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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Chemical in F00003A

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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 Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

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

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

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
BASF Australia Ltd (ABN 62 008 437 867)
Level 12, 28 Freshwater Place SOUTHBANK VIC 3006

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

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
New Zealand Inventory of Chemicals (November 2009)
US: Released, listed in confidential section of TSCA; Section 5 PMN commenced May 2010

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
F00003A (containing the notified chemical at < 10%)

OTHER NAME(S)
Kerocom FM38

MOLECULAR WEIGHT
< 1000 Da

ANALYTICAL DATA
Reference ^{1}H-NMR, IR and potentiographic titration spectra were provided.

3. COMPOSITION

DEGREE OF PURITY > 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: low viscous, clear, amber liquid

Property	Value	Data Source/Justification
Melting Point	2°C	Measured
Boiling Point	Not determined	The test substance showed strongly increasing vapour pressure values above 120°C.
Density	967.5 kg/m^3 at 20°C	Measured
Vapour Pressure	0.11 kPa at 20°C 0.14 kPa at 25°C 0.39 kPa at 50°C	Measured
Water Solubility	Not determined	The water solubility could not be

		accurately determined due to the notified chemical's tendency to form emulsions in water. The notified chemical is expected to be water dispersible due to its surface activity.
Hydrolysis as a Function of pH	Not determined	The hydrolysability of the notified chemical could not be determined due to its low solubility in water. Based on its limited water solubility, hydrolysis is expected to be slow in the environmental pH range (4–9) at ambient temperature.
Partition Coefficient (n-octanol/water)	Not determined	The partition coefficient could not be determined due to the notified chemical's surface activity. The notified chemical is expected to partition to n-octanol due to its predominately hydrophobic structure and miscibility with n-octanol.
Adsorption/Desorption	log Koc ~ 2.8 – 3.7	The log Koc range of the notified chemical is reported. The notified chemical is expected to adsorb to soil and sediment due to its hydrophobic structure and potential cationic functions.
Dissociation Constant	Not determined	The notified chemical contains potentially cationic functionality and may be ionised in the environmental pH range (4-9).
Particle Size	Not determined	Liquid
Flash Point	114ºC (pressure unknown)	Measured
Flammability	Non flammable upon ignition.	The notified chemical has no pyrophoric properties and does not liberate flammable gases on contact with water (statement provided the notifier).
Autoignition Temperature	330ºC (100.7-102.3 kPa)	Measured
Explosive Properties	Not determined	The exothermic decomposition energy, determined by differential scanning calorimetry, is less than 500 J/g.

DISCUSSION OF PROPERTIES

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

Reactivity

The notified chemical is considered to be stable under normal conditions of use due to no condition being identified which would contribute to the instability of the notified chemical. No incompatible substances have been identified with the notified chemical, although oxidising substances are likely to be incompatible. Typical decomposition products are oxides of carbon and oxides of nitrogen. No other toxic gases/vapours have been identified.

Dangerous Goods classification

Based on the submitted physical-chemical data in the above table the notified chemical is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However the data above do not address all Dangerous Goods endpoints. Therefore consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of F00003A (< 10%). It will be imported by ship in 200 L drums, 1000 L intermediate bulk containers and in bulk shipments.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	100-1000	100-1000	100-1000	100-1000	100-1000

PORT OF ENTRY
Melbourne, Sydney, Brisbane, Perth, Adelaide and Hobart

IDENTITY OF RECIPIENTS
BASF Australia Ltd

TRANSPORTATION AND PACKAGING
The notified chemical as a component of F00003A is imported into Australia by ship in 200 L robust UN approved steel drums, 1000 L intermediate bulk containers or in bulk. F00003A is transported from the dockside to the contracted warehouse in Laverton North or direct to the customer sites, where it is stored until required.

USE
The notified chemical will be used as a friction modifier in a fuel additive for cleaning and keeping clean the inlet system of spark ignition engines.

OPERATION DESCRIPTION
The notified chemical as a component of F00003A will be reformulated by blending with petrol and other additives, in batches of 10,000 to 100,000 L. Reformulation will occur only at refineries or bulk fuel storage facilities. The finished fuel will contain up to 0.01% (w/w) notified chemical. The finished product will be transferred by road tanker to retail outlets.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage of F00003A	3-10	6-8	12-15
Blender	1-5 per site	6-8	125-225
QA analysis	1-2 per site	6-8	125-225
Transport and storage of fuel	1-10	6-8	125-225
End-use fuelling	> 10,000	10-30 minutes	200

EXPOSURE DETAILS
The potential routes of occupational exposure are dermal, ocular and inhalation. However inhalation exposure is not expected as the chemical has low vapour pressure, and the generation of mists/aerosols is not expected.

Transport and storage of F00003A
Transport workers are not expected to be exposed to the imported F00003A containing the notified chemical at < 10%, as they will be handling closed containers. Dermal or ocular exposure is possible in the event of an accident where the packaging is breached.

Blending
F00003A containing the notified chemical at < 10% will be reformulated by blending with fuel and other

additives to produce the finished fuel product. The blending will be mostly an automated enclosed process. The container for F00003A will be connected to the blending system by flexible transfer hose and the contents pumped into the blending vessel. Upon completion of the transfer, the container, transfer hose and pump are cleaned by flushing with fuel before the transfer hose is disconnected. Dermal contact with the notified chemical in F00003A (up to 10% notified chemical) is possible during the transfer operation. However, little loss of F00003A is expected upon connection and disconnection of the transfer hose due to the flushing process. Exposure is expected to be low and further reduced by workers wearing personal protective equipment, such as industrial overalls and footwear, safety glasses and protective gloves plus goggles and face shields if required, during these processes.

