File No: STD/1594

February 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Phosphoric acid, mixed esters with Bu alc. and ethylene glycol

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 and Energy.

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/1594	Clariant (Australia) Pty Ltd	Phosphoric acid, mixed esters with Bu alc. and ethylene glycol	Yes	20 tonnes per annum	Additive in automotive fluids

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is 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 recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Flammable Liquids (Category 4)	H227 – Combustible liquid
Eye damage (Category 1)	H318 – Causes serious eye damage

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 assessed use pattern and the expected low hazard to aquatic life, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Flammable Liquids (Category 4): H227 – Combustible liquid
  - 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 and the intended use/exposure scenario.

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself. However, these should be selected on the basis of all ingredients in the formulation.

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

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

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

Disposal

- The notified chemical should be disposed of in accordance with local regulations for recycling, re-use or recovery of calorific content.

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.

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(1) of the Act; if
  - the notified chemical is imported at ≥ 1%;
  - the notified chemical is imported for reformulation in Australia;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from an additive in automotive 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.

(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)
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 claimed exempt from publication: use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Adsorption/desorption, hydrolysis as a function of pH, flammability limits, acute dermal toxicity, acute inhalation toxicity, genotoxic damage in vivo, ready biodegradability, bioaccumulation, fish toxicity, daphnia toxicity, algal toxicity and inhibition of the respiration of activated sludge.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Europe (REACH-2015), China (IESC-2013)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Hordaphos MDGB

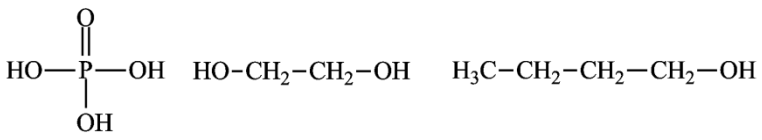
CAS NUMBER
84962-20-9

CHEMICAL NAME
Phosphoric acid, mixed esters with Bu alc. and ethylene glycol

OTHER NAMES
Reaction mass of phosphoric acid mono-(2-hydroxy-ethyl) ester and phosphoric acid monobutyl ester and phosphoric acid bis-(2-hydroxy-ethyl) ester
Phosphoric acid, mixed esters with ethylene glycol and butanol, mixed butyl ethylene glycol phosphates

MOLECULAR FORMULA
$C_4H_{10}O.C_2H_6O_2.H_3O_4P$

STRUCTURAL FORMULA



Component 1 Component 2 Component 3

MOLECULAR WEIGHT
142 – 256 Da
The molecular weight will depend on the degree of esterification

ANALYTICAL DATA
Reference NMR, IR, GC and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	1-Butanol		
CAS No.	71-36-3	*Weight %*	1-5
Hazardous Properties	Flammable liquid – Cat 3 (H226) Acute toxicity- Cat 4 (H302) STOT- SE (H335) Skin irritation – Cat 2 (H315) Eye damage – Cat 1 (H318) STOT – SE (H336)		
Chemical Name	1,2-Ethanediol		
CAS No.	107-21-1	*Weight %*	1-2
Hazardous Properties	Acute toxicity – Cat 4 (H302)		
Chemical Name	Phosphoric acid tributyl ester		
CAS No.	126-73-8	*Weight %*	< 0.1
Hazardous Properties	Acute toxicity – Cat 4 (H302) Acute toxicity – Cat 3 (H331) Carcinogenicity – Cat 2 (H351) Skin irritation – Cat 2 (H315)		
Chemical Name	Phosphoric acid		
CAS No.	7664-38-2	*Weight %*	5-15
Hazardous Properties	Skin corrosion – Cat 1B (H314)		
Chemical Name	Phosphoric acid butyl ester bis-(2-hydroxy-ethyl) ester		
CAS No.	-	*Weight %*	< 1
Chemical Name	Phosphoric acid dibutyl ester 2-hydroxy-ethyl ester		
CAS No.	-	*Weight %*	≤ 1

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)

Chemical Name	Water		
CAS No.	7732-18-5	*Weight %*	2-10

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: clear to yellowish viscous liquid with ester like odour

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -50 °C	Measured
Boiling Point	50 -230 °C	Measured
Density	1,358 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.8×10^{-6} kPa at 20 °C	Measured
Water Solubility	> 1000 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Hydrolytically stable	Measured (analogue)

Partition Coefficient (n-octanol/water)	log Pow < -0.8 at 22 °C	Measured
Adsorption/Desorption	Not determined	The chemical is not expected to partition to soil from water based on the high water solubility
Dissociation Constant	pKa_1 = 2.6 and pKa_2 = 7.3 at 25 °C	Measured
Surface tension	52.7 mN/m 20 °C	Measured
Flash Point	71.5 °C at 101.3 kPa	Measured
Autoignition Temperature	315 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not oxidising	Measured

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 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 recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Flammable Liquids (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured and/or reformulated in Australia. It will imported into Australia in automotive fluids at concentrations < 0.01%.

