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**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
(NICNAS)**

PUBLIC REPORT

Ethanol, 2,2'-(octylimino)bis-

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 in the NICNAS *Chemical Gazette*:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1709	Clariant (Australia) Pty Ltd	Ethanol, 2,2'-(octylimino)bis-	Yes	< 5 tonnes per annum	Component of brake fluids

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification

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

<i>Hazard Classification</i>	<i>Hazard Statement</i>
Acute toxicity oral (Category 4)	H302 – Harmful if swallowed
Skin irritation (Category 2)	H315 – Causes skin irritation
Eye damage (Category 1)	H318 – Causes serious eye damage

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.

<i>Hazard Classification</i>	<i>Hazard Statement</i>
Acute Category 2	H401 – Toxic to aquatic life
Chronic Category 3	H412 – Harmful 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 proposed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
 - Acute toxicity oral (Category 4): H302 – Harmful if swallowed
 - Skin irritation (Category 2): H315 – Causes skin irritation
 - Eye damage (Category 1): H318 – Causes serious eye damage

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

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical during repackaging:
 - Enclosed/automated processes if possible
- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during repackaging or final use:
 - 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 repackaging or final use:
 - Impervious gloves
 - Safety glasses or goggles
 - 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.

Storage

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

Emergency procedures

- Spills or accidental release of the 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 brake fluids, 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 SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)

Clariant (Australia) Pty Ltd (ABN 30 069 435 552)
Level 3, 3 Acacia Place
296-324 Ferntree Gully Road
NOTTING HILL VIC 3168

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: other names, analytical data, degree of purity, impurities, additives/adjuvants and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)

Schedule data requirements are varied for hydrolysis as a function of pH, adsorption/desorption, flammability, explosive properties, oxidising properties, acute inhalation toxicity and bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)

None

NOTIFICATION IN OTHER COUNTRIES

EU

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)

Genamin 3920

CAS NUMBER

15520-05-5

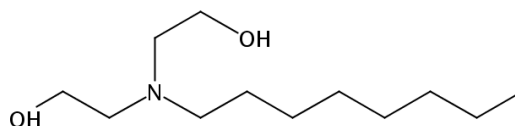
CHEMICAL NAME

Ethanol, 2,2'-(octylimino)bis-

MOLECULAR FORMULA

C₁₂H₂₇NO₂

STRUCTURAL FORMULA



MOLECULAR WEIGHT

217.35 g/mol

ANALYTICAL DATA

Reference NMR, IR, GC, UV-Vis spectra were provided.

3. COMPOSITION

DEGREE OF PURITY

90 - 99 %

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear, light brown liquid

<i>Property</i>	<i>Value</i>	<i>Data Source/Justification</i>
Melting Point/Freezing Point	≤ -20 °C at 100.2 kPa	Measured
Boiling Point	319 ± 5 °C at 102.1 kPa	Measured
Density	931 kg/m ³ at 20 °C	Measured
Viscosity	78.6 ± 2.0 mPa.s at 20°C 32.3 ± 0.8 mPa.s at 40°C	Measured
Vapour Pressure	8.1 × 10 ⁻⁶ kPa at 20 °C	Measured
Water Solubility	1.4 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains no readily hydrolysable groups
Partition Coefficient (n-octanol/water)	log Pow = 2.8 at 20 °C	Measured
Surface Tension	27.5 mN/m at 20 °C	Measured
Adsorption/Desorption	Not determined	Not expected to sorb to soil
Dissociation Constant	pKa = 8.9 at 22 °C	Measured
Flash Point	161 ± 2 °C at 101.3 kPa	Measured
Flammability	Not determined	Not expected to be highly flammable based on the measured flash point
Autoignition Temperature	270 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that imply oxidising 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 submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*, as adopted for industrial chemicals in Australia.

The notified chemical has a flash point of 161 °C which is greater than 93 °C. Based on *Australian Standard AS1940* definitions for combustible liquid, the notified chemical may be considered as a Class C2 combustible liquid if the chemical has a fire point below the boiling point.

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will not be manufactured in Australia. It will be imported into Australia in finished brake fluid at < 3% concentration.

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

<i>Year</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
<i>Tonnes</i>	< 1	< 2	< 3	< 4	< 5

PORT OF ENTRY

Melbourne and Sydney

IDENTITY OF MANUFACTURER

Clariant Produkte (Deutschland) GmbH

TRANSPORTATION AND PACKAGING

The brake fluids containing the notified chemical will be imported in 1,000 L IBCs, 200 L steel or plastic drums or in 0.25 – 40 L plastic containers. The imported products will be transported by road or rail to local repackaging facilities or retailer warehouses for further distribution nationwide.

USE

The notified chemical will be used as a component of brake fluid (at < 3% concentration) for use in automobiles.

OPERATION DESCRIPTION

The notified chemical will not be manufactured or reformulated in Australia. Repackaging of the imported brake fluids in 1000 L IBCs or 200 L drums into smaller containers will occur.

Repackaging

Repackaging will be carried out using 3 or 4 packing lines. The packing lines are automated and operators are involved in attaching and detaching suction nozzles that pump the brake fluids either directly into the filling line or into header tanks.

End use

Workers will add the brake fluids containing the notified chemical into car reservoirs where hoses are connected to the drum and fluids pumped via an automated system. Workers or do-it-yourself (DIY) users may also pour fluids manually from the container into the car reservoir.