Exposure to the notified chemical may also occur during sampling and analysis of blended fuel at the refinery or during maintenance of refinery plant or pipelines. The exposure would be limited by appropriate personal protective equipment worn by workers, such as industrial overalls and footwear, safety glasses and protective gloves plus goggles and face shields if required.

Transport and storage of fuel
Dermal or ocular exposure to drips and spills of fuel containing the notified chemical at < 0.01% is possible during the connection and disconnection of transfer hoses. Exposure is expected to be limited during transportation as the protocols of loading and unloading are done with minimal spills. The drivers also usually wear gloves and long sleeves shirts when unloading the fuel.

End users of fuel
Personnel from commercial trucking fleet, marine tugs or small ships, agriculture users, railroads, service stations, truck stops and construction companies may be exposed to fuel during handling and fueling of the vehicles.

6.1.2. Public exposure
The public does not typically have exposure to F00003A containing the notified chemical at < 10%.

The public may have incidental skin or eye contact with fuel containing the notified chemical at < 0.01% through operations such as refilling vehicles.

Other exposures to F00003A or fuel could only occur in the extremely unlikely event of an accident where import containers or the tank trucks are ruptured, liberating F00003A or fuel containing the notified chemical.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Epiderm in vitro skin model	non-corrosive
HET-CAM in vitro assay	severely irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 300 mg/kg bw/day NOAEL= 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test	non genotoxic
Genotoxicity – in vivo micronucleus test with bone marrow cells of the mouse	non genotoxic

Toxicokinetics, metabolism and distribution
Given the high lipophilicity, uptake into the stratum corneum is expected to be high. Given the possible low water solubility with the increased molecular weight of the notified chemical the rate of transfer from the stratum corneum to the epidermis is expected to be slow and hence percutaneous absorption would be limited. However, the surface activity and irritating effects of the notified chemical would likely increase dermal absorption. The fact that the notified chemical was shown to be a skin sensitiser when applied to the skin of mice (see below) is evidence of dermal absorption having occurred.

Given the high lipophilicity, oral and respiratory absorption may occur through micellular solubilisation.

Acute toxicity
The notified chemical is of low acute toxicity *via* the oral and dermal routes.

Irritation
Based on the in vitro skin corrosion study using human skin model provided, the notified chemical is not considered to be corrosive to the skin. However, due to structural alerts (and in the absence of test data on skin irritation) the notified chemical should be considered as irritating to the skin.

A Hen's Egg Test Chorioallantoic Membrane (HET-CAM) assay was performed in place of an *in vivo* acute eye irritation/corrosion test because the notified chemical was suspected to be strongly irritating and/or corrosive. Treatment with the notified chemical caused moderate intravascular coagulation and slight haemorrhagia in all eggs after 51-64 seconds. Based on these effects the notified chemical was considered to have the potential to cause severe ocular irritation and therefore an *in vivo* study was not performed due to animal welfare concerns. Although not a formally validated assay, positive outcomes from the HET-CAM assay are considered to be sufficient for classifying substances as severe eye irritants.

Sensitisation
The notified chemical is expected to have the potential to cause skin sensitisation based on the lymphocyte proliferative response observed in the Mouse Local Lymph Node Assay.

Repeated dose toxicity
The only test-substance related findings in the repeat dose toxicity study were inflammatory effects on the forestomach. These were most likely related to the irritating potential of the test substance as it was directly administered into the forestomach by gavage. The No Observed Adverse Effect Level (NOAEL) for local effects was therefore established as 300 mg/kg bw/day with regard to effects in the forestomach in the 1000 mg/kg bw/day group. As the forestomach of rats does not have a protective mucus lining and there is no direct counterpart of the rat forestomach in humans, the forestomach findings may not be of relevance to humans. Nonetheless, this NOAEL may be appropriate to evaluate the risk of possible local effects from oral exposure.

As no signs of general systemic toxicity were observed in the repeat dose toxicity study the NOAEL for systemic effects was established as 1000 mg/kg bw/day (the highest dose tested).

Mutagenicity
The notified chemical tested was not mutagenic in a bacterial reverse mutation study and not genotoxic in an *in vitro* mammalian chromosome aberration test. There was also no indication of clastogenicity in an *in vivo* micronucleus test with bone marrow cells of the mouse, however there was no indication from this study that the notified chemical was reaching the target organ bone marrow.

Health hazard classification
Based on the data provided the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

Xi; R38 Irritating to skin
Xi; R41 Risk of serious eye damage
Xi; R43 May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

The notified chemical is a skin sensitiser and is irritating to the skin and severely irritating to eyes.

Workers most at risk will be those workers handling the notified chemical as introduced where the concentration of the notified chemical is up to 10%. Given that exposure should be limited by the expected use of personal protective equipment and engineering controls in place (largely an automated and closed system), the risk to workers from use of the notified chemical is not considered unreasonable.

The risk to all other workers is considered negligible given the low concentration of the notified chemical (< 0.01%) in the fuel.

6.3.2. Public health

The risk to the public from exposure to the notified chemical in fuel is expected to be negligible based on the low concentration of the notified chemical in the fuel (< 0.01%) and the public's expected low exposure to fuel during vehicle refilling.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical is not expected to be released to the environment when it is blended into fuels, as blending will take place in closed systems. Minor spills will be contained and collected for safe disposal at an approved facility. Residues in drums will be destroyed during metals reclamation or disposed of to landfill with the empty drums.