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

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

PORT OF ENTRY
Melbourne

IDENTITY OF RECIPIENT
Clariant Australia Pty Ltd
Level 3, 3 Acacia Place, 296-324 Ferntree Gully Road
NOTTING HILL VIC 3168

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in automotive fluids at < 0.01% concentration in containers of various sizes including 1 L or 20 L steel cans and 75 kg PE tight headed drums, and transported by road in Australia.

USE
The notified chemical will be used as an additive in automotive fluids at < 0.01% concentration, that may be used by professional workers and the public.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia as an additive in automotive fluids at < 0.01% concentration.

At end-use sites, the automotive fluids containing the notified chemical at < 0.01% concentration will be transferred (by automated or manual means) to the vehicles.

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)*
Stevedores	2-3	10-15
Transport	6	260
Warehousing	6	260
Industrial workers	1	260
Professional workers	1	260

EXPOSURE DETAILS

Transport and storage
Stevedores, transport workers, and workers at warehousing facilities are not expected to be exposed to the notified chemical in automotive fluids except in the unlikely event of an accident.

Industrial and professional workers
Workers may be exposed to automotive fluids containing the notified chemical at < 0.01% concentration during use, for example, at automotive service centres or car dealerships during transfer, charging or top-up activities. Professional users such as mechanics may experience dermal or ocular exposure to the automotive fluids containing the notified chemical (< 0.01% concentration) when transferring automotive fluids to vehicles. The potential for dermal and ocular exposure may be mitigated through the use of PPE, such as suitable protective clothing, goggles and impervious gloves.

Overall, worker exposure to the notified chemical (< 0.01% concentration in finished automotive fluids) is not expected to be significant.

6.1.2. Public Exposure
The finished automotive fluids containing the notified chemical at < 0.01 concentration will be available to the general public. DIY users may experience inadvertent dermal and ocular exposure to automotive fluids containing < 0.01% of the notified chemical when maintaining their vehicles. However, once automotive fluids containing the notified chemical are added to the vehicles, further exposure is not expected.

Overall, public exposure is expected to be very low due to the infrequent use and the very low concentration of the notified chemical in finished automotive fluids.

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 3,575 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	severely irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, reproductive/developmental/repeat dose toxicity – Dose range finding study	inconclusive
Rat, repeat dose oral toxicity combined with reproductive/ developmental toxicity screening	NOAEL 200 mg/kg bw/day (parental toxicity) NOEL 500 mg/kg bw/day (reproduction and developmental toxicity)

Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non clastogenic
Genotoxicity – in vitro mammalian cell gene mutation test	non genotoxic

Toxicokinetics, metabolism and distribution
No information on toxicokinetics, metabolism and distribution of the notified chemical was provided. Based on the molecular weight (142-256 Da), water solubility (> 1000g/L) and partition coefficient (log Pow <-0.8) the notified chemical is likely to cross biological membranes. The repeated dose toxicity study conducted on the notified chemical below confirms this.

Acute toxicity
The notified chemical was found to be of low toxicity via the oral route with an LD50 of 3,575 mg/kg bw. No information on acute dermal and inhalation toxicity was provided.

Irritation and sensitisation
The notified chemical was slightly irritating to the skin when test on rabbits and severely irritating to the eyes of rabbits. Irreversible changes to the cornea were reported for all test animals.

The notified chemical was non sensitising when tested in a mouse LLNA study.

Repeated dose toxicity
A combined 28-day oral repeated dose toxicity study with the reproduction/developmental toxicity screening test was conducted using the notified chemical at 50, 200 and 500 mg/kg bw/day concentrations. Various test substance-related adverse effects including change in body weights, organ weights and clinical symptoms were observed in test animals exposed to 500 mg/kg test substance. Based on the findings a no observed adverse effect level (NOAEL) of 200 mg/kg bw/day was established by the study authors for repeated dose toxicity.

Mutagenicity/Genotoxicity
The notified chemical was reported to be non-mutagenic in a bacterial reverse mutation test and was reported to be non genotoxic in two *in vitro* studies.

Toxicity for reproduction
In the repeat dose oral toxicity combined with reproductive/developmental toxicity screening study on rats above, no adverse effects were observed on reproduction and development of foetus up to highest test concentration of 500 mg/kg bw test substance. A NOEL of 500 mg/kg bw/day can be established from the findings.