6. HUMAN HEALTH IMPLICATIONS**6.1. Exposure Assessment****6.1.1. Occupational Exposure**

CATEGORY OF WORKERS

<i>Category of Worker</i>	<i>Exposure Duration (hours/day)</i>	<i>Exposure Frequency (days/year)</i>
Packaging operators	2-3	< 10
New vehicle production workers	2-4	< 100
Service station workers/DIY users	1	< 50

EXPOSURE DETAILS

Transport and Storage

Transport and storage workers may come into contact with the notified chemical at < 3% concentration only in the event of accidental rupture of containers.

Repackaging

The repackaging process is expected to be automated in a closed system; however, plant operators may be exposed (dermal and ocular) to the notified chemical at < 3% concentration during opening of containers, connection and disconnection of hoses when pumping into filling lines. Workers may also come into contact with the notified chemical during maintenance and cleaning.

Dermal and ocular exposure to workers will be mitigated through the use of personal protective equipment (PPE) including protective clothing, gloves and goggles as anticipated by the notifier. Inhalation exposure is not expected given the low vapour pressure of the notified chemical.

End-use

Workers at automotive service stations may experience dermal (with possibility of accidental ocular exposure) to the brake fluids containing the notified chemical (at < 3% concentration) when transferring the fluids into car reservoirs. The potential for dermal and ocular exposure may be mitigated through the use of PPE (e.g. gloves, protective clothing and goggles).

6.1.2. Public Exposure

DIY users may experience dermal (with possibility of accidental ocular exposure) to the brake fluids containing the notified chemical (at < 3% concentration) when transferring the fluids into car reservoirs. PPE may not be used by DIY users; however, exposure is not expected to be significant given the low frequency of use.

6.2. Human Health Effects Assessment

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

<i>Endpoint</i>	<i>Result and Assessment Conclusion</i>
Acute oral toxicity – rat	LD50 = 1,157 mg/kg bw; harmful
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation – rabbit	irritating
Eye irritation – rabbit	severely irritating
Skin sensitisation – guinea pig, Magnusson and Kligman	no evidence of sensitisation
Repeat dose oral toxicity – rat, 28 days	NOAEL = 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – <i>in vitro</i> mammalian gene mutation test	non mutagenic
Genotoxicity – <i>in vitro</i> chromosome aberration test	genotoxic*
Genotoxicity – <i>in vivo</i> mammalian erythrocyte micronucleus test	non genotoxic
Reproductive and developmental toxicity – rat, up to 54 days	NOAEL > 320 mg/kg bw/day

* Equivocally positive only at the highest clearly cytotoxic tested concentration

Toxicokinetics

Based on the low molecular weight (< 500 g/mol) and partition coefficient (log Pow = 2.8 at 20 °C) of the notified chemical, there is potential for the chemical to cross biological membranes.

Acute Toxicity

The notified chemical was found to be harmful via the oral route in a study conducted in rats. Oral administration of the notified chemical resulted in the deaths of 3/5 male animals treated at 2,000 mg/kg bw, 1/5 female animals treated at 800 mg/kg bw, 3/5 female animals treated at 1,250 mg/kg bw and 4/5 female animals treated at 1,600 mg/kg bw and 2,000 mg/kg bw respectively. The LD50 (female rats) was established as 1,157 mg/kg bw.

The notified chemical was found to be of low acute toxicity (LD50 > 2,000 mg/kg bw) via the dermal route in a study conducted in rats.

Irritation and Sensitisation

In an acute skin irritation study conducted on rabbits (n = 3), the notified chemical was found to be a skin irritant, with erythema and slight oedema being observed on the skin of the test animals. The effects were reversible within seven days.

The notified chemical was found to be severely irritating to eyes in an acute eye irritation study conducted using one rabbit. Application of the notified chemical resulted in redness in the conjunctiva and iris with the cornea displaying diffuse to pearly-like opacity. In addition, a clear-colourless or white to mucous effluent and bleeding of the nictitating membrane was observed. The adverse effects were not reversible by the end of 7-day observation period.

The notified chemical was found to be non-sensitising in a guinea pig maximisation test when tested at 0.5% (intradermal) and 25% (topical) at induction and 1% at challenge.

Repeated Dose Toxicity

A repeated dose oral (gavage) toxicity study on the notified chemical was conducted in rats (n = 5/sex/dose), in which the test substance was administered at 20, 100 and 500 mg/kg bw/day for 28 consecutive days, with a 14-day recovery period for high dose and control animals.

The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 500 mg/kg bw/day, based on no adverse systemic toxicity at all doses tested.

Mutagenicity/Genotoxicity

The notified chemical was found to be negative in a bacterial reverse mutation assay and an *in vitro* mammalian cell gene mutation test using Chinese hamster V79 cells. The notified chemical was equivocally positive in an *in vitro* mammalian chromosome aberration test using Chinese hamster V79 cells. The positive response was only observed at the highest clearly cytotoxic tested concentration. This result was not reproducible when tested in an *in vivo* mammalian erythrocyte micronucleus test in mouse up to 100 mg/kg bw. Based on the results of all tests conducted, the notified chemical is expected to be non-mutagenic and non-genotoxic.

Reproductive/developmental toxicity

In a repeated dose reproductive/developmental toxicity screening test in rats, the notified chemical was administered at 20, 80 or 320 mg/kg bw/day for 4 weeks minimum (males) or up to 54 days (females). The NOAEL for both systemic toxicity of animals (male and female) and reproductive/developmental toxicity was established as > 320 mg/kg bw/day, based on the absence of toxicologically relevant adverse effects at all dose levels tested.