RELEASE OF CHEMICAL FROM USE

Small amounts may be spilt to the ground when vehicles are refuelled, but the notified chemical will otherwise be consumed with fuel during automobile combustion engine operation.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of the notified chemical is expected to be consumed by combustion in automobile combustion engines. Any significant spill is expected to be contained and disposed of to landfill.

7.1.2 Environmental fate

In a study submitted by the notifier, the notified chemical was shown to be biodegradable. However, the notified chemical cannot be formally classified as readily biodegradable according to the strict definition of the test guidelines. Bioaccumulation of the notified chemical is not expected due to its biodegradability and limited potential for exposure to the aquatic compartment. Most of the notified chemical will share the fate of the fuel containing it and be consumed in automotive engines. The limited amount of notified chemical anticipated to be disposed of to landfill (e.g. residues in drums) is expected to bind to soil due to the presence of cationic functionality, and undergo slow degradation processes via biotic and abiotic pathways. Whether by thermal decomposition in engines or degradation in landfill, the notified chemical is expected to decompose into water and oxides of carbon and nitrogen. For the details of the environmental fate study refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

It is neither necessary nor meaningful to determine the PEC as the notified chemical is not expected to be released to aquatic environments when it is used as proposed as a fuel additive.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity (96 h)	LC50 = 15.2 mg/L	Harmful
Daphnia Toxicity (48 h)	EC50 = 2.25 mg/L	Toxic
Algal Toxicity (96 h)	E_rC50 = 2.14 mg/L	Toxic

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemical is acutely harmful to fish and acutely toxic to aquatic invertebrates and algae. The notified chemical is therefore formally classified 'Acute Category 2; Toxic to aquatic life'. As the notified chemical is not readily biodegradable and has an acute EC50 ≤ 10 mg/L, it is formally classified under the GHS as 'Chronic Category 2; Toxic to aquatic life with long lasting effects'.

7.2.1 Predicted No-Effect Concentration

The PNEC has not been calculated as no significant aquatic exposure is expected based on the reported use pattern.

7.3. Environmental risk assessment

The Risk Quotient, Q (= PEC/PNEC), has not been determined due to the notified chemical's low potential for release to the aquatic compartment. The majority of the notified chemical is expected to be thermally decomposed in automobile engines. If the notified chemical is spilt to soil or disposed of to landfill, it is expected to degrade biotically and abiotically. Based on its reported use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification and labelling details are:

Xi; R38 Irritating to skin
Xi; R41 Risk of serious eye damage
Xi; R43 May cause sensitisation by skin contact

and

The classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2009) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Skin irritation	2	Causes skin irritation
Eye irritation	2A	Cause serious eye irritation
Skin sensitisation	1	May cause sensitisation by skin contact
Aquatic Environment	Acute Category 2	Toxic to aquatic life
	Chronic Category 2	Toxic to aquatic life with long lasting effects

Human health risk assessment

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

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

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

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - Xi; R38 Irritating to skin
  - Xi; R41 Risk of serious eye damage
  - Xi; R43 May cause sensitisation by skin contact

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - concentration ≥ 20%: R38, R41, R43
  - ≥ 10% concentration < 20%: R41, R43
  - ≥ 5% concentration < 10%: R36, R43
  - ≥ 1% concentration < 5%: R43

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker (exposed to the imported fuel additive containing the notified chemical at < 10%) who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - Automation of blending processes
  - Closed blending vessels

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Avoid contact with skin and eyes
  - Avoid generation and inhalation of aerosols

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Overalls
  - Gloves

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

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

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(2) of the Act; if
- the function or use of the chemical has changed from a fuel additive at < 0.01%, or is likely to change significantly;
- the amount of chemical being introduced has increased from 1000 tonne per annum, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet
The MSDS of the product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 2ºC (peak temperature)

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Measured using differential scanning calorimetry. The test substance showed a broad melting range from about -50ºC up to 10ºC.
Test Facility	BASF SE (2009b)

Boiling Point Not determined

Method	OECD TG 104 Vapour Pressure.
Remarks	The test substance is a mixture of substances of different volatility, in consequence the dynamic method according to OECD TG 102 is not suitable. Therefore the determination of the Normal Boiling Point was carried out via vapour pressure measurement by the static method according to OECD TG 104. At temperatures above 120ºC, the static method showed strongly increasing vapour pressure values. An extrapolation from about 3.7 kPa to the normal boiling point at 101.325 kPa is not feasible, reliable data could not be determined. The test substance is liquid in the temperature range used for the vapour pressure measurements.
Test Facility	BASF SE (2009b)

Density 967.5 kg/m^3 at 20ºC

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Oscillating densitometer was used.
Test Facility	BASF SE (2009b)

Vapour Pressure 0.11 kPa at 20ºC
0.14 kPa at 25ºC
0.39 kPa at 50ºC

Method	OECD TG 104 Vapour Pressure.
Remarks	Static method was used. The vapour pressure at 20°C, 25°C and 50°C was calculated from the regression equation. The test substance is liquid in the temperature range used for the vapour pressure measurements.
Test Facility	BASF SE (2009b)

Water Solubility Not determined

Method	OECD TG 105 Water Solubility
Remarks	Flask Method. The test substance was partly miscible in water depending on the ratios of the test substance to water applied. While low concentrations (e.g. 11 mg/L) of the test substance were not completely soluble in water, the test substance was able to form clear and homogenous mixtures with small amounts of water (up to mixing ratio 8:2). Intermediate mixture ratios resulted in turbid emulsions which partly separated in two phases with different homogeneity. Due to this behaviour, it was not possible to determine the water solubility of the test substance.
Test Facility	BASF SE (2009b)