An earlier dose range finding study for reproduction/development/repeated dose toxicity using the notified chemical at 100, 300 and 1000 mg/kg bw/day was carried out on 6 test animals (3M and 3F) at each dose. The results were inconclusive due to the high mortality of test animals at 300 and 1000 mg concentration and the low number of test animals in each group.

Health hazard classification
Based on the available information, the notified chemical is 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 recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
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 slightly irritating to skin, and severely irritating to the eyes with irreversible effects.

The notified chemical will be available only in end use products at concentration < 0.01% at which concentration the irritation potential is expected to be greatly mitigated. The workers may come into contact with the notified chemical when transferring/adding the automotive fluid(s), top-ups and cleaning and maintenance of vehicles. Automated fillers may be employed in large scale use. Small business such as

mechanics may add the fluids manually and are at greater risk of exposure. Use of PPE including impervious gloves, coveralls and eye protection would further reduce exposure to the notified chemical. Overall the risk to workers from use of the notified chemical is not considered unreasonable.

6.3.2. Public Health

Functional automotive fluids containing the notified chemical at concentrations < 0.01% will be available to public for DIY purposes. The public may come in contact with the notified chemical via dermal and accidental ocular route during top-up of the functional fluids. The exposure is anticipated to be infrequent. Considering the very low concentration, infrequent and short term exposure, the risk to the public 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 as an ingredient of finished products. Environmental release of the notified chemical may only occur during importation, storage and transportation in the event of accidental spills or leaks. Spills or leaks are expected to be collected with inert material and disposed in accordance with State/Territory regulation or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used as an additive in automotive fluids. During use, release of the notified chemical may occur mainly through accidental spills or leaks. These spills and leaks are expected to be collected with inert material and disposed in accordance with state/ territory regulation or disposed of to landfill.

The notified chemical may enter the sewer from container cleaning and recycling. This release is expected to be limited and the concentration of the notified chemical in the environment is expected to be further diluted.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in empty containers are expected to be discarded to domestic garbage and disposed of to landfill or be collected by approved facilities for reuse. Used automotive fluid is likely to be recycled for further reuse or for the use of the calorific value.

7.1.2. Environmental Fate

The notified chemical is expected to be rapidly biodegradable. For the details of the environmental fate studies on the notified chemical and an acceptable analogue, please refer to Appendix C.

A bioaccumulation study was not provided. The notified chemical is not expected to bioaccumulate given its high water solubility and low log P_{OW}.

Most of the notified chemical is expected be recycled or reused for the calorific value at the end of its useful life. The notified chemical is expected to be either thermally decomposed during the recycling or to be reused for the calorific value as a component of the reused automotive fluid. In either case, the notified chemical is expected to be decomposed into water and oxides of carbon and phosphorus.

A small amount of the notified chemical may be sent to landfill as residues in empty containers, leaks or spills. In landfill, the notified chemical has potential to leach into public water due to the high water solubility. In water, the notified chemical is expected to biodegrade rapidly.

The notified chemical may be released to sewer as residues from container cleaning and recycling. Given the high water solubility, the notified chemical is expected to remain in the effluent water from the sewage treatment plants where the notified chemical is expected to degrade rapidly.

In water or landfill, the notified chemical is expected to undergo abiotic and biotic degradation processes, forming water and oxides of carbon and phosphorus.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical is not expected to be released to water compartments significantly based on its use pattern in Australia. Moreover, the notified chemical is expected to dissipate quickly via biodegradation in water. In addition, the notified chemical is considered to be of low concern to aquatic organisms based on the analogue

data as shown below. Therefore, the calculation of Predicted Environmental Concentration (PEC) is not considered to be necessary.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on analogues are summarised in the table below. Details of these studies can be found in Appendix C. These analogues are phosphoric acid alcohol esters that are structurally similar to the notified chemical. They are expected to have similar ecotoxicological profiles. Therefore, analogue data has been used to predict the potential environmental effects of the notified chemical for the purpose of risk assessment.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 hours LC50 >100 mg/L (Analogue 2) 96 hours LC50 >100 mg/L (Analogue 3)	Not harmful to fish
Daphnia Toxicity	48 hours ECC50 >100 mg/L (Analogue 4)	Not harmful to aquatic invertebrates
Algal Toxicity	72 hours ErC50 > 100 mg/L (Analogue 5) 72 hours ErC50 > 100 mg/L (Analogue 3)	Not harmful to algae
Inhibition of Bacterial Respiration	3 hours EC50 > 1000 mg/L (Analogue 3)	Not inhibitory to bacterial respiration

Based on the above analogue data, the notified chemical is not expected to be harmful to aquatic life. It is acceptable to predict the environmental effects of the notified chemical based on these analogue data given their structural similarity.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) has not been calculated given no PEC was calculated and the expected low concern of the notified chemical to aquatic organisms.