Health Hazard Classification

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

Hazard Classification	Hazard Statement
Acute toxicity oral (Category 4)	H302 – Harmful if swallowed
Skin irritation (Category 2)	H315 – Causes skin irritation
Eye damage (Category 1)	H318 – Causes serious eye damage

6.3. Human Health Risk Characterisation**6.3.1. Occupational Health and Safety**

The notified chemical is expected to be of low systemic toxicity while presenting as a skin irritant and severe eye irritant. Given workers will only be exposed to the notified chemical at < 3% concentration, the risk of irritation effects is expected to be reduced. Furthermore, during repackaging and end uses, exposure of workers to the notified chemical is expected to be low given the use of engineering controls (such as enclosed and automated systems) and PPE (including protective clothing, impervious gloves, goggles).

Under the conditions of the occupational settings described and assessed use patterns, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health

Brake fluids containing the notified chemical (at < 3% concentration) may be used by DIY users on an infrequent basis. The potential risk to the DIY users is expected to be minimised by following safe use practices.

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

7. ENVIRONMENTAL IMPLICATIONS**7.1. Environmental Exposure & Fate Assessment****7.1.1. Environmental Exposure****RELEASE OF CHEMICAL AT SITE**

The notified chemical will not be manufactured in Australia. Release may occur during the repackaging, filling and refilling of hydraulic lines from accidental spillage both in commercial and DIY use. The notifier estimates that up to 0.1% of the total import volume of the notified chemical may be released in this way.

RELEASE OF CHEMICAL FROM USE

The finished brake fluid product will be available to both commercial garages (80%) and DIY users (20%). Motor mechanics will pump the finished brake fluids containing the notified chemical from the 200 L drums or 1000 L IBC containers into the vehicle hydraulic lines. DIY users will manually transfer the finished brake fluid containing

the notified chemical from the smaller containers into the vehicle hydraulic lines. Both motor mechanics and DIY users will manually drain spent brake fluid containing the notified chemical from the hydraulic lines during servicing. Motor mechanics are expected to collect the drained brake fluid which will be mixed into on-site waste oils.

In a recent Australian survey, it was found that only 4% of households disposed of motor oil and approximately 70% of this motor oil was correctly disposed (Aither, 2013). Although there is some uncertainty, it may be estimated based on this data that approximately 1% (0.04×0.3) of all motor oil sold could be incorrectly disposed by DIY users. Improper release of brake fluid from DIY users is expected to follow a similar pattern to that of motor oil disposal. Additionally, it is expected that a smaller proportion of the population will participate in disposal of brake fluid at a lower frequency compared to motor oil. Therefore, the release from DIY usage is expected to be < 1%.

The notifier estimates that up to 0.1% of the total import volume of the notified chemical may be released into the environment from leaks from hydraulic lines, however this is expected to be very low in volume and widely diffuse.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical are expected to remain in the empty drums and containers. Empty drums, commercial containers and DIY containers are expected to be disposed of to landfill. Other than, small amounts of oil incorrectly disposed of, the used oil containing the notified chemical is expected to be collected and mixed with on-site waste oils which are to be recycled, re-refined, possibly used as low grade burner fuel or disposed of by approved waste management contractors, in accordance with local government regulations.

7.1.2. Environmental Fate

Most of the notified chemical is expected to be disposed of by licensed waste contractors as a part of the waste oil recycling process. A minority of the notified chemical may be released from accidental spills and leaks from vehicles and from improper disposal during DIY use. In the environment, the notified chemical is not expected to be mobile in soil due to its high water solubility. The notified chemical it is not expected to bioaccumulate based on the low log Kow (Log Kow = 2.8), is readily biodegradable (94.5% after 28 days) and will eventually be degraded by biotic and abiotic processes to form water and oxides of carbon, nitrogen. For the details of the environmental fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A Predicted Environmental Concentration (PEC) was not calculated as the proposed use pattern is expected to result in limited and diffuse dispersal from accidental leaks and will lead to minimal exposure in aquatic environments.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. The endpoints for fish and bacterial respiration were derived from translated study summaries. For further details on the daphnia and algal growth tests, see Appendix C.

<i>Endpoint</i>	<i>Result</i>	<i>Assessment Conclusion</i>
Fish Toxicity	LC50 = 22 mg/L	Harmful to fish
Daphnia Toxicity	EC50 = 19.1 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	ErC50 = 1.35 mg/L NOEC = 0.253 mg/L	Toxic to algal growth
Inhibition of Bacterial Respiration	IC50 = 328 mg/L	Not harmful to bacterial respiration

Based on the above ecotoxicological endpoints for the notified chemical, the notified chemical is expected to be toxic to algal growth. Therefore, the notified chemical is classified as Acute Category 2, Toxic to aquatic life according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* (United Nations, 2009). The notified chemical is readily biodegradable and is not expected to bioaccumulate, but due to the chronic toxicity to algae it is also formally classified under the GHS for its long-term hazard as Chronic Category 3, Harmful to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration was calculated using the most sensitive endpoint (Algal growth ErC50 = 1.35) and a safety factor of 100 as at least three acute trophic endpoints were provided.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment

EC50 (Alga).	1.35	mg/L
Assessment Factor	100	
Mitigation Factor	1	
PNEC:	13.50	µg/L