Hydrolysis as a Function of pH Not determined

Method	OECD TG 111 Hydrolysis as a Function of pH
Remarks	Mixtures of the test substance with different buffer solutions were prepared using 1% solubiliser (acetonitrile, THF or methanol). Preparations were checked visually immediately, after standing overnight at room temperature and after tempering for 2 hours at 50°C. In all mixtures the solubility of the test substance in buffer solutions was too low to determine the hydrolysis of the test substance.
Test Facility	BASF SE (2009c)

Partition Coefficient (n-octanol/water) Not determined

Method In-house method
Remarks The partition coefficient of the test substance could not be determined by OECD guidelines because it was not a pure substance and also due to its surface activity. The partition coefficient could not be determined from the solubilities of the notified substance in water and n-octanol since its water solubility could not be determined.

The test substance was mixed with n-octanol in ratios of 1:9, 1:1 and 9:1 and mixed by hand at room temperature (23°C). The test substance was miscible with n-octanol at all ratios tested.
Test Facility BASF SE (2009b)

Adsorption/Desorption log Koc ~ 2.8 – 3.7

Method OECD 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography
Remarks The test substance was found to consist of a mixture of compounds with log Koc values in the range from -1.7 to 6.0 at 23°C and pH = 6.2. The four main components with largest peak areas were found to have log Koc 2.8 to 3.7. A determination of the area percentage of the different components using a refractive index detector was reportedly not possible. No sufficiently concentrated solution could be prepared to obtain a quantifiable signal, due to the low solubility of the test substance in water and eluent.
Test Facility BASF SE (2009c)

Dissociation Constant Not determined

Method OECD TG 112 Dissociation Constants in Water.
Remarks The determination of the dissociation constant of an aqueous preparation of the test substance was not possible due to the low solubility of test substance in water.
Test Facility BASF SE (2009c)

Flash Point 114ºC (pressure known)

Method EC Directive 92/69/EEC A.9 Flash Point.
DIN EN ISO 2719
Remarks The test substance was placed in a test vessel, which was progressively heated until the vapour reached a sufficiently high concentration in air to produce a flammable mixture which could be ignited.
Test Facility BASF SE (2009d)

Autoignition Temperature 330ºC (100.7-102.3 kPa)

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks The apparatus described in EN 14522 was used and correction to the measurement was performed according to EN 14522.
Test Facility BASF SE (2009d)

Explosive Properties Not determined

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks The exothermic decomposition energy, determined by differential scanning calorimetry (DSC), is less than 500 J/g. cf. UN Recommendations on the transport of dangerous goods, Manual of test and criteria, Annex 6.
Test Facility BASF SE (2009d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
- Species/Strain: Rat/Wistar
- Vehicle: Olive oil Ph.Eur.
- Remarks - Method: No deviations from the protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	6 F	2000	0
2	3 F	300	0

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: No clinical signs and findings were observed in the 2000 and 300 mg/kg bw administration groups.
- Effects in Organs: There were no macroscopic pathological findings in the animals sacrificed at the end of observation period.
- Remarks - Results: The mean body weights of the test groups increased normally throughout the study period.

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

TEST FACILITY: Bioassay (2009a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
- Species/Strain: Rat/Wistar
- Vehicle: None
- Type of dressing: Semi-occlusive.
- Remarks - Method: No deviations from the protocol.

RESULTS

Number and Sex of Animals	*Dose (mg/kg bw)*	*Mortality*
5 per sex	2000	0

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: Skin effects at the application site comprised erythema (grade 1), scaling and incrustations and were observed from study day 1 until study day 8.
- Signs of Toxicity - Systemic: No systemic clinical signs were observed during clinical examination.
- Effects in Organs: No macroscopic pathologic abnormalities were noted in all animals examined on the last day of observation.
- Remarks - Results: The mean body weight of the male animals increased within the normal range throughout the study period.
  Mean body weight of the female animals slightly increased during the first post-exposure observation week, probably due to the bandage procedure, but increased during the second week within the normal range.

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

TEST FACILITY Bioassay (2009b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD
Vehicle None
Remarks - Method Due to ability of the test substance to reduce MTT ([3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) directly, a MTT-reduction control, (killed tissue control, KC) was introduced. However, the result of the KC did not indicate an increased MTT reduction, therefore the KC was not used for viability calculation.

RESULTS

Exposure: 3 min						
Test Article	OD_{570} tissue 1	OD_{570} tissue 2	OD_{570} KC	mean OD_{570}	mean OD_{570} KC corrected	Viability [% of NC]
NC	1.944	1.813	0.181	1.878	-	100
TS	2.091	1.868	0.151	1.980	-	105
PC	0.448	0.422	-	0.435	-	24

Exposure: 1 h						
Test Article	OD_{570} tissue 1	OD_{570} tissue 2	OD_{570} KC	mean OD_{570}	mean OD_{570} KC corrected	Viability [% of NC]
NC	1.888	1.759	0.167	1.823	-	100
TS	1.736	1.588	0.145	1.662	-	91
PC	0.108	0.148	-	0.128	-	7

OD: optical density; NC: negative control (highly deionised water); TS: test substance; PC: positive control (8 N potassium hydroxyde).

CONCLUSION The notified chemical does not show a corrosive potential in the EpiDerm skin corrosivity test under the test conditions chosen.

The test method used does not allow for the evaluation of skin irritation. The result does not exclude an irritation potential of the test substance. For final assignment of a risk phase for skin irritation, results from an in vivo study or an additional in vitro assay conducted according to OECD 439 would be needed.