7.3. Environmental Risk Assessment

The Risk Quotient (PEC/PNEC) has not been calculated since the PEC or PNEC were not calculated. The potential for rapid biodegradation and the expected low hazard of the notified chemical indicate that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its proposed use pattern.

The notified chemical is expected to have a low potential for bioaccumulation. Therefore, on the basis of the assessed 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 No melting point was shown above -50 ºC

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Differential scanning calorimetry was used.
Test Facility	Siemens AG (2012a)

Boiling Point 50-230 ºC, followed by decomposition

Method	OECD TG 103 Boiling Point.
Remarks	Differential scanning calorimetry was used.
Test Facility	Siemens AG (2012a)

Vapour Pressure 2.8×10^{-6} kPa at 20 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Vapour pressure balance and effusion method were used.
Test Facility	Siemens AG (2011)

Water Solubility > 1000 g/L at 20 °C

Method	OECD TG 105 Water Solubility.
Remarks	Flask Method. The test substance is miscible with water at every measured ratio.
Test Facility	Clariant (2011)

Hydrolysis as a Function of pH

Method	OECD TG 111 Hydrolysis as a Function of pH.
Results	No significant hydrolysis was observed at pH 2, 4, 7 and 9
Remarks	The test was conducted on an analogue containing 24% phosphoric acid, 70% phosphoric acid, monomethyl esters and 6% phosphoric acid, dimethyl ester
Test Facility	Infrapark (2011)

Partition Coefficient (n-octanol/water) log Pow < -0.8 at 22 °C

Method	OECD TG 107 Partition Coefficient (n-octanol/water).
Remarks	Flask Method
Test Facility	Siemens AG (2012c)

Surface Tension 52.7 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	The test substance was determined to be surface active at the test concentration 998 mg/L.
Test Facility	Siemens AG (2012d)

Dissociation Constant $pKa_1 = 2.6$ and $pKa_2 = 7.3$ at 25 °C

Method	OECD TG 112 Dissociation Constants in Water.
Remarks	Titration method
Test Facility	Siemens AG (2012e)

Flash Point 71.5 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Pensky-Martens method was used.
Test Facility	Cosilab (2011)

Autoignition Temperature 315°C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility Siemens AG (2013)

Oxidizing Properties Not oxidising

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Test Facility Siemens AG (2012b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 401 Acute Oral Toxicity.
Species/Strain	Rat/Wistar SPF
Vehicle	Water
Remarks - Method	The study was conducted before introduction of appropriate OECD test guideline. The observation period after exposure was 14 days. All the surviving animals were subject to necropsy. A detailed English summary of the German study report was provided.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	10 F	1,600	0/10
2	10 F	2,500	0/10
3	10 F	3,200	2/10
4	10 F	4,000	8/10
5	10 F	5,000	10/10

LD50	3,575 mg/kg bw
Signs of Toxicity	Clinical signs of toxicity reported were closed eye lid, abnormal breathing and crouched posture.
Effects in Organs	Necropsy of deceased animals revealed reddened stomach mucosa with the stomach filled with bloody content. No microscopic findings were noted in animals who survived the study.
Remarks - Results	The deaths occurred within 1-2 days administration of test substance.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Hoechst AG (1976)

B.2. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (1981).
Species/Strain	Rabbit/New Zealand White: Hy/Cr
Number of Animals	6
Vehicle	None
Observation Period	6 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant deviations from the OECD guideline. The test substance was applied undiluted for 4 hours.

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	2	0.33	1	2	2	1	2	6 days	0
Oedema	1	0	0	0	1.33	0	3	6 days	0

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

Remarks - Results	Scab formation was noted in one test animal 48 and 74 hours after application.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	CIT (1985a)

B.3. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion (1981).
Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Observation Period	21 days
Remarks - Method	No significant deviations from the OECD guideline.

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	2	2	2	2	2	2	2	15†	2
Conjunctiva: chemosis	4	4	4	4	4	4	4	15†	4
Conjunctiva: discharge	2	2	2	2	2	2	3	10	0
Corneal opacity	4	4	4	4	4	4	4	15†	4
Iridial inflammation#	-	-	-	-	-	-	1	-	-

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animals.
# Gradation was not possible because of the degree of corneal opacity.
† Evaluation of the cornea and conjunctiva was not possible because the eye lids could not be opened from day 15 onwards.

