File No: STD/1526

December 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Amides, coco, N,N-bis(hydroxyethyl), reaction products with coco monoglycerides and molybdenum oxide (MoO_3)

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

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

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

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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/1526	Chemical Compliance Australia Pty Ltd	Amides, coco, N,N-bis(hydroxyethyl), reaction products with coco monoglycerides and molybdenum oxide (MoO_3)	No	≤ 5 tonnes per annum	A component of engine oils and lubricants

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 for the Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

The environmental hazard classification according to the *Globally Harmonised System for the 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*
Acute (Category 2)	H401 - Toxic to aquatic life
Chronic (Category 2)	H411 - Toxic to aquatic life with long lasting effects

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

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

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

Recommendations

REGULATORY CONTROLS

(Material) Safety Data Sheet

- The (M)SDS provided by the notifier should be amended as follows:
  - When an importer is identified, the Australian contact details should be included on the (M)SDS and a copy of the revised (M)SDS should be provided to NICNAS for the publication purpose.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation:

  - Enclosed, automated processes, where possible
  - Local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation:
  - 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 reformulation:
  - Coveralls
  - Gloves
  - Safety goggles

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

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

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

Disposal

- Where reuse or recycling are 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 and/or accidental release of the notified chemical should be handled by physical containment, 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 engine oils and 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.

No additional secondary notification conditions are stipulated.

(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(S)
Chemical Compliance Australia Pty Ltd (ABN: 83 143 463 709)
5 Guinea Court
EPPING VIC 3076

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: other names, molecular and structural formulae, molecular weight, degree of purity, impurities, additives/adjuvants, use details and import volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Europe, Japan, Korea, Philippines, USA, Canada

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Molyvan® 855

CAS NUMBER
445409-27-8

CHEMICAL NAME
Amides, coco, N,N-bis(hydroxyethyl), reaction products with coco monoglycerides and molybdenum oxide (MoO_3)

MOLECULAR WEIGHT
Variable, 200-800 Da

ANALYTICAL DATA
Reference NMR, LC/MS, IR, HPLC and IC spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: dark brown paste

Property	Value	Data Source/Justification
Pour Point	22 °C	Measured
Boiling Point	Decomposes without boiling at > 92 °C at 102 kPa	Measured
Density	1,090 kg/m^3 at 21.5 °C	Measured
Vapour Pressure	4.2 × 10^{-7} kPa at 25 °C	Measured
Water Solubility	1.25 x 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of	t½ = 10.3 days at 15 °C (pH 7)	Measured

pH		
Partition Coefficient (n-octanol/water)	log Pow > 4.5	Estimated
Adsorption/Desorption	Not determined	The notified chemical is expected to partition to soil, sediment and sludge and have low mobility in soil based on its low water solubility.
Dissociation Constant	Not determined	No dissociable functionality
Particle Size	Not determined	Liquid
Flash Point	Not determined	Paste
Flammability	Not highly flammable	Measured
Autoignition Temperature	382 °C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Predicted negative	Estimated based on chemical structure

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a liquid at > 90% concentration.

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

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

PORT OF ENTRY
Melbourne

IDENTITY OF RECIPIENTS
Chemical Compliance Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported 0.9 L, 3.8 L, 18.9 L containers, 208 L drums and 1040 L and 1250 L intermediate bulk containers (IBCs) into Australia by sea and transported by road in Australia.

USE
The notified chemical will be used as an anti-wear additive for engine oils and lubricants at 0.1-1.5%.

OPERATION DESCRIPTION
After importation products containing the notified chemical will be blended with other components into additive packages in a predominantly closed system. After formulation, the additive packages will be pumped into containers for commercial use. End-use products are expected to contain 0.1-1.5% notified chemical.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	1	30-60
Blending operators	2	50-100
Quality technicians	1	50-100
Packaging workers	2	50-100
End use of lubricants	1	100-200

EXPOSURE DETAILS

Transport and storage workers may come into contact with the notified chemical at > 90% concentration only in the unlikely event of accidental rupture of containers.

The blending process will be automated in a closed system; however, plant operators may be exposed (dermal and ocular) to the notified chemical at > 90% concentration during opening of containers and connection of pipes when pumping into blending or storage tanks. Other workers may also come into contact with the notified chemical during maintenance, cleaning, sampling and repackaging.

Dermal and ocular exposure to workers should be mitigated through the use of personal protective equipment (PPE), such as aprons, gloves and safety glasses, as anticipated by the notifier in the application dossier. Inhalation exposure is not expected given the measured low vapour pressure of the notified chemical. The expected use of local exhaust ventilation should further reduce inhalation exposure.