7.3. Environmental Risk Assessment

A risk quotient ($Q = PEC/PNEC$) was not determined as while the notified chemical is toxic to aquatic life, the PEC is expected to be minimal. Therefore, on the basis of the low environmental exposure from the proposed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point	$\leq -20\text{ }^{\circ}\text{C}$ at 100.2 kPa
Method	OECD TG 102 Melting Point/Melting Range
Remarks	Determined using differential scanning calorimetry and through direct observation of the pour point.
Test Facility	Clariant (2012a)
Boiling Point	$319 \pm 5\text{ }^{\circ}\text{C}$ at 102.1 kPa
Method	OECD TG 103 Boiling Point
Remarks	Determined using differential scanning calorimetry
Test Facility	Clariant (2012b)
Density	931 kg/m^3 at $20\text{ }^{\circ}\text{C}$
Method	OECD TG 109 Density of Liquids and Solids
Remarks	Determined by changes of resonance frequency
Test Facility	Clariant (2012c)
Viscosity	$78.6 \pm 2.0\text{ mPa}\cdot\text{s}$ at $20\text{ }^{\circ}\text{C}$ $32.3 \pm 0.8\text{ mPa}\cdot\text{s}$ at $40\text{ }^{\circ}\text{C}$
Method	OECD TG 114 Viscosity of Liquids
Remarks	Determined using a rotational viscometer.
Test Facility	Clariant (2012d)
Vapour Pressure	$8.1 \times 10^{-6}\text{ kPa}$ at $20\text{ }^{\circ}\text{C}$ $1.3 \times 10^{-5}\text{ kPa}$ at $25\text{ }^{\circ}\text{C}$ $1.1 \times 10^{-4}\text{ kPa}$ at $50\text{ }^{\circ}\text{C}$
Method	OECD TG 104 Vapour Pressure
Remarks	Determined using a vapour pressure balance
Test Facility	Siemens (2011a)
Water Solubility	1.4 g/L at $20\text{ }^{\circ}\text{C}$
Method	OECD TG 105 Water Solubility
Remarks	Flask Method
Test Facility	Clariant (2012e)
Partition Coefficient (n-octanol/water)	$\log\text{ Pow} = 2.8$ at $20\text{ }^{\circ}\text{C}$
Method	OECD TG 117 Partition Coefficient (n-octanol/water)
Remarks	A partition coefficient was not able to be determined by the shake flask method, therefore the log Pow was calculated based on the test substances individual solubilities in water and n-octanol.
Test Facility	Clariant (2012f)
Surface Tension	$27.5 \pm 0.1\text{ mN/m}$ at $20\text{ }^{\circ}\text{C}$
Method	OECD TG 115 Surface Tension of Aqueous Solutions EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks	Concentration: 1 g/L
Test Facility	Clariant (2012g)

Dissociation Constant pKa = 8.9 at 22 °C

Method OECD TG 112 Dissociation Constants in Water
Remarks Potentiometric endpoint titration method
Test Facility Clariant (2012h)

Flash Point 161 ± 2 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point
Remarks Conducted using a petrotest PMA\$ (Pensky-Martens)
Test Facility Clariant (2012i)

Autoignition Temperature 270 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility Siemens (2011b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS**B.1. Acute Oral Toxicity – Rat**

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity
Species/Strain	Rat/Wistar
Vehicle	Sesame oil
Remarks – Method	No significant protocol deviations

RESULTS

<i>Group</i>	<i>Number and Sex of Animals</i>	<i>Dose (mg/kg bw)</i>	<i>Mortality</i>
1	5 F	800	1/5
2	5 F	1250	3/5
3	5 F	1600	4/5
4	5 F	2000	4/5
5	5 M	2000	3/5

LD50	1,157 mg/kg bw (female rats)
Signs of Toxicity	Clinical signs noted included reduced spontaneous activity, crouch, long-legged walk, uncoordinated/tumbled walk, face-down position, narrowed palpebral fissure, mydriasis, tonic clonic convulsions, abnormal breathing.
Effects in Organs	At necropsy, the male/female animals died showed ablation of the mucous membrane of the gastrointestinal tract. The small intestine of the female animals died was partial reddened and at one female animal treated at 2000 mg/kg bw it was filled with a red, mucous material (blood).
Remarks – Results	The animals showed expected body weight gains during the observation period.

CONCLUSION The notified chemical is harmful via the oral route.

TEST FACILITY Hoechst (1994a)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity
Species/Strain	Rat/Wistar CrI: WI(Han)
Vehicle	None
Type of dressing	Semi-occlusive
Remarks – Method	No significant protocol deviations

RESULTS

<i>Group</i>	<i>Number and Sex of Animals</i>	<i>Dose (mg/kg bw)</i>	<i>Mortality</i>
1	5 M/5 F	2000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Erythema and oedema (both up to grade 2) were noted in all animals, which were not reversible within the observation period.
Signs of Toxicity – Systemic	No treatment-related signs were noted.
Effects in Organs	On the skin and in the subcutis either scab or scab and one or more red or dark foci were noted in all animals.
Remarks – Results	4/5 female animals showed a slight weight loss during the first week and 1/5 female animals showed the same effect during the second week. The male animals showed weight gain during the observation period.

CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Eurofins & BSL (2016)

B.3. Skin Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	7 days
Type of Dressing	Semi-occlusive
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			
Erythema/Eschar	2	2.7	2.3	3	< 7 d	0
Oedema	0.3	0.3	1	1	< 7 d	0

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

Remarks – Results Erythema and slight oedema were observed on the skin of the test animals. The effects were reversible within seven days.

CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	Hoechst (1994b)

B.4. Eye Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	1
Observation Period	7 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
	Animal No.			
Conjunctiva – Redness	3	3	> 7 d	2
Conjunctiva – Chemosis	1.33	2	< 7 d	0
Conjunctiva – Discharge	1.33	3	< 7 d	0
Corneal Opacity	1.33	3	> 7 d	1
Iridial Inflammation	1	1	< 7 d	0

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

Remarks – Results Application of the test substance to one rabbit on one eye resulted in redness in the conjunctiva and iris. The cornea displayed diffuse to pearly-like opacity. In addition, a clear-colourless or white to mucous effluent and bleeding of the nictitating membrane was observed. The adverse effects were not fully reversible by the end of 7-day observation period.

CONCLUSION The notified chemical is severely irritating to the eye.