TEST FACILITY BASF SE (2009e)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD The potential of the notified chemical to cause serious damage to the eye/mucous membranes was assessed by a single topic application of 0.3 mL of the undiluted test substance and 0.3 mL of a 10% test substance solution in olive oil to the chorionallantoic membrane (CAM) of fertilised and incubated hen eggs.
Three eggs per test substance concentration were observed until unambiguous irritation reactions were detected. The occurrence of vascular injury or intravascular coagulation in response to the test substance was recorded.

RESULTS

Concentration:	Egg-No.:	Time (seconds) until appearance of:		Grading of effects:	
		Haemorrhagia:	Coagulation:	Haemorrhagia:	Coagulation:
Undiluted test substance	1	64	64	1	2
	2	53	53	1	2
	3	51	51	1	2
Mean:	n = 3	56	56	1	2

Concentration:	Egg-No.:	Time (seconds) until appearance of:		Grading of effects:	
		Haemorrhagia:	Coagulation:	Haemorrhagia:	Coagulation:
10% test substance in olive oil	1	30	30	1	2
	2	36	36	1	2
	3	41	41	1	2
Mean:	n = 3	36	36	1	2

Positive controls

Concentration:	Egg-No.:	Time (seconds) until appearance of:		Grading of effects:	
		Haemorrhagia:	Coagulation:	Haemorrhagia:	Coagulation:
0.1 M NaOH	1	20	40	2	2
	2	28	42	2	2
Mean:	n = 2	24	41	2	2

Concentration:	Egg-No.:	Time (seconds) until appearance of:		Grading of effects:	
		Haemorrhagia:	Coagulation:	Haemorrhagia:	Coagulation:
10% sodium dodecyl sulfate	1	22	41	2	2
	2	21	35	2	2
Mean:	n = 2	22	38	2	2

Remarks - Results: The undiluted test substance caused moderate (grade 2) intravascular coagulation and slight (grade 1) haemorrhagia in all eggs after 51-64 seconds.
The 10% test substance solution in olive oil caused moderate intravascular coagulation and slight haemorrhagia in all eggs after 30-41 seconds.

CONCLUSION: The notified chemical is severely irritating to the eye under the test conditions.

TEST FACILITY: BASF SE (2009f)

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B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain: Mouse/CBA/J (female)
Vehicle: Methyl ethyl ketone (MEK)
Remarks - Method: No deviations from the protocol.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		

0 (vehicle control)	543.3	1.00
3%	1562.1	2.88
10%	2466.5	4.54
30%	9759.6	17.96

Remarks - Results

No signs of systemic toxicity were noted.
The test substance induced a concentration dependent response in the auricular lymph node cell counts, which was biologically relevant (increase to 1.5 fold or above control value = stimulation index (SI) ≥ 1.5) when applied as 10% and 30% preparations in MEK. There was a concentration dependent increase in lymph node weights as well.
The increase of ^{3}H-thymidine incorporation into the cells was concentration dependent and biologically relevant (increase above the cut off stimulation index of 3) at concentrations of 10% and 30%.
The 3% and 10% test substance preparations caused some increase and the 30% preparation a severe increase in ear weights. Scaling on ears was observed on the day of lymph node removal in the 10% and 30% test substance groups.
Although the magnitude of ear skin irritation in the 30% test substance group might have influenced the lymph node reaction, the considerable increase in the cell count and ^{3}H-thymidine incorporation indices cannot be explained by irritation alone.
The threshold concentration for sensitisation induction was >3%<10%. The estimated concentration that leads to the SI of 1.5 for cell count (EC 1.5) and the estimated concentration that leads to the SI of 3.0 for ^{3}H-thymidine incorporation (EC3) were calculated by linear regression from the results of the 3% and 10% concentrations to be 6.8% and 3.5%, respectively.

CONCLUSION

There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY

BASF SE (2009g)

B.6. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical

METHOD

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

Species/Strain: Rats/Wistar

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: not applicable

Vehicle: Drinking water

Remarks - Method: No deviations from the protocol.

RESULTS

Dose (mg/kg bw/day)	*Number and Sex of Animals*	*Mortality*
0	5 per sex	0
100	5 per sex	0
300	5 per sex	0
1000	5 per sex	0

Mortality and Time to Death

No animals died prematurely in the study.

Clinical Observations

Regarding clinical examinations, signs of general systemic toxicity were not observed up to a dose level of 1000 mg/kg bw/day.

Slight and moderate salivation after treatment was seen in all animals of both sexes of 1000 mg/kg bw/day group. Slight salivation after treatment was observed in all males and 3 females of 300 mg/kg bw/day group and 1 male animal of 100 mg/kg bw/day group. This finding was observed on day 2 for the first time and afterwards on several days of the study. Salivation was considered to be related to either the bad taste of the test substance or local affection of the upper digestive tract. Therefore this finding was not considered to be an adverse and toxicologically relevant effect.

Detailed clinical observations, which were performed in all animals at weekly intervals did not reveal additional findings.

No test substance-related effects on food consumption were observed.

No test substance-related changes of body weight and body weight change were observed in any test group.

Body weight change was significantly increased in female animals of 100 mg/kg bw/day group on day 21 (+26%). These changes were assessed as being incidental and not related to treatment.

For functional observational battery, deviations from “zero values” were observed in several rats. However, as most findings were equally distributed between test substance treated groups and controls, were without a dose-response relationship or occurred in single rats only, these observations were considered to have been incidental. No test substance related or spontaneous findings during the home cage and open field observations or for sensorimotor test/reflexes and quantitative parameters in male and female animals of all test groups were observed.