There is potential for dermal and possibly ocular exposure to the notified chemical at ≤ 1.5% concentration by workers at end user sites. Exposure will be reduced if PPE is used by workers.

6.1.2. Public Exposure

The public may come into contact with end-use lubricants containing ≤ 1.5% notified chemical in the case of a do-it-yourself (DIY) motor oil change. In these cases, dermal and ocular exposure may occur; however, such exposure is expected to be of a short-duration and infrequent basis.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical 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 > 5,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOEL = 150 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic
Genotoxicity – in vitro mammalian cell gene mutation test	non genotoxic

Toxicokinetics

No toxicokinetic data on the notified chemical were submitted. The notified chemical has a variable composition and contains a percentage of low molecular weight (< 500 Da) components; therefore absorption across biological membranes may occur. This is supported by systemic effects observed in the 28-day repeated dose

oral toxicity study. Absorption by the dermal route is expected to be limited by the highly lipophilic nature (log Pow > 4.5) of the notified chemical.

Acute toxicity
The notified chemical was of low acute oral and dermal toxicity in rats.

Irritation and sensitisation
The notified chemical was slightly irritating to the skin and eyes. The notified chemical was not a skin sensitiser in a guinea pig skin sensitisation study using the Magnusson and Kligman method. It is also noted that the notified chemical does not contain any structural alerts for sensitisation.

Repeated dose toxicity
In a 28-day repeat dose toxicity study, rats were administered the notified chemical by gavage at 0, 15, 150 or 1,000 mg/kg bw/day. The No Observed Effect Level (NOEL) was established as 150 mg/kg bw/day, based on biological effects observed for animals treated at 1,000 mg/kg bw/day, including clinical observations, kidney weights and renal changes.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay. The notified chemical was not clastogenic in an *in vitro* mammalian chromosome aberration test and an *in vitro* mammalian cell gene mutation test.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the 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

6.3.1. Occupational Health and Safety
The notified chemical is expected to be slightly irritating to the skin and eyes. There is the potential to cause systemic toxicity, although this is expected to be limited by the dermal route given the highly lipophilic nature of the notified chemical. Workers most at risk of irritation effects will be reformulation workers handling the notified chemical at > 90% concentration during reformulation processes. Given the toxicological profile of the notified chemical adverse effects from the notified chemical are not expected when handling finished engine oils and lubricants containing the notified chemical at concentrations of 1.5% or less. During reformulation exposure should be minimised through the stated use of enclosed, automated processes, local exhaust ventilation and PPE.

Overall, the risk to workers from exposure to the notified chemical is not considered to be unreasonable, given the toxicological profile of the notified chemical and the expected use of engineering controls and PPE during reformulation.

6.3.2. Public Health
The public may have dermal or ocular exposure to engine oils and lubricants containing the notified chemical at concentrations of 1.5% or less, at much less frequency than workers. Given the toxicological profile of the notified chemical the risk of adverse effects from the notified chemical at these concentrations is considered to be low, even in the absence of PPE.

Overall, the risk to public health associated with the proposed use of the notified chemical in engine oils and lubricants 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 into Australia for repackaging and reformulation into an anti-wear additive for engine oil and lubricants. Significant release of the notified chemical to the environment is not expected during transport and storage except in the unlikely event of accidental spills or leaks.

The blending operation occurs in closed pipes and vessels, where the notified chemical is expected to be blended with mineral oil and other additives and automatically pumped out for distribution to customers in Australia. If incidental spillage of the additive package occurs during normal blending procedures, it will be contained and soaked up with earth or sand before being transported off-site to an approved industrial facility for appropriate disposal.

RELEASE OF CHEMICAL FROM USE

The finished products containing the notified chemical will be used as a component of engine oil and lubricants. Release during its use may come from spills when pouring the fluid into engines or leaks from the engines, which is expected to be negligible.

RELEASE OF CHEMICAL FROM DISPOSAL

After reformulation, empty import drums containing residues of the notified chemical (< 0.1% of the total import volume) are expected to be steam cleaned, with the residual waste sent to on-site wastewater treatment facilities. Assuming < 0.1% of the notified chemical remains in the empty drums after use, 5 kg/yr (5 tonnes/yr × 0.1%) of the notified chemical will be sent to the on-site waste treatment. It is estimated that greater than 90% of the notified chemical may be removed during waste treatment processes. Therefore, the amount of the notified chemical released to sewer from the cleaning of empty drums is estimated to be 0.5 kg/yr. The wastewater will be further treated at sewage treatment plants. Therefore, the release of the notified chemical to surface waters is expected to be limited from the cleaning of empty drums.

