the aqueous stream by the Australian Petroleum Industry (API) process. As estimated by the notifier, empty import containers may contain up to 1-2% of the import volume of the notified chemical (corresponding to 400 kg/annum). The empty containers will be steamed clean and the residual waste containing the notified chemical is also expected to be sent to on-site treatment facilities.

RELEASE OF CHEMICAL FROM USE

Minimal losses of the notified chemical are expected while adding the finished grease to machinery. These losses are expected to be cleaned up using an adsorbent/absorbent material (such as a rag and newspaper etc.) which is expected to be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

Residual greases within the machinery will have the same fate as the machinery which may be recycled as scrap metal or disposed of to landfill. A small proportion of the notified chemical may also remain in end-use containers. Residues of the notified chemical in empty containers are likely either to share the fate of the container and be disposed of to landfill, or be recycled through an approved waste management facility.

7.1.2. Environmental Fate

A biodegradability test conducted on an analogue chemical (identity is Exempt Information) shows that it is not readily biodegradable (17% degraded over 28 days in an OECD 301 B test). For details see appendix C. The notified chemical will be present within a grease matrix throughout its lifecycle. The grease products are intended to have a long life on the metal parts (within the equipment being lubricated) to which they have been applied. Thus, replenishment of the grease reservoir with the equipment will occur infrequently. The majority of the notified chemical is expected to share the fate of the metal objects onto which it is adhering at the end of their useful lifetime. The metal objects are likely to be recycled, during which the notified chemical will be thermally decomposed to water vapour and oxides of carbon and nitrogen. A small amount of the total import volume may be also thermally decomposed during engine operation. Based on its low water solubility and high log Pow (> 9), the notified chemical is expected to have low mobility in soil. The notified chemical in landfill or soil is expected to slowly degrade through the biotic and abiotic processes, resulting in the formation of water, and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated. The concentration of the notified chemical in the aquatic environment is expected to be limited.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on an analogue chemical (identity is Exempt Information) are summarised in the table below. Details of three studies submitted can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity*	96 hr LL50 > 100 mg/L (WAF)	Not harmful to fish up to its water solubility limit
Daphnia Toxicity*	48 hr EL50 > 100 mg/L (WAF)	Not harmful to aquatic invertebrates up to its water solubility limit
Algal Toxicity*	72 hr EL50 > 100 mg/L (WAF)	Not harmful to algae up to its water solubility limit

* Analogue chemical

WSF: Water Accommodated Fraction

Based on the above ecotoxicological data, the notified chemical is not expected to be acutely toxic up to the limit of water solubility. However, the notified chemical has the potential to bioaccumulate (Pow > 9), is not readily biodegradable and therefore is formally classified as “Chronic Category 4; May cause long lasting harmful effects

to aquatic life" under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for chronic toxicities. This classification has been assigned on the basis of the notified chemical's lack of biodegradability and persistence in the environment.

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated, since the analogue chemical is not harmful to aquatic life up to the limit of its solubility in water.

7.3. Environmental Risk Assessment

The risk quotient (Q = PEC/PNEC) for the notified chemical has not been calculated as a PNEC value is not available and release of the notified chemical to the aquatic environment in ecotoxicologically significant concentration is not expected based on its reported use pattern. The notified chemical is not readily biodegradable. The notified chemical has the potential to bioaccumulate but is not expected to be significantly bioavailable in the aquatic environment due to low water solubility. On the basis of the low aquatic hazard and reported use pattern as an additive in lubricating greases, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point > 250 °C

Method	ASTM E324-99
Remarks	Capillary tube method
Test Facility	SRL (2016)

Density 1,060 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Determined using a pycnometer
Test Facility	ACE (2018)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Remarks	The test substance was observed to ignite and burn. The time for propagation was longer than the test limits of 4 mins.
Test Facility	ACE (2018)

Autoignition Temperature 395 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Remarks	A small but sharp exothermic temperature increase was observed at a furnace temperature of 258 °C which was not determined to be ignition. Large ignition was detected at 395 °C.
Test Facility	Envigo (2017)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure EC Council Regulation No 440/2008 B.1 Acute Toxicity (Oral)
Species/Strain	Rat/Wistar
Vehicle	Arachis oil BP
Remarks – Method	No significant deviations from the test guidelines

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	All animals showed expected body weight gains.
CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	Harlan (2012a)

B.2. Skin Irritation – Rabbit

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	Water
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks – Method	No significant deviations from the test guidelines
RESULTS	
Remarks – Results	No skin reactions were noted. All animals showed expected body weight gains.
CONCLUSION	The test substance is non-irritating to the skin.
TEST FACILITY	Harlan (2011a)

B.3. Eye Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 females
Observation Period	72 hours
Remarks – Method	No significant deviations from the test guidelines

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva – Redness	0.33	0.33	0	1	< 48 h	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 completely resolved within 48 hours in all animals. Iridial irritation was noted for two animals after 1 hour only.