Remarks - Results	Irreversible effect in conjunctiva and cornea were observed.
CONCLUSION	The notified chemical is severely irritating to the eye.
TEST FACILITY	CIT (1985b)

B.4. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	94.5% Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2010).
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	Dimethylformamide
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde in acetone:olive oil in December 2011..
Remarks - Method	No significant deviations from the OECD guideline. A preliminary study was conducted using 2 test animals exposed to the test substance at 50% and 100% concentrations. Effects observed in the test animal exposed to 100% test substance included local skin irritation, ear swelling >25% based on ear punch weight, hyperactivity, and open wound in the neck region.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	4 F	318.1	1.00
10	4 F	484.9	1.52
25	4 F	385.9	1.21
50	4 F	419.7	1.32

Remarks - Results: All test animals treated with the test substance showed an erythema of the ear skin (score 1) on days 3 and 4.

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY: Harlan CCR (2012a)

B.5. Repeat dose toxicity combined with reproductive/developmental toxicity screening

TEST SUBSTANCE: 94.5% Notified chemical

METHOD: OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996).

Species/Strain: Rat/Wistar Crl:WI SPF(Han)

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: Male – 28-30 days
Female – up to54 days (post natal day 3)
Dose regimen: 7 days per week

Vehicle: Sterile water

Remarks - Method: No significant deviations from the OECD guideline. The study was conducted after obtaining inconclusive results in a prenatal developmental toxicity study (OECD TG 414) carried out using test substance concentrations 100, 300 and 1000 mg/kg bw/day (see study B9 below).

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 F & 10M	0	0/10
low dose	10 F & 10M	50	0/10
mid dose	10 F & 10M	200	0/10
high dose	10 F & 10M	500	6 /10 (4 F & 2 M)

Mortality and Time to Death

Two male rats from high dose were found dead or euthanized on days 8 and 15 during the mating/post mating stage. Four female rats from high dose group were found dead or euthanized. Two were on premating day 13 and the others were on gestation days 17 and 19. No mortality occurred in other groups. The mortality observed in high dose group was considered to be test substance related by the study authors.

Clinical Observations

Several clinical symptoms were noted in majority of male and female rats from high dose group including piloerection, vocalization, moving the bedding, abnormal breathing, salivation and reddish nasal discharge. The effects were considered to be test substance related.

Test animals from mid dose group also showed slight to moderate piloerection, abnormal breathing (1 male) and eschar (1 female). The effects were considered to be test substance related.

Clinical symptoms observed in low dose group animals were reddish nasal discharge (2 males and 1 female), slight piloerection (1 male and 2 females), exophthalamus (1female) and eschar (1 female). Similar effects to various extents were also observed in control rats and thus were considered not to be test substance related.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Ten animals (5 females and 5 males) were randomly selected from each group of investigation. Increase in total

white blood cell count was observed in males from high dose group. A dose dependent increase in monocytes count was noted in female rats but did not reach statistical significance. Percentage of eosinophils of females was significantly increased in mid dose group. This was also seen in high dose group but did not reach statistical significant.

Increase in cholesterol levels were observed in male rats. The increase reached statistical significance in low and high dose group. Due to dose dependent increase, the effect is considered to be test substance related. Significant increase in total bile acids was observed in low dose group males. This was considered not to be test substance related by the study authors due to no dose dependence. In female rats, values of SGOT (ASAT) and phosphate were slightly decreased in treatment group but the change was minor did not reach statistical significance.

Increase in blood content in urine of female rats from high dose group was observed. Due to lack of dose response and no other associated histopathological changes, the effects were considered not to be test substance related by the study authors. No changes in urine were observed in other treated animals when compared to control animals.

Effects in Organs

Small gross pathological changes in the gastrointestinal tract and lungs were observed in rats from high dose group. This included gased stomach, duodenum, jejunum, mesenteric lymph nodes, small size spleen, thymus and discoloured red stomach. The association of these effects to test substance cannot be ruled out.

In male rats absolute and relative weight of spleen was slightly increased in mid dose group and was significantly increased ($p<0.05$) in high dose group. Similarly in females increased in spleen weight was observed in high dose groups.

Minor increase in absolute thymus weight was observed in male rats from high dose group. Decreases in absolute and relative thymus weight were observed in test substance treated female rats.

Decrease in absolute and relative weight of prostate (plus seminal vesicles and coagulating glands) was observed in male rats from high dose group, but was not considered test item related due to variability and absence of histopathological changes. In female rats, absolute weights of uteri (with cervix) showed a slight tendency to a dose dependent decrease. Relative weight changes of uteri did not confirm the absolute weight changes and did not reach statistical significance.

Effects in Reproduction and Offspring

There were no treatment related effects on mating performance, fertility, gestation length, litter size, sex ratio, viability, or the number of corpora lutea or implantation sites.