For motor activity measurement, there were no significant deviations with regard to the overall motor activity (summation of all intervals) in the male and female animals of all test groups in comparison to the concurrent control group. Comparing the single intervals, values of interval 3 for female animals in 100 and 300 mg/kg bw/day groups were significantly increased. These findings were assessed as spontaneous in nature and not related to test substance administration as the other interval values did not show any changes in these test groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No treatment related changes among haematological parameters were observed. In males of 100 and 300 mg/kg bw/day groups the haemoglobin and haematocrit values were significantly decreased. These decreases were marginal and not dose-dependent and, therefore, regarded as incidental rather than treatment related.

No treatment related adverse changes among clinical chemistry parameters were observed. In females of 100 mg/kg bw/day group the alkaline phosphatase (ALP) activity was higher compared to controls. The ALP was not dose-dependently increased. Therefore, this change was regarded as incidental and not treatment related.

No treatment related changes among urinalyses parameters were observed.

Effects in Organs

When compared to control group (set to 100%), the mean absolute weights of kidneys and testes were significantly increased:

	Male animals		
Test group (mg/kg bw/d)	1 (100)	2 (300)	3 (1000)
Kidneys	107%*	103%	112%**
Testes	107%	111%*	111%**

*P ≤ 0.05; **P ≤ 0.01

All other mean absolute weight parameters did not show significant differences when compared to the control group. In females, there were no significant weight deviations.

The significantly increased mean absolute kidney and testes weights were considered to be incidental.

When compared to control group (set to 100%), the mean relative weights of kidneys and testes were significantly increased:

	Male animals		
Test group (mg/kg bw/d)	1 (100)	2 (300)	3 (1000)
Kidneys	103%	105%	112%*
Testes	102%	113%	110%*

*P ≤ 0.05

All other mean relative weight parameters did not show significant differences when compared to the control group. In females, there were no significant weight deviations.

Because there were no histopathological correlates for the significantly increased mean relative kidney and testes weights in males of the 1000 mg/kg bw/day group and because the increase of testes weights was not dose-related, the increased organ weights were considered to be incidental.

The single gross lesions noted were considered to be incidental and spontaneous in nature and not related to treatment.

In the 1000 mg/kg bw/day group findings in the forestomach were observed in 3 out of 5 males and 1 female: in 1 male animal a focal squamous hyperplasia was observed, in 1 male and in 1 female animal an erosion/ulcer occurred in the 3rd male, a minimal focal inflammation was noted. The erosion or ulcer and the inflammation were located in the region of margo plicatus. All these findings were assessed as being related to treatment and were considered to be adverse.

All other findings noted were either single observations or they were biologically equally distributed between the control group and treatment groups. All of them were considered to be incidental or spontaneous in origin and without any relation to treatment.

Remarks – Results

The findings observed for the forestomach were most likely related to the irritating potential of the test substance as it was directly administered into the forestomach by gavage with the consequence of a local irritation.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) concerning systemic toxicity was established as 1000 mg/kg bw/day since only local signs of toxicity in the forestomach were observed.

The No Observed Adverse Effect Level (NOAEL) for local effects was established as 300 mg/kg bw/day with regard to irritation effects in the forestomach in the 1000 mg/kg bw/day group.

TEST FACILITY BASF SE (2010)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.

	Plate incorporation procedure (tests 1 and 2) and pre incubation procedure (test 3)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 mix was prepared from the livers of phenobarbital/β-naphthoflavone induced male Wistar rats.
Concentration Range in Main Test	Test 1) With and without metabolic activation: 0, 20, 100, 500, 2500, 5000 µg/plate Test 2) With and without metabolic activation: 0, 31.3, 62.5, 125, 250 and 500 µg/plate for TA1535, TA1537, TA98, TA100 Test 3) Without metabolic activation: 0, 15.6, 31.3, 62.5, 125, 250 µg/plate for TA1535, TA1537, TA100 With metabolic activation: 0, 31.3, 62.5, 125, 250, 500 µg/plate for TA1535, TA1537, TA100 With and without metabolic activation: 0, 31.3, 62.5, 125, 250, 500 µg/plate for TA98 With and without metabolic activation: 0, 20, 100, 500, 2500, 5000 µg/plate for *E. coli*: WP2uvrA
Vehicle	Dimethylsulfoxide (DMSO)
Remarks - Method	No preliminary toxicity test was conducted. However, dose selection and evaluation as well as the number of plates used in repeat studies or further experiments are based on findings of the Test 1.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Tests 1 and 2	≥ 250	≥ 500	negative
Test 3	≥ 250	≥ 500	negative
Present			
Tests 1 and 2	≥ 250	≥ 500	negative
Test 3	≥ 250	≥ 500	negative

Remarks - Results	According to the results of the study, the test substance did not lead to an increase in the number of revertant colonies either without S9 mix or after adding a metabolising system in three experiments carried out independently. In addition, the results of negative and positive controls performed in parallel corroborated the validity of the study, since the values fulfilled the acceptance criteria.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BASF SE (2009h)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	V79 Cells of the Chinese hamster
Metabolic Activation System	S9 mix was prepared from the livers of phenobarbital/β-naphthoflavone induced male Wistar rats.
Vehicle	Suspended in deionised water
Remarks - Method	Minor deviations had no detrimental impact on the outcome of the study.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*