Formulated products containing the notified chemical will be used in an enclosed system. These systems will require initial loading and occasional top-up. At the end of life, the fluids will be drained from the machinery for disposal. The main method of disposal will be recycling or thermal decomposition.

7.1.2. Environmental Fate

A ready biodegradability study for the notified chemical showed a 28 day biodegradation of 56%. Therefore, the notified chemical is not considered readily biodegradable. For details of the environmental fate studies please refer to Appendix C. Most of the notified chemical will be thermally decomposed during use, recycled or re-refined. Bioaccumulation and bioavailability of the notified chemical is not expected due to its biodegradability and limited potential for exposure to the aquatic compartment.

The notified chemical has very low water solubility at $< 1.25 \times 10^{-3}$ g/L. With a high partition coefficient (log Pow > 4.5), the notified chemical is expected to partition to organic matter and to sediments and soils in the environment, and is therefore considered highly immobile within a landfill environment. Notified chemical released to surface water is expected to partition to sediment based on its limited water solubility. The notified chemical has a tendency to partition to organic phases as indicated by its n-octanol/water partition coefficient (log Pow > 4.5), which indicates a potential to bioaccumulate. However, given the notified chemical is expected to be biodegradable, not significantly bioavailable and is not expected to be released to the aquatic environment, the notified chemical has limited potential for bioaccumulation.

Despite the presence of a hydrolysable functionality, the potential for hydrolysis is low due to poor water solubility of the notified chemical. However, a reasonable level of biotic degradation (57% in 28 days) has been detected. Therefore, the notified chemical is expected to quickly break down in either a landfill or aquatic environment to form water and oxides of carbon, nitrogen and molybdenum.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) was not calculated since no significant release to the aquatic environment is expected based on the proposed use pattern.

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*
Acute toxicity		
Fish Toxicity	96 h LC50 > 10 mg/L	Not harmful to fish up to the limit of water solubility
Daphnia Toxicity	48 h EL50 = 1.5 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	72 h EL50 = 4 mg/L	Toxic to algae
Earthworm Toxicity	14 d LC50 > 1000 mg/kg dry soil	Not harmful to earthworm
Chronic toxicity		
Daphnia Toxicity	21 d NOEL = 0.47 mg/L	Toxic to daphnia

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is considered to be toxic to aquatic invertebrates and algae and is formally classified as 'Acute Category 2: Toxic to aquatic life'. On the basis of the chronic toxicity and the lack of ready biodegradability, the notified chemical is classified 'Chronic Category 2: Toxic to aquatic life with long lasting effects'.

7.2.1. Predicted No-Effect Concentration

It is not necessary to calculate the predicted no-effect concentration (PNEC) since no significant release of the notified chemical is expected from the proposed use pattern.

7.3. Environmental Risk Assessment

The risk quotient (RQ = PEC/PNEC) has not been calculated since no PEC or PNEC was available. The notified chemical has a tendency to partition to organic phases as indicated by its n-octanol/water partition coefficient (log Pow > 4.5), which indicates a potential to bioaccumulate. However, given the notified chemical is expected to be biodegradable, not significantly bioavailable and is not expected to be released to the aquatic environment, the notified chemical has limited potential for bioaccumulation. Notified chemical released to surface water is expected to partition to sediment based on its limited water solubility. Therefore, based on the assessed use pattern and low potential for aquatic exposure, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point 22 ± 3 °C

Method EC Council Regulation No 92/69/EEC A.1 Melting/Freezing Temperature.
Test Facility Safepharm (2011)

Boiling Point Decomposes without boiling at > 92 °C at 102 kPa

Method EC Council Regulation No 92/69/EEC A.2 Boiling Temperature.
Remarks Determined using differential scanning calorimetry. The test substance decomposed from 92 °C, prior to any boiling.
Test Facility Safepharm (2011)

Density 1,090 kg/m^3 at 21.5 ± 0.5 °C

Method EC Council Regulation No 92/69/EEC A.3 Relative Density.
Remarks Determined using a gas comparison pycnometer.
Test Facility Safepharm (2011)

Vapour Pressure 4.2×10^{-7} kPa at 25 °C

Method EC Council Regulation No 92/69/EEC A.4 Vapour Pressure.
Remarks Determined using a vapour pressure balance.
Test Facility Safepharm (1997a)