All animals showed expected body weight gains.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: CRL (2019)

B.4. Skin Sensitisation – LLNA

TEST SUBSTANCE: Analogue chemical (identity is Exempt Information)

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain: Mouse/CBA/Ca

Vehicle: 1% pluronic L92 in distilled water

Preliminary study: Yes

Positive control: Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.

Remarks – Method: No significant deviations from test guidelines. The dose selection in the main test was based on a preliminary test carried out at 10% concentration.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (test/control ratio)*
0 (vehicle control)	4F	765.39	1.00
Test Substance			
2.5	4F	725.22	0.95
5	4F	947.75	1.24
10	4F	884.02	1.15

Remarks – Results: No signs of systemic toxicity and visual local skin irritation were noted in the preliminary test. No signs of systemic toxicity were observed the main test.

The test substance did not elicit a stimulation index greater than or equal to 3.

All animals showed expected body weight changes.

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance up to 10% concentration.

TEST FACILITY: Harlan (2012b)

B.5. Combined Repeat Dose Toxicity with Reproductive/Developmental Toxicity Screening Test

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: up to 6 weeks for males and up to 8 weeks for females (including a 2-week pre-pairing phase, pairing, gestation and early lactation) Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	MOL WO M 46 medicinal white oil
Remarks – Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	12M/12F	0	0/24
Low Dose	12M/12F	375	0/24
Mid Dose	12M/12F	750	0/24
High Dose	12M/12F	1,500	0/24

Mortality and Time to Death
There were no unscheduled deaths.

Effects on parental animals
No toxicologically relevant clinical signs were observed. There were no test substance-related changes in behavioural parameters, functional performance, sensory reactivity, body weight development, or food and water consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No test substance related effects were observed in haematological parameters.

The following statistically significant changes observed in treated animals (compared to control animals) were not considered by the study authors to be of toxicological significance as the majority if individual values were within the normal range for rats of the strain and age and/or there were no true dose related response or histological correlation:
- All treated males showed statistically significant reductions in neutrophil and eosinophil counts.
- Male and female rats treated at 1500 mg/kg bw/day showed statistically significantly increased clotting times.
- Males treated at 1500 mg/kg bw/day showed a statistically significant reduction in mean creatinine level.
- Females treated at 1500 mg/kg bw/day showed a statistically significant increase in glucose level and a statistically significant reduction in albumin level and albumin/globulin ratio.
- Females treated at 375 mg/kg bw/day showed a statistically significant increase in mean aspartate aminotransferase level.

Effects in Organs
There were no treatment related macroscopic abnormalities or effects in organ weights in animals. No test substance-related microscopic abnormalities were observed at necropsy.

The following changes were not considered by the study authors to be toxicologically relevant as there were no true dose related responses or histological correlation:
- One male treated at 375 mg/kg bw/day had increased pelvic space in the right kidney.
- Another male treated at 375 mg/kg bw/day had a small right seminal vesicle.
- One female treated at 375 mg/kg bw/day had reddened lungs at necropsy.
- Males treated at 1500 or 750 mg/kg bw/day showed a statistically significant increase in adrenal weight, both absolute and relative to terminal body weight.

- Males from all treatment groups showed a statistically significant increase in thyroid weight, both absolute (37.9% for low dose group, 27.2% for mid dose group and 13.5% for high dose group) and relative to terminal body weight (25% for each dose group).
- Females treated at 375 mg/kg bw/day showed a statistically significant reduction in absolute (12.4%) and relative thyroid weight (28.5%).

Reproductive effects

There were no test substance-related effects on mating performance, conception rates, fertility, gestation lengths, litter size at birth and subsequently on Days 1 and 4 *post partum* or sex ratio.