Although there was an increase in the percentage of pre-implantation loss of the low dose group and post-implantation loss in mid dose group, the lack of statistical significance and dose-dependency, these effects were not attributed to the treatment by the study authors. A reduced copulation index was observed in treated animals when compared to control. This was not considered to be test substance related by the study authors.

Remarks – Results

One female rat from low dose group bit the cannula and swallowed it. The animal was observed more frequently to detect and report any abnormalities.

Body temperature of female rats from high dose group was slightly increased at the end of the treatment.

Body weight gain was attenuated in rats from high dose group and reached statistical significance ($p< 0.05$) in females between gestation day 0 and 7 when compared to control. Reduction in food intake was also observed in in high dose group rats and reached statistical significant in males during pre-mating days 7-14 and in females during gestation day 7-14. A decrease in food consumption was also noted in females from mid dose group during gestation but did not reach statistical significance.

Histopathological studies on the rats that were found dead or euthanized before scheduled necropsy showed prominent changes in the gastro-intestinal tract and the respiratory system indicative of local irritation effects. Degenerative/atrophic changes of the lymphoid organs and hypocellularity of the bone marrow was also observed in all of these animals and were considered to be secondary to bad general condition and/or agonal

stress.

CONCLUSION
The parental No Observed Adverse Effect Level (NOAEL) was established as 200 mg/kg bw/day by the study author in this study, based on the adverse test substance related clinical and body weight changes observed in rats from 500 mg/kg bw/day group.

The reproductive and developmental No Observed Effect Level (NOEL) was established as 500 mg/kg bw/day by the study author in this study, based on no treatment related effects observed for reproductive and developmental parameters at the highest test concentration of 500 mg/kg bw/day.

TEST FACILITY BSL (2013a)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE 94.5% Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1997).
Plate incorporation procedure (Test 1) & Pre incubation procedure (Test 2)

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 3-5,000 µg/plate b) Without metabolic activation: 3-5,000 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant deviations from the OECD guideline. Dosage was adjusted to take account of purity. A pre-test was conducted using all five bacterial strains to assess toxicity of the test substance. The pre-test has been reported as test 1 due to no toxic effects.

RESULTS

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

Remarks - Results The positive controls gave the expected increase in the number of revertant colonies, confirming the activity of S9 fraction and the integrity of the assay system.

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

TEST FACILITY Harlan CCR (2012b)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE 94.5% Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test (1998).

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from Phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant deviations from the OECD guideline. Test concentrations

were adjusted to take account of the purity. The pH was adjusted to physiological values. A preliminary test was conducted for cytotoxicity. Due to no cytotoxicity at highest test concentration of the test substance, the preliminary test was reported as test 1.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	35.8, 62.7, 109.7, 191.9, 335.9, 587.8, 1028.7, 1800.2*, 3150.3*, 5513.0*	4 h	22 h
Test 2	35.8, 62.7, 109.7, 191.9, 335.9, 587.8, 1028.7, 1800.2*, 3150.3*, 5513.0*	22 h	22 h
Present			
Test 1	35.8, 62.7, 109.7, 191.9, 335.9, 587.8, 1028.7, 1800.2*, 3150.3*, 5513.0*	4 h	22 h
Test 2	587.8, 1028.7, 1800.2*, 3150.3*, 5513.0*	4 h	22 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 5513.0	> 5513.0	Negative
Test 2	> 5513.0	> 5513.0	Negative
Present			
Test 1	> 5513.0	> 5513.0	Negative
Test 2	> 5513.0	> 5513.0	Negative

Remarks - Results

Exposure to positive controls ethyl methane sulfonate and cyclophosphamide resulted in significant increase in the number of cells carrying structural chromosomal aberrations confirming the activity of S9 fraction and the integrity of the assay system.
No biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed in cells treated with the test substance at any test concentrations. No increase in polyploidy was seen.

CONCLUSION

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

TEST FACILITY

Harlan CCR (2012c)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE

94.5% Notified chemical

METHOD

OECD TG 476 In vitro Mammalian Cell Gene Mutation Test (1998).

Species/Strain: *Chinese hamster*
Cell Type/Cell Line: V79
Metabolic Activation System: S9 fraction from Phenobarbital/β-naphthoflavone induced rat liver
Vehicle: Dimethyl sulfoxide
Remarks - Method: No significant deviations from the OECD guideline. Dosage was adjusted to compensate for purity. Test1 was repeated due to bacterial contamination. The results of new test 1 are reported below.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	21.6, 43.2*, 86.3*, 172.5*, 345.0*, 517.5*	4 h	7 days	8 days
Test 2	43.2, 86.3*, 172.5*, 345.0*, 517.5*, 690.0*	24 h	7 days	8 days
Present				
Test 1	172.5, 345.0*, 690.0*, 1380.0*, 2760.0*, 5520.0*	4 h	7 days	8 days
Test 2	172.5, 345.0*, 690.0*, 1380.0*, 2760.0*, 5520.0*	4 h	7 days	8 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	≥ 345.0	≥ 517.5	> 517.5	Negative
Test 2	≥ 690.0	> 517.5	> 690.0	Negative
Present				
Test 1	> 5520.0	> 5520.0	> 5520.0	Negative
Test 2		> 5520.0	> 5520.0	Negative

Remarks - Results

The positive controls showed distinct increase in the number of mutant colonies confirming the integrity of the assay and S9 mix.