Water Solubility < 1.25×10^{-3} g/L at 20 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method. A definitive study could not be conducted for the water solubility, due to the unstable nature of the test substance in aqueous media. Therefore, only preliminary estimate by visual inspection was performed.
Test Facility Safepharm (2011)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.
EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ days*
7	15	10.3

Remarks The test substance is a complex mixture of coco diethanolamide and monoglycerides with molybdenum trioxide. The total concentration of molybdenum ion was determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).
Test Facility Safepharm (2004a)

Partition Coefficient (n-octanol/water) log Pow > 4.5

Method Visual inspection
Remarks The shake flask method could not be used as the test substance was found to be unstable in water. The HPLC method could not be used as this method is not applicable to metal complexes. Therefore, the partition coefficient for the test substance was estimated by visual inspection.
Test Facility Safepharm (2011)

Flammability Not highly flammable

Method EC Council Regulation No 92/69/EEC A.10 Flammability (Solids).
Test Facility Safepharm (1997b)

Autoignition Temperature 382 ± 5°C

Method EC Council Regulation No 92/699/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility Safepharm (1997b)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The BAM fall hammer, BAM friction and Korean steel tube test were used.
Test Facility Safepharm (1997b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE Notified Chemical

METHOD Similar to OECD TG 401 Acute Oral Toxicity – Limit Test.
Species/Strain Rat/Sprague-Dawley
Vehicle None
Remarks – Method No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5 M	5000	0
II	5 F	5000	0

LD50 > 5000 mg/kg bw
Signs of Toxicity There were no deaths or remarkable bodyweight changes. Clinical signs such as salivation, diarrhoea, decreased activity were observed in some of the animals in both groups. Decreased respiratory rate was observed in two female rats in the group.
Effects in Organs There were no remarkable necropsy findings.
Remarks - Results All animals showed expected body weight gains during the study.

CONCLUSION The notified chemical is of low toxicity via the oral route.

TEST FACILITY Food and Drug Research (1985a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 402 Acute Dermal Toxicity – Limit Test.

Species/Strain Rabbits/New Zealand White
Vehicle None
Type of dressing Occlusive
Remarks - Method No significant protocol deviations.

RESULTS

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

LD50 > 2000 mg/kg bw
Signs of Toxicity - Local Test substance application site exhibited well defined erythema and slight oedema, at the time of removal of the test material from the skin.
Signs of Toxicity - Systemic There were no deaths or remarkable bodyweight changes. Clinical signs such as soft stools, nasal discharge and anorexia were observed in some of the male rabbits.
Effects in Organs There were no remarkable necropsy findings.
Remarks - Results All animals showed expected body weight gains during the study.

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

TEST FACILITY Food and Drug Research (1985b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals Six
Vehicle None
Observation Period 14 days
Remarks - Method No significant protocol deviations.

RESULTS

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Erythema/Eschar	1	1	0.8	0.7	0.3	0.3	1	14 days	1
Oedema	0	0.2	0	0	0	0	1	14 days	1

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

Remarks - Results All test animals showed very slight erythema that was resolved by the Day 7 observation period in 5 animals. Very slight oedema was observed in 3 test animals which was resolved in two of the test animals by the 48 hour observation period. One test animal displayed very slight erythema and oedema at the end of the observation period. However these signs of irritation were not observed in this animal on the Day 7 and Day 10 observation period.

Two animals were reported dead during the 14 day test. Animals died on day 7 and day 8 of the test period.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Food and Drug Research (1985c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 (rinsed); 6 (unrinsed)
Observation Period 72 hours
Remarks - Method Nine animals were used in the study. The treated eye of six rabbits was not washed after instillation with the test substance. The treated eye of the remaining three rabbits was washed with lukewarm water 30 seconds after instillation.

RESULTS

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Conjunctiva: redness	0.7	0.3	0.3	0.3	0	0.3	2	< 72 h	0
Conjunctiva: chemosis	0.3	0	0	0.3	0	0	1	< 48 h	0
Conjunctiva: discharge	0	0	0	0	0	0	2	< 24 h	0
Corneal opacity	0	0	0	0	0	0	0	None	0
Iridial inflammation	0	0	0	0	0	0	0	None	0

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

Remarks - Results

The table above shows the irritation scores for the unrinsed eyes only.

For the unrinsed eyes, slight to moderate conjunctival irritation was observed in all six animals. All signs of irritation were resolved at the 72 hour observation period.

For the rinsed eyes, only very slight conjunctival irritation was observed in all three animals. All signs of irritation were resolved by the 48-hour observation period.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Food and Drug Research (1985d)

B.5. Skin sensitisation

TEST SUBSTANCE

Notified Chemical

METHOD

OECD TG 406 Skin Sensitisation – Guinea pig maximisation test (Magnusson and Kligman).