TEST FACILITY Hoechst (1994c)

B.5. Skin Sensitisation – Guinea Pig Maximisation Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 406 Skin Sensitisation – Magnusson and Kligman

Species/Strain Guinea pig/Crl: HA

PRELIMINARY STUDY Maximum non-irritating concentration:

Intradermal: < 0.5%

Topical: 1%

MAIN STUDY

Number of Animals Test Group: 10 F Control Group: 5 F (vehicle): 5 F (positive)

Vehicle Physiological saline 0.9% NaCl (for intradermal induction)

Vaseline (for topical induction and challenge)

Positive Control Conducted in parallel with the test substance using 2-mercaptobenzothiazole

INDUCTION PHASE Induction concentration:

Intradermal: 0.5%

Topical: 25%

Signs of Irritation Following intradermal induction, slight erythema (in 10/10) and slight oedema (in 10/10) were noted at the 24- and 48-hour readings. No signs of irritation following topical induction.

CHALLENGE PHASE

Challenge Topical: 1%

Remarks – Method No significant protocol deviations

RESULTS

<i>Animal</i>	<i>Challenge Concentration (%)</i>	<i>Number of Animals Showing Skin Reactions after Challenge</i>	
		<i>24 h</i>	<i>48 h</i>
<i>Test Group</i>	1	0	0
<i>Control Group</i>	1	0	0

Remarks – Results Visual observation following challenge did not reveal any positive skin responses at the 24- and 48-hour observations for the treatment or control group.

The validity of the test method was confirmed by the satisfactory results with the vehicle and positive controls conducted in parallel.

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

TEST FACILITY BSL (2012a)

B.6. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE Notified chemical

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

Species/Strain Rat/Wistar Crl: WI(Han) (Full Barrier)

Route of Administration Oral – gavage

Exposure Information Total exposure days: 28 days

Dose regimen: 7 days per week

Post-exposure observation period: 14 days

Vehicle Sterile water

Remarks – Method No significant protocol deviations

RESULTS

<i>Group</i>	<i>Number and Sex of Animals</i>	<i>Dose (mg/kg bw/day)</i>	<i>Mortality</i>
Control	5 per sex	0	0/10
Low Dose	5 per sex	20	0/10
Mid Dose	5 per sex	100	0/10
High Dose	5 per sex	500	5/10
Control Recovery	5 per sex	0	0/10
High Dose Recovery	5 per sex	500	0/10

Mortality and Time to Death

Five female animals treated at 500 mg/kg bw/day died during treatment. The study authors stated that mortality was the result of local irritant effects due to the physicochemical properties of the test substance and not the result of a systemic effect of the test substance. No mortality was noted in the recovery group of animals.

Clinical Observations

Dose dependent symptoms included moving the nose through the bedding, salivation, piloerection, respiratory noise, a reduction in spontaneous activity, and slow movement. These findings were considered by the study authors to be related to the local irritant effect of the test substance, which was supported by pathological alterations found microscopically.

Reduced body weight gain and food consumption for animals treated at 100 and 500 mg/kg bw/day were considered by the study authors to be related to the local irritant effect of the test substance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Mean corpuscular volume showed a statistically significant decrease in male animals dosed at 100 and 500 mg/kg bw/day at the end of the treatment period. Male animals dosed at 500 mg/kg bw/day also showed a statistically significant increase in the urea concentration. All other statistically significant changes were either not dose dependent or occurred in the recovery group only. The study authors ascribed the effects seen to the local irritant properties of the test substance.

Effects in Organs

Thymus weights showed statistically significant decreases for male animals treated at 500 mg/kg bw/day, for both the absolute values and relative to brain weight. This was considered by the study authors to be a secondary stress-related response to the local irritant effect of the test substance. The kidneys, liver and adrenal glands of male animals treated at 500 mg/kg bw/day showed statistically significant mean weight increases (of 12.82%, 16.24% and 28.10% respectively, compared to the mean weight of control group) relatively to body weights but the absolute increase, and the increase relative to brain weight did not achieve statistical significance.

Necrotizing inflammation in the forestomach was noted in animals treated at 500 mg/kg bw/day, due to irritation from the test substance.

Necrotic inflammatory lesions and the relating reactive changes noted in trachea and lung of animals treated at 500 mg/kg bw/day were considered by the study authors to be secondary events due to accidental regurgitation/aspiration.

Lymphoid atrophy of the spleen, increased incidence of thymic atrophy, and/or adrenocortical diffuse hypertrophy noted in animals treated at 500 mg/kg bw/day were considered by the study authors to be secondary responses to a stressful condition and not adverse.

The lesions described above disappeared or showed a tendency to recover after 14 days of recovery period.

For the animals that died prior to the completion of the study, necrotizing inflammation of trachea was considered by the study authors to be the direct cause of the deaths rather than them being a result of systemic exposure to the test substance. The irritating nature of the test substance was considered to have great relevancy to the accidental regurgitation/aspiration and to the eliciting of the local lesions.

Remarks – Results

All histopathological lesions related to the test substance were considered by the study authors to be the results of local irritant effects of the test substance.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 500 mg/kg bw/day in this study, based on no adverse systemic toxicity at from the test substance.

TEST FACILITY BSL (2015)

B.7. Genotoxicity – Bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test
Plate incorporation procedure (Test 1) and Pre incubation procedure (Test 2)

Species/Strain *Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, TA102

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

Concentration Range in Main Test
Test 1:
a) With metabolic activation: 3.16-5000 μ g/plate
b) Without metabolic activation: 3.16-5000 μ g/plate
Test 2:
a) With metabolic activation: 1-5000 μ g/plate
b) Without metabolic activation: 1-2500 μ g/plate

Vehicle Dimethyl sulfoxide

Remarks – Method No significant protocol deviations. The dose selection for the main tests was based on the toxicity results in a preliminary test.