None of the test concentrations exceeded the induction factor of 3 established by the study laboratory as an indication of test substance being a mutagen. Although an induction factor between 2 and 3 was observed in culture II, this did not occur in culture I and there was no clear dose related effect.

The highest solvent control values exceed the historical control values in test 2. However due to the average being still within the historical range, the test was considered acceptable by the study authors.

CONCLUSION

The notified chemical was not mutagenic to Chinese hamster V79 cell lines treated in vitro under the conditions of the test.

TEST FACILITY

Harlan CCR (2012d)

B.9. Reproductive/Developmental/Repeat Dose Toxicity – range finding study

TEST SUBSTANCE

94.5% Notified chemical

METHOD

OECD TG 414 Prenatal developmental toxicity study (2001) & OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996).

Species/Strain: Rat/Wistar Crl:WI SPF(Han)

Route of Administration: Oral – gavage

Exposure Information: Exposure days: Male – 28 days
Female – up to gestation day 19

Vehicle: Sterile water

Remarks - Method: Minor deviations were reported. Two female rats from control group inadvertently received test substance formulation of the low dose group at one single day during gestation. This could impact the study outcome due to the use of low number of test animals. The doses were adjusted regarding the purity of the test item to 100%.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	3 F & 3M	0	1(F)/6
low dose	3 F & 3M	100	0/6
mid dose	3 F & 3M	300	0/6
high dose	3 F & 3M	1000	5(2 F & 3 M)/6

Mortality and Time to Death

All 3 males and 2 females from high dose group died during the study. The male rats died during the mating and post-mating days 2, 7 and 8. 1 female rat died on pre-mating day 3 and the other died on gestation day 2. One female rat from control group was euthanized due to littering.

Effects on Dams

Clinical signs such as abnormal breathing were recorded for animals from mid dose group. Several clinical signs were observed in test animals from high dose group including abnormal breathing, moving the bedding, vocalization, piloerection and salivation.

Effects on Foetus

Gross external abnormalities were recorded in foetuses from treatment and control groups. This included hematoma on various body locations with slightly increased number of incidence in mid dose group.

Skeletal examination of the Alizarin red stained foetuses revealed a range of abnormalities which were mostly of a type or which occurred at an incidence comparably lower in treated groups when compared to the control group. Some were at higher incidence in mid dose group. In particular, fused frontal parietal suture, fused parietal-interparietal suture, rudimentary 14^{th} rib etc.

Craniofacial examination by razor blade serial sectioning conducted only on control and mid dose group revealed increased amount of haemorrhagic positions in treated animals. These were considered to be test item related by the study author under the light of the increased haemorrhagic positions observed externally.

Remarks - Results

Due to insufficient number of surviving dams in high dose group, the findings were not evaluated.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) could not be established due to insufficient data. It was suggested by the study authors that a study with lower test substance concentrations be conducted. This was done and the results are reported above (B.5. Repeat dose toxicity combined with reproductive/developmental toxicity screening).

TEST FACILITY BSL (2013b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Chemical oxygen demand
Remarks - Method	The test was conducted according to the test guideline above with no significant deviation from the protocol.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	31.5	7	59
14	49.0	14	79
21	61.5	21	84
28	68.0	28	91

Remarks - Results	The test substance attained > 60% biodegradation in 28 days, indicating a rapid biodegradation. However, the 10-days window for being readily biodegradable is not met. Therefore, the test substance is considered to rapidly biodegradable but not readily biodegradable. The biodegradation of toxicity controls was 65% after 14 days and reached to 78% after 28 days, suggesting the test substance does not inhibit the activity of the microorganisms in sludge. All validation criteria for the test were met.
CONCLUSION	The notified chemical is considered to be rapidly biodegradable
TEST FACILITY	Aventis (2002)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 301 B CO_2 evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical CO_2 production ($ThCO_2$)
Remarks - Method	The test was conducted according to the test guideline above with no significant deviation from the protocol.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	16.5	6	67
14	65.5	14	83
21	88.0	21	89