Species/Strain

Guinea pig/ Dunkin Hartley

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
topical: 75% (v/v in liquid paraffin BP)

MAIN STUDY

Number of Animals

Test Group: 10 Control Group: 5

INDUCTION PHASE

Induction Concentration:
intradermal: 1% w/v in liquid paraffin BP
topical: 100%

Signs of Irritation

Intradermal induction resulted in a well-defined erythema in test groups at 24 hours followed by very slight to well defined erythema at 48 hours. Very slight erythema was noted at 24 and 48 hours for control groups.

Topical induction resulted in well-defined erythema with or without very slight to slight oedema at the induction sites for 8 test group animals at 24 hours of observation. Other reactions such as bleeding from the intradermal induction sites, hardened dark brown/black coloured scabs and small superficial scattered scabs were observed. Two animals were not measured due to adverse reactions.

CHALLENGE PHASE

1st challenge

topical: 75% and 50% v/v in liquid paraffin BP

Remarks - Method

Only one challenge test with two different concentrations was tested.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	75%	0	0
	50%	0	0
Control Group	75%	0	0
	50%	0	0

Remarks - Results: Brown/green-coloured staining was noted at the challenge sites of all test and control animals at the 24- and 48-hour observations. The staining did not affect evaluation of skin reactions.

No skin reactions were noted at the challenge sites of the test or control group animals at the 24- or 48-hour observations.

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

TEST FACILITY: Safepharm (1997c)

B.6. Repeat dose toxicity

TEST SUBSTANCE: Notified chemical

METHOD: EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain: Rat/Sprague- Dawley Crl:CD BR strain
Route of Administration: Oral – gavage
Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week, once daily
Post-exposure observation period: None
Vehicle: Arachis oil BP
Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 (5 M/ 5F)	0	0
low dose	10 (5 M/ 5F)	15	0
mid dose	10 (5 M/ 5F)	150	0
high dose	10 (5 M/ 5F)	1000	0

Mortality and Time to Death
There were no deaths during the study.

Clinical Observations
No clinical signs of toxicity were detected in test or control animals throughout the study period.

Incidents of increased salivation, fur staining and wetness, together with one incident of noisy respiration were observed at 1000 mg/kg bw/day but all were considered to be attributable to the unpalatable or slightly irritant nature of the test material formulation and, in isolation, were considered not to be indicative of toxicity.

Bodyweight of males treated with 1000 mg/kg bw/day showed a statistically significant reduction in bodyweight gain from week 2 onwards with one male showing a loss in bodyweight at day 28. No such effects were observed for 1000 mg/kg bw/day females or for animals of either sex treated with 150 or 15 mg/kg/day throughout the study.

Males treated with 1000 mg/kg bw/day showed a slightly reduced dietary intake and food efficiency compared with that of controls from week 2 onwards, while the females were not affected. No such effect was observed for the other concentrations on both sex animals.

Increased water intake for animals of either sex treated with 1000 mg/kg bw/day, the effect slightly more severe for males than females. No such effect was observed with other dose groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No treatment related effects were reported.

Effects in Organs
Kidney weights, both absolute and relative to bodyweight were substantially elevated for animals of either sex treated with 1000 mg/kg bw/day compared with controls. No such effect was observed with dose 150 or 15 mg/kg bw/day. Treatment renal changes were observed. The incidence and severity of groups of basophilic cortical tubules were increased in both sex groups when treated with 1000 mg/kg bw/day. Necropsy showed pale kidneys in three males and three females treated with 1000 mg/kg bw/day.

Remarks –Results
Oral administration of the test material showed significant toxicological effects at 1000 mg/kg bw/day. No such effects were detected at 150 or 15 mg/kg bw/day.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 150 mg/kg bw/day in this study, based on toxicological effects at the highest dose tested.