RESULTS

Metabolic Activation	Test Substance Concentration (μ g/plate) Resulting in:			
	Cytotoxicity in Preliminary Test	Cytotoxicity in Main Test	Precipitation	Genotoxic Effect
<i>Absent</i>				
Test 1	≥ 1000	≥ 1000	> 5000	negative
Test 2		≥ 316	> 2500	negative
<i>Present</i>				
Test 1	≥ 2500	≥ 2500	> 5000	negative
Test 2		≥ 2500	> 5000	negative

Remarks – Results No significant increases in the frequency of revertant colonies were noted for any of the bacterial strains, with any concentration of the test substance, either 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 BSL (2012b)

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

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test

Species/Strain Chinese hamster

Cell Type/Cell Line V79

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

Vehicle
Remarks – Method

Cell culture medium
No significant protocol deviations. The dose selection for the main tests was based on the toxicity results in a preliminary test.

<i>Metabolic Activation</i>	<i>Test Substance Concentration (mM)</i>	<i>Exposure Period</i>	<i>Harvest Time</i>
<i>Absent</i>			
Test 1	0*, 0.05*, 0.1*, 0.2*, 0.4*, 0.6*, 0.8*, 1*, 1.25*, 1.5*, 1.75*	4 h	48-72 h
Test 2	0*, 0.005*, 0.0075*, 0.01*, 0.05*, 0.075*, 0.1*, 0.25*, 0.5*, 0.6*, 0.7*	20 h	48-72 h
<i>Present</i>			
Test 1	0*, 0.025*, 0.05*, 0.1*, 0.25*, 0.5*, 1*, 1.5*, 1.75*, 2*, 2.5*	4 h	48-72 h
Test 2	0*, 0.3*, 0.9*, 1.2*, 1.5*, 1.8*, 2.1*, 2.4*, 2.7*, 3*, 4*	4 h	48-72 h

*Cultures selected for metaphase analysis.

RESULTS

<i>Metabolic Activation</i>	<i>Test Substance Concentration (mM) Resulting in:</i>			
	<i>Cytotoxicity in Preliminary Test</i>	<i>Cytotoxicity in Main Test</i>	<i>Precipitation</i>	<i>Genotoxic Effect</i>
<i>Absent</i>				
Test 1	≥ 1	≥ 1.5	> 1.75	negative
Test 2	-	≥ 0.5	> 0.7	negative
<i>Present</i>				
Test 1	≥ 0.5	≥ 2.5	> 2.5	negative
Test 2	-	≥ 2.7	≥ 4	negative

Remarks – Results

The test substance did not induce any biologically relevant increases in the mutant frequency at any tested concentration in each exposure group, 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 Chinese hamster V79 cells treated *in vitro* under the conditions of the test.

TEST FACILITY

BSL (2012c)

B.9. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test

Species/Strain

Chinese hamster

Cell Type/Cell Line

V79

Metabolic Activation System

S9 mix from β-naphthoflavone/phenobarbitone induced rat liver

Vehicle

Remarks – Method

No significant protocol deviations. The dose selection for the main tests was based on the toxicity results in a preliminary test.

<i>Metabolic Activation</i>	<i>Test Substance Concentration (mM)</i>	<i>Exposure Period</i>	<i>Harvest Time</i>
<i>Absent</i>			
Test 1	0*, 0.25, 0.5, 1*, 1.5*, 2.5*, 3.2, 4, 5	4 h	24 h
<i>Present</i>			
Test 1	0*, 0.25, 0.5, 1*, 2*, 3.5*, 5, 6.5, 8, 10	4 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

<i>Metabolic Activation</i>	<i>Test Substance Concentration (µg/mL) Resulting in:</i>			
	<i>Cytotoxicity in Preliminary Test</i>	<i>Cytotoxicity in Main Test</i>	<i>Precipitation</i>	<i>Genotoxic Effect</i>
<i>Absent</i>				
Test 1	≥ 2.5	≥ 2.5	> 5	positive
<i>Present</i>				
Test 1	≥ 10	≥ 10	> 10	positive

Remarks – Results An increase of aberrant cells was noted at 2.5 mM without metabolic activation (6.5%) and at 3.5 mM with metabolic activation (7.5%). These values were outside of the historical control values for the laboratory, which are 0-4.0%. However, the positive response was only observed at the highest clearly cytotoxic tested concentration.

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

CONCLUSION The notified chemical was clastogenic to Chinese hamster V79 cells treated *in vitro* under the conditions of the test.

TEST FACILITY BSL (2013a)

B.10. Genotoxicity – *In Vivo* Mammalian Erythrocyte Micronucleus Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test
 Species/Strain Mouse/NMRI
 Route of Administration Intraperitoneal injection
 Vehicle Cottonseed oil
 Remarks – Method No significant protocol deviations. The dose selection for the main test was based on the toxicity results in a preliminary test.

<i>Group</i>	<i>Number and Sex of Animals</i>	<i>Dose (mg/kg bw)</i>	<i>Sacrifice Time (hours)</i>
I (vehicle control)	5 M/5 F	0	44
II (low dose)	5 M/5 F	20	44
III (mid dose)	5 M/5 F	50	44
IV (high dose)	5 M/5 F	100	44
V (positive control, CP)	5 M/5 F	40	44
VI (vehicle control)	5 M/5 F	0	68
VII (high dose)	5 M/5 F	100	68

CP = cyclophosphamide

RESULTS

Doses Producing Toxicity Animals treated at 20 mg/kg bw or 50 mg/kg bw showed no or slight signs of systemic toxicity. Signs of systemic toxicity noted in animals treated at 100 mg/kg bw included reduction of spontaneous activity, constricted abdomen, half eyelid closure, recumbency, opisthotonos and tremor.