28	97.5	28	90

Remarks - Results	The test substance attained > 90% biodegradation in 28 days and met the 10-days window. Therefore, the test substance is considered to be readily biodegradable. The biodegradation of toxicity controls was 66% in 14 days and reached to 78% after 28 days, suggesting the test substance does not inhibit the activity of the microorganisms in sludge. All validation criteria for the test were met.
CONCLUSION	The analogue chemical is considered to be readily biodegradable
TEST FACILITY	Noack-Laboratorien (2012)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static
Species	*Danio rerio* (Zebrafish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	40 -180 mg $CaCO_3$/L
Analytical Monitoring	Dissolved organic carbon (DOC)
Remarks – Method	A limit test was conducted at the test concentration of 100 mg/L according to the test guideline above. There is no significant deviation from the protocol.

RESULTS

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

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The dissolved oxygen concentration was greater than 60% and the mortality in blank control was 0%. Therefore, all the validation criteria for the test were satisfied.
CONCLUSION	The analogue and therefore, the notified chemical are not harmful to fish
TEST FACILITY	Noack-Laboratorium (2002)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static
Species	*Danio rerio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	108 mg $CaCO_3$/L
Analytical Monitoring	Spectral Photometer

Remarks – Method	A limit test with a single test concentration of 100 mg/L was conducted according to the test guideline above with no significant deviation from the protocol.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	-	7	0	0	0	0
100	101.2	7	0	0	0	0

LC50	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The dissolved oxygen stay above 79% during the test and there is no mortality in blank control. Therefore, all the validation criteria for the test are satisfied.

CONCLUSION	The analogue and therefore, the notified chemical are not harmful to fish
TEST FACILITY	LAUS (2007a)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Analogue 4
METHOD	OECD TG 202 *Daphnia sp*. Acute Immobilisation Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	210 mg $CaCO_3$/L
Analytical Monitoring	Spectrophotometrical determination
Remarks - Method	A limit test with a single test concentration of 100 mg/L was conducted according to the test guideline above with no significant deviation from the protocol.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	-	20	0	0
100	103	20	0	0

EC50	> 100 mg/L at 48 hours
Remarks - Results	Dissolved oxygen concentration in all the test solutions was greater than 3 mg/L and the immobilisation in blank control was less than 10%. Therefore, all validation criteria for the test are satisfied.
CONCLUSION	The analogue and therefore, the notified chemical are not harmful o aquatic invertebrates.
TEST FACILITY	Aventis (2001)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Analogue 5
METHOD	OECD TG 201 Alga, Growth Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: Control, 6.25, 12.5, 25, 50 and 100 mg/L Actual: Not reported
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Dissolved Organic Carbon (DOC)
Remarks - Method	The test was conducted according to the test guideline above with no significant deviation from the protocol.

RESULTS

Biomass		*Growth*	
EC50 *mg/L at 72 h*	*NOEC* *mg/L at 72 h*	*EC50* *mg/L at 72 h*	*NOEC* *mg/L at 72 h*
> 100	< 100	>100	100

Remarks - Results	The test concentrations during the test are verified by DOC analysis and the correlation between nominal and measured concentration was good. Therefore, the test results were based on nominal concentrations. All validation criteria for the test were satisfied.

CONCLUSION The analogue and therefore, the notified chemical are not harmful to algae

TEST FACILITY Noack-Laboratorien (2013)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE Analogue3

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: Control, 100 mg/L Actual: 6.8, 104 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Spectral photometer
Remarks - Method	The test was conducted with a single test concentration of 100 mg/L following the test guideline above. There is no significant deviation from the protocol.

RESULTS

Biomass		*Growth*	
EC50 *mg/L at 72 h*	*NOEC* *mg/L at 72 h*	*EC50* *mg/L at 72 h*	*NOEC* *mg/L at 72 h*
> 100	100	>100	100

Remarks - Results	The test results are based on nominal concentrations as the recovery of the test substance was 98% and the correlation between nominal and measured concentration was good. Therefore, the test results were based on nominal concentrations. All validation criteria for the test were satisfied.

CONCLUSION The analogue and therefore, the notified chemical are not harmful to algae

TEST FACILITY	LAUS (2007b)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Analogue 3
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: Control, 1, 10, 100 and 1000 mg/L Actual: NA
Remarks – Method	The test was conducted according to the test guideline above with no significant deviation from the test.
RESULTS	
EC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	The reference substance has a determined 3 hours EC50 = 5.1 mg/L, within the range of 5-30 mg/L. The oxygen consumption rate of the blank control is 8%, less than the recommended value of 15% All the validation criteria for the test were met.
CONCLUSION	The analogue and therefore, the notified chemical are not inhibitory to micro-organisms respiration activity.
TEST FACILITY	LAUS (2007c)

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