TEST FACILITY Safepharm (1997d)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Species/Strain *S. typhimurium*: TA1535, TA1537, TA98 and TA100
E. coli: WP2uvrA$^-$
Metabolic Activation System S9 fraction from Aroclor 1254 induced rat liver
Concentration Range in Main Test a) With metabolic activation: 0, 5,15, 50, 150, 500, 1500, 5000 µg/plate
b) Without metabolic activation:0, 5,15, 50, 150, 500, 1500, 5000 µg/plate
Vehicle Dimethyl formamide

Remarks - Method No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1: TA100	≥ 500	≥ 1500	5000	Negative
TA 1535		≥ 1500	5000	Negative
WP2uvrA	≥ 5000	> 5000	5000	Negative
TA 98		≥ 1500	5000	Negative
TA 1537		≥ 1500	5000	Negative
Present				
Test 1: TA100		≥ 500	5000	Negative
TA 1535		≥ 1500	5000	Negative
WP2uvrA		> 5000	5000	Negative
TA 98		≥ 1500	> 5000	Negative
TA 1537		≥ 1500	> 5000	Negative

Remarks - Results	No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material either with or without metabolic activation.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Harlan (2012)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Acetone
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	20, 40*, 80*, 120*, 160	4 h	20 h
Test 2	20, 40*, 80*, 120*, 160	4 h	20 h
Test 2	120*, 160, 240	4 h	44 h
Present			
Test 1	20, 40*, 80*, 120*, 160	4 h	20 h
Test 2	20, 40, 80, 120*, 160*, 240*	4 h	20 h
Test 2	120, 160*, 240	4 h	44 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	≥ 312.5	≥ 160	> 160	Negative
Test 2		≥ 120	> 160	Negative
Test 2		≥ 120	> 160	Negative
Present				
Test 1	≥ 312.5	≥ 120	> 160	Negative
Test 2		≥ 240	> 240	Negative
Test 2		≥ 240	> 240	Negative

Remarks - Results	The test substance did not induce a significant increase in the numbers of polyploidy cells at any dose level in any of the treatment cases.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Harlan (2010a)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma cell line (L5178Y cells)
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks – Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	5*, 10*, 20*, 40*, 60*, 80	4 h	2 days
Test 2	2.5*, 5*, 10*, 20*, 30*, 40*	24 h	2 days
Present			
Test 1	10*, 20*, 40*, 60*, 80*, 160*, 240	4 h	2 days
Test 2	20*, 40*, 80*, 160*, 200, 240	4 h	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	≥ 39.06	≥ 40	> 80	Negative
Test 2	≥ 19.53	≥ 30	> 40	Negative
Present				
Test 1	≥ 156.25	≥ 160	> 240	Negative
Test 2		≥ 160	> 240	Negative

Remarks – Results	The test substance did not induce any toxicologically significant dose-related increases in the mutant frequency with and without metabolic activation.
CONCLUSION	The notified chemical was non mutagenic to L5178Y cells treated in vitro under the conditions of the test.
TEST FACILITY	Safepharm (2004b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	MHLW/PFSB Notification No. 1121003 dated November 21, 2003, METI/MIB Notification No. 3 dated November 17, 2003, and MOE/EPB Notification No. 031121004, last revised on July 4, 2008, equivalent to OECD TG 301 Ready Biodegradability (stated by the notifier)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	
Analytical Monitoring	The biochemical oxygen demand (BOD)
Remarks - Method	The test was conducted according to the above mentioned test guidelines. No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	34	7	88
14	43	14	99
21	50	21	100
28	56	28	100

Remarks - Results	All test validation criteria were satisfied. The test substance attained 56% degradation after 28 days. However, despite attaining in excess of 50% biodegradation, the test substance failed to satisfy the 10 day window validation criterion by which 60% degradation must be attained within 10 days of the degradation exceeding 10%. Therefore, the test substance is not considered to be readily biodegradable under the strict terms of the test. During analysis, molybdenum was not detected in the organic phase of the test substance degradation system and all amounts were detected from the aqueous phase. Based on this it was concluded that the test substance disappeared during degradation with molybdenum trioxide converted to the corresponding acid and water soluble structurally modified substances were produced.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	MCMC (2009)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	New Chemical Substances Bioconcentration test of chemical substances in fish and shellfish (Yakushokuhatsu No.1121002, Heisei 15.11.13 Seikyoku No.2, Kanpokihatsu No.031121002, November 21, 2003) : Continuous flow-through dilution system. The method is equivalent to OECD Guidelines 305 (1996) “Bioconcentration: Flow-through Fish Test”.
Species	Carp *(Cyprinus carpio)*

Exposure Period	Exposure: 61 days
Auxiliary Solvent	Tetrahydrofuran (25 ppm)
Concentration Range	Nominal: 0.2 mg/L Actual: 0.02 mg/L
Analytical Monitoring	Liquid Chromatography- Mass Spectrometry (LCMS)
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Taking into consideration the complex composition of the test substance, five components referred to as A (m/z: 477.4), B (m/z: 488.4), C (m/z: 498.4) D (m/z: 535.5) and E (m/z: 540.4) were analysed and evaluated for their bioconcentration potential. During the exposure period, the concentration of the test substance in water and fish was measured periodically. There was no depuration period. The bioconcentration factor (BCF) was determined by comparing the concentration of the test substance in the fish to the mean concentration of test substance in the test water. Test conditions were 24 ± 2 °C, pH 7.2-7.6 and 7.4-8.5 mg O_2/L.
RESULTS	High concentration = 6-9
Bioconcentration Factor	Low concentration = 52-78
CT50	Not determined

The bioconcentration factors (BCF) during exposure period are shown in the table below.