A statistically significant increase in post-implantation loss was observed in females treated at 1500 mg/kg bw/day, when compared to control females. This effect was not considered by the study authors to be toxicologically significant as only one of the individual values were outside of the normal historical control range and there were no any associated changes in the number of implantation sites or the number of offspring born.

Effects on pups

There were no treatment related effects observed in offspring body weight gain or litter weights at birth or subsequently on Days 1 and 4 *post partum*.

Statistically significant increases in mean body weights on Day 1 *post partum* were observed in female offspring from litters of dams treated at 1500 mg/kg bw/day (10.7%) and 750 mg/kg bw/day (10.9%) and in male offspring from litters of dams treated at 750 mg/kg bw/day (10.2%). This effect was not considered by the study authors to be an adverse effect of treatment.

Remarks – Results

The test substance was not considered to elicit toxicologically relevant or dose related effects in the rat adults or pups of the study.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 1,500 mg/kg bw/day by the study authors for systemic and reproductive toxicity, the highest dose tested in this study, based on no toxicologically relevant effects observed up to this dose level.

TEST FACILITY	Harlan (2013a)

B.6. Mutagenicity – Bacterial Reverse Mutation Test

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	Test 1 and 2: a) With metabolic activation: 50 – 5,000 µg/plate b) Without metabolic activation: 50 – 5,000 µg/plate
Vehicle	DMSO
Remarks – Method	No significant deviations from the test guidelines. A preliminary assay carried out at 0.15-5000 µg/plate established the dose range chosen for the main experiments. Positive control: i) without S9: 4-nitroquinoline-1-oxide (TA98), 9-aminoacridine (TA1537), N-ethyl-N-nitro-N-nitrosoguanidine (TA100, TA1535, WP2uvrA); ii) with S9: 2-Aminoanthracene (TA1535, TA1537, TA100, WP2uvrA), benzo(a)pyrene Benzo(a)pyrene (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	≥ 1,500	negative
Test 2	> 5,000	> 5,000	≥ 1,500	negative
Present				
Test 1	> 5,000	> 5,000	≥ 1,500	negative
Test 2	> 5,000	> 5,000	≥ 1,500	negative

Remarks – Results: No significant increases in the frequency of revertants were observed for any of the bacterial strains, with any concentration of the test substance, with or without metabolic activation.

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: Harlan (2010)

B.7. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE: Analogue chemical (identity is Exempt Information)

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

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

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

Vehicle: THF

Remarks – Method: No Significant deviations from the test guidelines. A preliminary assay carried out at 2-500 µg/mL established the dose range chosen for the main experiments.

Positive control: i) without metabolic activation - Mitomycin C; ii) with metabolic activation: cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 2, 4*, 8, 16*, 24, 32*	4 hr	24 hr
Test 2	0*, 2, 4, 8*, 12*, 16*, 24*	24 hr	24 hr
Present			
Test 1	0*, 2, 4*, 8, 16*, 24, 32*	4 hr	24 hr
Test 2	0*, 2, 4*, 8*, 16*, 24*, 32	4 hr	24 hr

*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	> 500	> 32	≥ 4	negative
Test 2	> 500	> 24	24	negative
Present				
Test 1	> 500	> 32	≥ 8	negative
Test 2		> 24	≥ 16	negative

Remarks – Results	In both main tests, no statistically significant increases in the frequency of cells with structural chromosome aberrations were observed in the presence or absence of metabolic activation. The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The test substance was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2012c)

B.8. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test EC Directive 2000/32/EC B.17 Mutagenicity – *In vitro* Mammalian Cell Gene Mutation Test
Species/Strain	Mouse
Cell Type/Cell Line	L5178Y lymphoma
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Vehicle	THF
Remarks – Method	No significant deviations from test guidelines. A preliminary assay carried out at 2-128 µg/mL established the dose range chosen for the main tests. Positive controls: i) without S9 activation: ethylmethansulphonate; ii) with S9 activation: cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	0*, 1*, 2*, 4*, 8*, 16*, 32*	4 hr	2 days
Test 2	0*, 1*, 2*, 4*, 8*, 16*, 32*	24 hr	2 days
Present			
Test 1	0*, 1*, 2*, 4*, 8*, 16*, 32*	4 hr	2 days
Test 2	0*, 1*, 2*, 4*, 8*, 16*, 32*	4 hr	2 days