Genotoxic Effects No biologically relevant increase of micronuclei was found after treatment with the test substance in any of the dose groups evaluated.

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

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* mammalian erythrocyte micronucleus test.

TEST FACILITY BSL (2013b)

B.11. Developmental Toxicity – Rats

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 421 Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Exposure days: Males - 4 weeks minimum (14 days prior to pairing and 14 days through the pairing) Females – up to 54 days (14 days prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum) Post-exposure observation period: none
Vehicle	<i>aqua ad iniectabilia</i>
Remarks – Method	No significant protocol deviations

RESULTS

Group	Number of Animals	Dose (mg/kg bw/day)	Mortality
1	10 per sex	0	0/20
2	10 per sex	20	1/20
3	10 per sex	80	0/20
4	10 per sex	320	0/20

Mortality and Time to Death

There was no test substance-related mortalities. One female treated at 20 mg/kg bw/day was found dead on pre-mating day 5 and the cause was considered by the study authors to be accidental influx (regurgitation or aspiration) into the respiratory tract.

Effects on Dams

There were no adverse clinical signs caused by systemic exposure to the test substance. Clinical signs including salivation, piloerection and abnormal breathing in most animals treated at 80 or 320 mg/kg bw/day were considered by the study authors to be due to discomfort or the irritancy of the test substance.

There were no adverse changes in body weights, body weight gain and food consumption. The decreases in body weight gain and food consumption in animals treated at 320 mg/kg bw/day were minimal and transient.

There were no statistically significant or toxicologically relevant changes for litter data (including total number of pups born, still birth and runts on post natal day (PND) 0 and number of live pups, number of male and female pups and sex ratio on PND 0 and PND 4), litter weight data (including pup mean weight, total litter weight and male and female litter weight on PND 0 and 4), the duration of pre-coital or gestation, pre and post natal data (including number of corpora lutea, number of implantation sites, percent pre and post implantation loss and number of live pups).

There were no toxicologically relevant changes for reproductive and viability indices and absolute and relative reproductive organ weights.

Fluid content of trachea, red spots of thymus and discoloured red of axillary lymph node were recorded in the female animal that died prematurely at necropsy. It was considered by the study authors that the tracheal fluid content was associated with accidental influx (regurgitation or aspiration) of the dosing solution into the respiratory tract and the red spots of thymus and discoloured (red) axillary lymph node (both correlated microscopically with congestion) were non-specific changes which are commonly recorded in the dead animals.

There were no histological changes related to treatment in the organs and tissues of the reproductive system (i.e. testes, epididymides, prostate, seminal vesicles, coagulating glands, ovaries, uterus and cervix, and vagina).

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO ₂ Evolution Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC)
Remarks – Method	No deviations were noted. A toxicity control was also conducted using 8 mg/L test substance and 20 mg/L reference substance (sodium benzoate).

RESULTS

<i>Test Substance</i>		<i>Sodium benzoate</i>		<i>Toxicity control</i>	
<i>Day</i>	<i>% Degradation</i>	<i>Day</i>	<i>% Degradation</i>	<i>Day</i>	<i>% Degradation</i>
6	22.4	6	67	6	49
14	59	14	83	14	81
21	74	21	89	21	91
28	94.5	28	90	28	96

Remarks – Results The toxicity control reached > 25% degradation by day 14 and therefore the test substance is not considered toxic to the inoculum. All validity criteria were met. The difference in extremes of the test samples was < 20% and the reference substance reached pass levels by day 6, the CO₂ evolution in the inoculum blank was 29.7 mg/L.

CONCLUSION The test substance is readily biodegradable

TEST FACILITY Noack (2012)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	<i>Daphnia magna</i>
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	160 - 180 mg CaCO ₃ /L
Analytical Monitoring	LC-MS
Remarks – Method	No deviations were noted. A reference test using potassium dichromate was conducted within a month of the definitive test.

RESULTS

<i>Concentration (mg/L)</i>		<i>Number of D. magna</i>	<i>Number Immobilised</i>	
<i>Nominal</i>	<i>Actual</i>		<i>24 h</i>	<i>48 h</i>
Control	-	20	0	0
6.25	6.05	20	1	1
12.5	13.3	20	3	8

25	26.1	20	2	11
50	49.7	20	16	20
100	101.5	20	20	20

LC50 40.7 mg/L at 24 hours
19.1 mg/L at 48 hours
LOEC 12.5 mg/L at 48 hours
Remarks – Results All validity criteria were met. Temperature was maintained between 19 – 20°C, pH was maintained between 7.76 – 8.97 and the dissolved oxygen was maintained at > 8.49 mg O₂/L
The reference test showed an EC50 of 1.74 mg/L which is within the expected range for potassium dichromate (0.6 – 2.1 mg/L)

CONCLUSION The test substance is harmful to aquatic invertebrates.

TEST FACILITY Noack (2013a)

C.2.2. Algal Growth Inhibition Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test

Species

Exposure Period 72 hours

Concentration Range Nominal: 0.08 – 8.0 mg/L

Actual: 0.087 - 8.39 mg/L

Auxiliary Solvent None

Analytical Monitoring LC-MS

Remarks – Method No deviations were noted. Potassium dichromate was used as a positive control sample.

RESULTS

<i>ErC50</i> <i>(mg/L)</i>	<i>Growth</i>	<i>NOEC</i> <i>(mg/L)</i>	<i>EyC50</i> <i>(mg/L)</i>	<i>Yield</i>	<i>NOEC</i> <i>(mg/L)</i>
1.35		0.253	0.397		0.08

Remarks – Results All validity criteria were met. The control sample experienced a 175-fold growth rate, the coefficients of variation for section-by-section specific growth rates of the control sample was 20.4% and the coefficient of variation of average specific growth rate for the control sample was 1.99%.