Absent			
Test 1	0, 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5*, 25.0*, 50.0*	4 h	18 h
Test 2a	0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5*, 25.0*, 50.0*, 100.0	18 h	18 h
Test 2b	0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5*, 25.0*, 50.0*, 100.0	28 h	28 h
Present			
Test 1	0, 19.5, 39.1, 78.1*, 156.3*, 312.5*, 625.0, 1250.0, 2500.0, 5000.0	4 h	18 h
Test 2	0, 2.4, 4.9, 9.8, 19.5, 39.1, 78.1*, 156.3*, 312.5*, 625.0	4 h	28 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 100.0			
Test 1		≥ 50.0	> 50.0	negative
Test 2a		≥ 100	> 100.0	negative
Test 2b		≥ 100	> 100.0	negative
Present	> 5000.0			
Test 1		> 625.0	≥ 312.5	negative
Test 2		≥ 625.0	≥ 156.3	negative

Remarks - Results

No relevant influence of the test substance on pH value or osmolarity was observed.
In both tests, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test substance (1.0-4.0% aberrant cells, excluding gaps) were close to the range of the solvent control values (1.0-3.5% aberrant cells, excluding gaps) and within the range of the laboratory's historical control data regarding all possible solvents (0.0-4.0% aberrant cells, excluding gaps).
In both tests, no biologically relevant increase in the rate of polyploidy metaphases was found after treatment with the test substance (1.6-5.2%) as compared to the rates of the solvent controls (1.8-5.7%).
In Test 1 in the absence as well as in the presence of S9 mix statistically significant increases in endomitotic metaphases were observed. In the absence of metabolic activation 0.7% of endomitotic cells were observed after treatment with a test substance concentration of 12.5 µg/mL. In the presence of S9 mix 1.3 and 0.4% endomitotic cells occurred after treatment with 156.3 and 312.5 µg/mL. As these findings were not dose dependent and could not be confirmed in Test 2 they are regarded as biologically irrelevant.
In both tests, either ethylmethane sulfonate (EMS, 550.0 or 1000.0 µg/mL) or cyclophosphamide (CPA, 1.4 or 2.0 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

CONCLUSION

The notified chemical was not clastogenic to V79 cells (Chinese hamster cell line) treated *in vitro* under the conditions of the test.

TEST FACILITY

Harlan CCR (2010a)

B.9. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain	Mouse/NMRI
Route of Administration	Oral – gavage
Vehicle	Corn oil
Remarks - Method	Minor deviations had no detrimental impact on the outcome of the study. As estimated by a pre-test 2000 mg/kg bw (the maximum guideline-recommended dose) was suitable. Since no gender specific differences concerning signs of toxicity in the pre-test were observed, the main study was performed using males only.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
vehicle control	5 M	0	24
	5 M		48
low dose	7 M	500	24
mid dose	7 M	1000	24
high dose	7 M	2000	24
	7 M		48
positive control, CPA	5 M	40	24

CPA=cyclophosphamide.

RESULTS

Doses Producing Toxicity	The mean number of polychromatic erythrocytes was not decreased after treatment with the test substance as compared to the mean value of PCEs of the vehicle control indicating that the notified chemical did not have any cytotoxic properties in the bone marrow.
Genotoxic Effects	In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test substance. Although a slight dose-related increase in the mean values of micronuclei was observed after treatment with the notified chemical all values were below or near to the value of the corresponding vehicle group and clearly within the historical negative control data range.
Remarks - Results	40 mg/kg bw CPA administered orally was used as positive control which showed a substantial increase of induced micronucleus frequency. There was no indication, either from bone marrow cytotoxicity or clinical observations of toxicity, that the test substance was reaching the target organ bone marrow.

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* micronucleus test with bone marrow cells of the mouse, although there may not have been significant bone marrow exposure to the notified chemical.

TEST FACILITY Harlan CCR (2010b)

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 from a domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	TOC
Remarks - Method	The test was conducted for 28 days in accordance with the above guidelines. The test substance (20 mg/L TOC) was added to a liquid medium inoculated with sewage microorganisms. CO_2 production was analysed. A reference (aniline, 20 mg/L TOC) and toxicity control were run in parallel.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation**	*Day*	*% Degradation*
0	0	0	0
3	20	3	4
7	32	7	49
14	50	14	71
28	65	28	87

*Mean of 2 values

Remarks - Results	All validity criteria were satisfied. The reference compound reached the 60% pass level by day 14 indicating the suitability of the inoculum. The toxicity control attained 67% degradation after 14 days indicating the notified chemical is not toxic to the inoculum. The test substance was found to be biodegradable (65%) under the conditions of the test. However, as biodegradation did not reach the pass level of > 60% CO_2 production within the 10 day window, it cannot be classed as readily biodegradable.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	BASF SE (2009i)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Flow through
Species	Bluegill Sunfish (*Lepomis macrochirus*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Approx. 100 mg $CaCO_3$/L
Analytical Monitoring	TOC
Remarks – Method	Based on a preliminary test under static conditions a test was conducted with concentrations from 0 (control) to 56 mg/L of test substance under flow through conditions (3.75 L/hr, 5 volume changes/day). The test substance was mixed with stock solution using an ultra turrax high shear mixer. The median lethal concentration (LC50) at 96 hours was

calculated using the probit method.