*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	> 128	> 32	> 32	negative
Test 2	> 128	> 32	32	negative
Present				
Test 1	> 128	> 32	≥ 16	negative
Test 2		> 32	≥ 16	negative

Remarks – Results	No statistically significant dose-dependent increases in the mutation frequency were observed, with or without metabolic activation. The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The test substance was not clastogenic to mouse lymphoma L5178Y cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Harlan (2013b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge from a local STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC)
Remarks - Method	No significant deviations from the test guidelines were reported. The test substance at a concentration of 10 mg carbon/L was exposed to activated sludge.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	7	6	76
14	7	14	77
21	9	21	75
28	17	28	89

Remarks - Results	All validity criteria for the test were satisfied. The total CO_2 evolution in the control vessels on day 28 was 26.75 mg/L. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the notified chemical after 28 days was 17%.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Harlan (2012d)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-Static
Species	*Oncorhynchus mykiss* (Rainbow Trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	TOC analyser
Remarks – Method	The study was carried out in accordance with the test guidelines and GLP. Following a preliminary range-finding test, fish were exposed to a water accommodated fraction (WAF) of the test substance which was prepared by stirring 100 mg test substance in deionised water for 23 hours and allowing the mixture to stand for 1 hour. The aqueous phase was removed by mid-depth siphoning to give the 100 mg/L loading rate WAF.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0

100	Not determined	7	0	0	0	0	0

LL50	> 100 mg/L (WAF) at 96 hours
NOEL	100 mg/L at 96 hours
Remarks – Results	The test was conducted at approximately 14°C. All validity criteria for the test were satisfied. The dissolved oxygen (DO) concentration was 7.8 mg/L [equivalent to 76% oxygen saturation in fresh water at 14°C (USGS, 2011)].
CONCLUSION	The test substance is not harmful to fish up to its water solubility limit.
TEST FACILITY	Harlan (2011b)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Total Organic Carbon (TOC)
Remarks – Method	The study was carried out in accordance with the test guidelines and GLP. Following a preliminary range-finding test, Daphnia were exposed to a water accommodated fraction (WAF) of the test substance which was prepared by stirring 100 mg test substance in deionised water for 23 hours and allowing the mixture to stand for 1 hour. The aqueous phase was removed by mid-depth siphoning to give the 100 mg/L loading rate WAF. A positive control was also run, less than six months prior to the definitive study.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
100	Not determined	20	0	0

EL50	> 100 mg/L (WAF) at 48 hours
NOEL	100 mg/L at 48 hours
Remarks – Results	The validity criteria of the test were met. The dissolved O_2 was maintained at 8.6 mg/L. The pH of the control replicates was 8.0 ± 0.2, which is slightly above the range for the protocol. This was considered not to have affected the results or validity of the test as no immobilisation was observed throughout the test. The results from the positive control with potassium dichromate was EC50 = 0.99 mg/L within the normal range for this reference substance.
CONCLUSION	The test substance is not harmful to aquatic invertebrates up to its water solubility limit.
TEST FACILITY	Harlan (2011c)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Analogue chemical (identity is Exempt Information)
METHOD	OECD TG 201 Alga, Growth Inhibition Test

Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L (WAF) Actual: Not determined
Auxiliary Solvent	None
Water Hardness	Not measured
Analytical Monitoring	TOC analyser
Remarks – Method	Following a preliminary range-finding test, algae were exposed to a water accommodated fraction (WAF) of the test substance which was prepared by stirring 100 mg test substance in culture medium for 23 hours and allowing the mixture to stand for 1 hour. The aqueous phase was removed by mid-depth siphoning to give the 100 mg/l loading rate WAF. A positive control was also run less than six months prior to the definitive study.

RESULTS

Biomass		*Growth*	
EbL50 *mg/L at 72 h*	*NOEL* *mg/L*	*ErL50* *mg/L at 72 h*	*NOEL* *mg/L*
> 100	100	> 100	100

Remarks – Results	All validity criteria for the test were satisfied. The mean cell density in the control increased by a factor of 39 times after 72 hours. The mean coefficient of variation by section specific growth rate for the control cultures was 7%. The coefficient of variation for average growth rate for the control culture over the test period was 4%. The results from the positive control with potassium dichromate was EC50 =1.4 mg/L (within the normal range for this reference substance).
CONCLUSION	The test substance is not harmful to algae up to its water solubility limit.
TEST FACILITY	Harlan (2012e)

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