The ErC50 for potassium dichromate was determined to be 0.686 mg/L.

CONCLUSION The test substance is toxic to algal growth

TEST FACILITY Noack (2013b)

BIBLIOGRAPHY

- BSL (2012a) Test for Sensitisation (Guinea Pig Maximisation Test) with Genamin 3920 (Study No. 120516, May, 2012). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- BSL (2012b) Reverse Mutation Assay using Bacterial (*Salmonella typhimurium*) with Genamin 3920 (Study No. 120517, April, 2012). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- BSL (2012c) *In vitro* Mammalian Cell Gene Mutation test (HPRT-Locus) in Chinese Hamster V79 Cells with Genamin 3920 (Study No. 120519, June, 2012). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- BSL (2013a) *In vitro* Mammalian Chromosome Aberration Test in Chinese Hamster V79 Cells with Genamin 3920 (Study No. 120518, January, 2013). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- BSL (2013b) Mammalian Micronucleus Test of Murine Peripheral Blood Cells with Genamin 3920 (Study No. 130288, June, 2013). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- BSL (2014) Reproduction/Developmental Toxicity Screening Test after Oral Administration in Wistar Rats with Genamin 3920 (Study No. 134234, August, 2014). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- BSL (2015) 28-Day Repeated Dose Oral Toxicity Study in Wistar Rats with Genamin 3920 including a 14-day Recovery Period (Study No. 134233, November, 2015). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).
- Clariant (2012a) Genamin 3920: Melting Point (Study No. 11-105747.2, February, 2012). Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished report submitted by the notifier).
- Clariant (2012b) Genamin 3920: Boiling Point (Study No. 11-105747.1, February, 2012). Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished report submitted by the notifier).
- Clariant (2012c) Genamin 3920: Density (Study No. 11-105747.3, February, 2012). Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished report submitted by the notifier).
- Clariant (2012d) Genamin 3920: Viscosity (Study No. 11-105747.6, February, 2012). Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished report submitted by the notifier).
- Clariant (2012e) [Notified Chemical]: Water Solubility (Report No. 11-105638-2, February 2012), Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished document submitted by the notifier).
- Clariant (2012f) Genamin 3920: Estimation of the Partition Coefficient (n-Octanol/Water) (Report No. 11-105747.7, March 2012), Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished document submitted by the notifier).
- Clariant (2012g) Genamin 3920: Surface Tension (Study No. 11-105747.4, February, 2012). Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished report submitted by the notifier).
- Clariant (2012h) [Notified Chemical]: pKa (Report No. 11-105638-4, 22 May 2012), Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished document submitted by the notifier).
- Clariant (2012i) Genamin 3920: Flash Point (Study No. 11-105747.5, February, 2012). Frankfurt am Main, Germany, Clariant Produkte (Deutschland) GmbH (Unpublished report submitted by the notifier).
- Eurofins & BSL (2016) Acute Dermal Toxicity (Limit Test) in the Rat with Genamin 3920 (Study No. 157154, April, 2016). Munich, Germany, Eurofins BioPharma Product Testing Munich GmbH & Bioservice Scientific Laboratories Munich GmbH (Unpublished report submitted by the notifier).
- Hoechst (1994a) Caprylamin + 2 EO: Prüfung der akuten oralen Toxizität an der Wistar-Ratte (Study No. 94.0777, November, 1994). Frankfurt am Main, Germany, Hoechst Aktiengesellschaft (Unpublished report submitted by the notifier) (Report submitted in German with English translation).
- Hoechst (1994b) Caprylamin + 2 EO: Prüfung auf Hautreizung am Kaninchen (Study No. 94.0744, October, 1994). Frankfurt am Main, Germany, Hoechst Aktiengesellschaft (Unpublished report submitted by the notifier) (Report submitted in German with English translation).

- Hoechst (1994c) Caprylamin + 2 EO: Prüfung auf Augenreizung am Kaninchen (Study No. 94.0800, November, 1994). Frankfurt am Main, Germany, Hoechst Aktiengesellschaft (Unpublished report submitted by the notifier) (Report submitted in German with English translation).
- Noack (2012) [Notified Chemical]: Ready Biodegradability Modified Sturm Test (Study No. AST14822, May 2012), Sarstedt, Germany, Dr U Noack-Laboratorien, (Unpublished document submitted by the notifier).
- Noack (2013a) [Notified Chemical]: Acute Immobilisation Test to Daphnia magna, Static, 48 h (Study No. DA114822, August 2013), Sarstedt, Germany, Dr U Noack-Laboratorien, (Unpublished document submitted by the notifier).
- Noack (2013b) [Notified Chemical]: Alga, Growth Inhibition Test with Desmodesmus subspicatus, 72 hours (Study No. SSO14822, August 2013), Sarstedt, Germany, Dr U Noack-Laboratorien, (Unpublished document submitted by the notifier).
- Siemens (2011a) Genamin 3920: Vapour Pressure (Study No. 20110306.01, October, 2011). Frankfurt am Main, Germany, Siemens AG (Unpublished report submitted by the notifier).
- Siemens (2011b) Genamin 3920: Auto-Ignition Temperature (Liquids and Gases) (Study No. 20110306.02, September, 2011). Frankfurt am Main, Germany, Siemens AG (Unpublished report submitted by the notifier).
- SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, <https://www.safeworkaustralia.gov.au/doc/model-code-practice-managing-risks-hazardous-chemicals-workplace>
- United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html>