Component	BCF at high Concentration level	BCF at low concentration level
A	< 8	< 72 - < 78
B	< 7 - < 8	< 76 - < 84
C	< 5 - 9	< 52 - < 53
D	< 6 - < 7	< 61 - < 63

Measured concentrations of the components A and B were widely varied and could not be maintained within ±20% indicating that one of the validity criteria was not full filled. However, this could be attributed to the instability of these components in water. Additionally, it was found in the preliminary analysis that the concentrations of the components A and B decreased to 44% and 30% as compared to the initial concentrations. Therefore, it is concluded that this variation could be technically inevitable.

Conclusion	Under the conditions of this test, the notified chemical is not considered to be bioaccumulative to fish.
Test Facility	MCMC (2011)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test –Semi - Static.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Dimethylformamide (DMF) 100 µl/L
Water Hardness	100 mg $CaCO_3$/L

Analytical Monitoring	ICP-AES for molybdenum concentration
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test solution was prepared by dissolving 1 g test substance in DMF and the volume was adjusted to give a 1.0 g/10 mL stock solution. The stock solution was diluted to give the required test concentrations. A range finding test indicated that the test substance precipitates at a concentration in excess of 10 mg/L. Based on this a single test concentration of 10 mg/L was selected for the definitive study.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*			
Nominal		*24 h*	*48 h*	*72 h*	*96 h*
Control	20	0	0	0	0
Solvent control	20	0	0	0	0
10	20	0	0	0	0

LC50	>10 mg/L at 96 hours.
NOEC	10 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. The test sample analysis at 0, 24 and 96 h showed that the measured test concentration of molybdenum was in excess of 80% of the nominal concentration. Therefore, the results are based on nominal concentrations only.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Harlan (2010b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test - Static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Dimethylformamide (DMF) 100 µl/L
Water Hardness	270 mg $CaCO_3$/L
Analytical Monitoring	ICP-AES for molybdenum concentration
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test solution was prepared by dissolving 2.5 g test substance in DMF and the volume was adjusted to give a 2.5 g/25 mL stock solution. The stock solution was diluted to give the required test concentrations.

RESULTS

Concentration mg/L	*Number of D. magna*	*Number Immobilised*	
Nominal		*24 h*	*48 h*

Control	10	0	0
Solvent control	10	0	0
0.1	10	0	0
0.18	10	0	0
0.32	10	0	0
0.56	10	0	0
1.0	10	0	0
1.8	10	0	8
3.2	10	3	10
5.6	10	7	10
10	10	10	10

EC50	1.5 mg/L at 48 hours
NOEC	1.0 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The test sample analysis at 0 and 48 h showed that the measured test concentration of molybdenum was in excess of 80% of the nominal concentration. Therefore, the results are based on nominal concentrations only.

CONCLUSION The notified chemical is not toxic to Daphnia.

TEST FACILITY Harlan (2010c)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction test Flow - Through.

Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	Dimethylformamide (DMF)
Water Hardness	242-260 mg/L
Analytical Monitoring	High Performance Liquid Chromatography with mass selective detection (HPLC-MSD)
Remarks – Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. The test substances (375 and 235 mg) were each separately dissolved in DMF and the volume adjusted to 25 and 50 mL respectively to give the stock solution. The stock solution was each dissolved in 5 litre of reconstituted water to give the required test concentrations.

Results

Concentration tested, cumulative mean number of offspring released, number of offspring released per female daphnid (*Daphnia magna*), mean length and survival of parental daphnids.

			Concentration (mg/L)				
Test Day 21	*Control*	*Solvent*	*0.015*	*0.047*	*0.15*	*0.47*	*1.5*
Total no. of survived *Daphnia*	210	206	210	203	210	206	40
Total no. of offspring released per survived daphnid	738	722	755	729	729	756	0

Mean length (mm)	3.9	3.8	3.9	3.9	4.0	4.0	
No. of adult daphnids Immobilised	1	0	2	2	0	0	0
% Survival	100	90	100	90	100	90	0

21 day NOEC 0.47 mg/L

Remarks – Results

The validity criteria for the test were met.