RESULTS

Concentration mg/L		*Number of Fish*	*Cumulative Mortality*					
Nominal	*Actual**		*1 h*	*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	20	0	0	0	0	0	0
5.6	4.8	20	0	0	0	0	0	0
10	6.8	20	0	0	0	0	0	0
18	15.2	20	0	0	0	2	8	10
32	28.8	20	0	17	20	20	20	20
56	47.4	20	0	20	20	20	20	20

*Mean of measured concentrations at 0, 48 and 96 h

LC50	15.2 mg/L at 96 hours (based on mean measured concentrations)
NOEC	6.8 mg/L at 96 hours (based on mean measured concentrations)
Remarks – Results	All validity criteria for the test were satisfied. However, for one concentration the mean measured concentration was 68% of the nominal concentration (guideline states it should be at least 80% of the nominal value). In accordance with the guideline the mean measured concentrations were used to calculate the LC50. The notified chemical is a complex mixture that forms emulsions in water and it was found that LC/MS could not be used for quantification of the test substance due to a shift in signal over time indicating a shift in relative composition favouring polar compounds. Hence, TOC values were used to calculate mean measured concentrations over the test period. Analyses confirmed that the test substance was stable in the test media for up to 48 hours and that the test substance was present in the test vessels over the exposure period.
CONCLUSION	The notified chemical is harmful to fish.
TEST FACILITY	BASF SE (2009j)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Flow-through
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	2.20 – 3.20 mmol/L
Analytical Monitoring	TOC
Remarks - Method	The test was conducted under flow-through conditions (flow rate 0.6 mL/min; 6 volume changes/day) with fresh stock solutions prepared daily. Each concentration was prepared separately by directly adding the test substance to test medium and stirring for approximately 1 hour at 20 ± 2°C. Solutions were pumped into each test replicate. A positive control was conducted monthly with potassium dichromate as the reference material. The EC50 at 96 hours was calculated using the probit method.

RESULTS

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

0.56	< LOQ**	20	0	0
1.0	< LOQ**	20	0	0
1.8	1.7	20	0	1
3.2	2.0	20	0	5
5.6	3.2	20	3	20
10	6.1	20	9	20

*Mean measured concentrations at 0 and 48 h
**LOQ (limit of quantification) = 1.2 mg/L

EC50	2.25 mg/L at 48 hours (based on mean measured concentrations) (95% CI 2.12 – 14.32 mg/L)
EC0	< 1.9 mg/L at 48 hours (based on mean measured concentrations)
Remarks - Results	Daphnia exhibited a normal toxic response to potassium dichromate, with an EC50(24 h) = 1.13 mg/L which was in the normal range (0.6 – 2.1 mg/L). All validity criteria for the test were satisfied. The test water was clear at all concentrations throughout the test. No significant deviations from the test guidelines were reported. The notified chemical is a complex mixture that forms emulsions in water and it was found that LC/MS could not be used for quantification of the test substance due to a shift in signal over time indicating a shift in relative composition favouring polar compounds. Hence TOC values were used to calculate mean measured concentrations over the test period. Analyses confirmed that the test substance was stable in the test media for up to 48 hours and that the test substance was present in the test vessels over the exposure period.
CONCLUSION	The notified chemical is toxic to aquatic invertebrates.
TEST FACILITY	BASF SE (2009k)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudeokirchneriella subcapitata*
Exposure Period	96 hours
Concentration Range	Nominal: 0, 0.56, 1.8, 3.2, 5.6 and 10 mg/L Actual§: 0.25*, 0.30*, 0.9*, 1.5, 3.0, 6.5 mg/L §Mean measured concentrations based on mean of 0 h inoculated and 96 uninoculated samples. *Values < LOQ (1.2 mg/L)
Auxiliary Solvent	None
Water Hardness	0.15 mmol/L (Ca^{2+} and Mg^{2+})
Analytical Monitoring	TOC
Remarks - Method	Based on a preliminary range finding test algae cells were exposed to notified chemical at nominal loading rates of 0, 0.56, 1.8, 3.2, 5.6 and 10 mg/L for a period of 96 hours. Algal growth was measured as *in vivo* chlorophyll-a fluorescence at 0, 24, 48, 72 and 96 h. A positive control test was performed by exposing algae to potassium dichromate under similar conditions to those in the main test. The EC50 was determined by linear interpolation on a plot of concentration *vs* % inhibition. LOEC was calculated by Dunnett's test (one-sided) at a 95% significance level and the NOEC was the next concentration tested below the LOEC. At the end of the definitive test, a regrowth test was performed to determine the algicidal or algistatic effect of the test substance.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h*	*NOEC* mg/L*	*E_rC_{50}* mg/L at 72 h*	*NOEC* mg/L*
1.47	0.9	2.14	0.9

*Based on the mean measured concentrations of the 0 h inoculated and 96 h uninoculated samples.

Remarks - Results

Algae exhibited a normal toxic response to potassium dichromate, with an E_rC50(24 h) = 1.36 mg/L which was in the normal range (0.92 – 1.46 mg/L). All validity criteria for the test guideline were satisfied.

The notified chemical is a complex mixture that forms emulsions in water and it was found that LC/MS could not be used for quantification of the test substance due to a shift in signal over time indicating a shift in relative composition favouring polar compounds. Hence TOC values were used to calculate mean measured concentrations over the test period.

The test substance was not stable in the test medium over the duration of the test so the results are considered as the effect of the toxicity due to both the test substance and degradation products. The measured concentrations of notified chemical were low due to reported degradation of notified chemical and adsorption of notified chemical to algae (which was removed by centrifugation). The toxicity endpoints derived from the measured concentrations were used because of the complications reported with notified chemical concentration and to ensure a conservative result as the actual notified chemical concentrations were most likely higher than those reported.

In the regrowth test, growth was observed on day 4 indicating that the test substance was algistatic.

CONCLUSION

The notified chemical is toxic to algae

TEST FACILITY

BASF SE (2009l)

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