Exposure of *Daphnia magna* to the test substance resulted in significant mortalities at the test concentration of 1.5 mg/L resulting in 100% mortalities by day 6.

The 14 and 21 day EC50 values based on nominal concentrations for the parental Daphnia generation (P1) were calculated to be 0.84 and 0.79 mg/L with 95% confidence limits of 0.47-1.5 mg/L and 0.67-0.93 mg/L respectively.

The first young offspring released from their parent animals were recorded in the control, solvent control and at all test concentrations except at 1.5 mg/L at day 7- 8. Thus, the time of first brood was not affected by the test substance up to 1.5 mg/L test concentration.

The NOEC was determined to be 0.47 mg/L as a mean measured concentration. All the endpoints were determined by the study author and are considered acceptable.

CONCLUSION

The notified chemical is considered toxic to daphnids on a chronic basis.

TEST FACILITY

Safepharm (2005)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 201 Alga, Growth Inhibition Test.

Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.625, 1.25, 2.5, 5.0 and 10 mg/L
Auxiliary Solvent	Dimethylformamide (DMF)
Water Hardness	Not reported
Analytical Monitoring	ICP-AES for molybdenum concentration

Remarks - Method

The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

The test solution was prepared by dissolving 1.0 g test substance in DMF and the volume was adjusted to give a 1 g/10 mL stock solution. The stock solution was diluted to give the required test concentrations.

RESULTS

Biomass		*Growth*	
EyC50 *mg/L at 72 h*	*NOEC* *mg/L*	*ErC50* *mg/L at 72 h*	*NOEC* *mg/L*
1.5	0.625	4.0	0.625

Remarks - Results: All validity criteria for the test were satisfied. The test sample analysis at 0 and 72 h showed that the measured test concentration of molybdenum was in excess of 80% of the nominal concentration. Therefore, the results are based on nominal concentrations only.

CONCLUSION: The notified chemical is toxic to algae.

TEST FACILITY: Harlan (2010d)

C.2.5. Acute study in earthworm

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 207 Earthworms, Acute toxicity test
EXPOSURE PERIOD: 14 days
Species: Earthworm (*Eisenia foetida)*
Remarks – Method: Test substance (5000 mg) was dissolved in an appropriate amount of tetrahydrofuran (THF) and the volume was adjusted to give a 5000 mg/50 mL stock solution. The stock solution was further diluted to give the required test concentrations. An aliquot (35 mL) of the 5000 mg/50 mL solution was added to approximately 200 g of artificial soil and the solvent was allowed to evaporate off prior to incorporation into 3.5 kg (dry weight) of formulated artificial soil to give the test concentrations of 1000 mg/kg dry weight soil.

Earthworms (10) were subjected to single exposure to nominal concentration of 1000 mg/kg dry weight. The animals were observed on day 7 and 14 for mortality and other visible behavioural or pathological signs.

RESULTS

Concentration mg/kg	Number of earthworms	Mortality 7 days	Mortality 14 days
Control	10	0	0
Solvent	10	0	0
1000	10	0	0

Remarks – Results: All validity criteria for the test were satisfied. The 14 d LC50 was out of the tested concentration range (> 1000 mg/kg dry weight).

CONCLUSION: The notified chemical is not toxic to earthworm.

TEST FACILITY: Harlan (2011a)

C.2.6. Nitrogen Transformation Test

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 216, Soil Microorganisms: Nitrogen transformation test
EXPOSURE PERIOD: 28 days
Remarks - Method: The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.

Test substance (5000 mg) was dissolved in an appropriate amount of tetrahydrofuran (THF) and the volume was adjusted to give a 5000 mg/50

	mL stock solution. An aliquot (1 mL) of the stock solution was added drop wise to 5 g of quartz sand and the solvent allowed to evaporate. To this dried mixture 2.5 g of powdered Lucerne-green-grass was added followed by 0.5 kg of dry soil. This stock dry soil was then used to prepare test concentrations of 10, 32, 100, 320 and 1000 mg/kg dry weight with a nominal moisture content of 40% of water.
RESULTS	
Remarks - Results	All validity criteria for the test were satisfied. The test substance showed no significant effect on the nitrogen transformation activity of soil organisms at a test concentration of 1000 mg/kg soil dry weight over a period of 28 days.
CONCLUSION	The notified chemical has no long term effects on nitrogen transformation in soil.
TEST FACILITY	Harlan (2011b)

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