File No: STD/1662 and STD/1663

October 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

STD/1662: Chemical 1 in GSID 3056-2 FF

STD/1663: Chemical 2 in GSID 3056-2 FF

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/1662 STD/1663	BASF Australia Limited	Chemical 1 in GSID 3056-2 FF Chemical 2 in GSID 3056-2 FF	No	< 30 tonnes per annum	Component of plastic films

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemicals are not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

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

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

Environmental risk assessment
On the basis of the assumed low hazard, and the assessed use pattern the notified chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemicals:
  - Avoid skin and eye contact

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemicals:
  - Impervious gloves
  - Safety glasses
  - Coveralls

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

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

- If products and mixtures containing the notified chemicals 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

- Where reuse or recycling are not appropriate, dispose of the notified chemicals in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Emergency procedures

- Prevent from entering into soil, ditches, sewers, waterways and/or groundwater.
- Spills or accidental release of the notified chemicals 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 chemicals are 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 chemicals have changed from a component of plastic films, or is likely to change significantly;
  - the amount of chemicals being introduced has increased, or is likely to increase, significantly;
  - the chemicals have begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemicals 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.

Safety Data Sheet
The SDS of the products containing the notified chemicals provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

NOTIFICATION CATEGORY
STD/1662 - Standard: Chemical other than polymer (more than 1 tonne per year)

STD/1663 - Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Chemical is being notified at the same time as a similar chemical.

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, use details and import volume

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Hydrolysis as a Function of pH, Partition Coefficient, Adsorption / desorption, Dissociation Constant, Flash Point, Flammability Limits, Reactivity, Acute inhalation toxicity, Genotoxic Damage in vivo.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
STD/1662: EU (2012), China (2018)
STD/1663: Japan (2016), EU (2017), Switzerland (2018), China (2018).

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
STD 1662: Chemical 1 in GSID 3056-2 FF
STD/1663: Chemical 2 in GSID 3056-2 FF

OTHER NAME(S)
Sterically Hindered Amine Light Stabiliser

MOLECULAR WEIGHT
STD/1662: Value for the notified chemical > 500 g/mol
STD/1663: Value for the notified chemical > 500 g/mol

ANALYTICAL DATA
Reference Elemental analysis, NMR, IR, MALDI-MS, HPLC, GPC and UV spectra were provided on each substance.

3. COMPOSITION

DEGREE OF PURITY
> 95 %

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Reddish brown solid granules

Property	Value STD/1662	Value STD/1663	Data Source STD/1662	Data Source STD/1663
Melting Point/Freezing Point	Not determined	Not determined	Measured, Glass transition temperature 105 °C.	Measured, glass transition temperature 87 °C
Boiling Point	Not determined	Not determined	Measured (decomposition starts from about 250 °C)	Measured (decomposition starts from about 250 °C)
Relative Density	1.169	0.926	Measured	Measured
Vapour Pressure	Not determined	< $1\times\cdot10^{-6}$ hPa at 20, 25 or 50 °C	Expected to be similar to STD/1663	Measured
Water Solubility	< 11 mg/L at 20 °C	Not determined	Measured	Expected to be similar to STD/1662
Hydrolysis as a Function of pH	Not determined	Not determined	Does not contain hydrolysable functionalities.	Does not contain hydrolysable functionalities.
Partition Coefficient (n-octanol/water)	Not determined	log Pow = > 5 at 23 °C	Expected to be similar to STD/1663	Estimated value
Adsorption/Desorption	log K_{oc} = > 5	Not determined	Expert statement	Not determined
Dissociation Constant	Not determined	Not determined	Does not contain dissociable functionalities in the environmentally relevant range (pH 4-9)	Does not contain dissociable functionalities in the environmentally relevant range (pH 4-9)
Particle Size	Inhalable fraction (< 100 µm): 29.35% Respirable fraction (< 10 µm): 2.85%	Not determined	Measured	Expected to be similar to STD1662
Flash Point	Not determined	Not determined	Expected to be similar to STD/1663	Expected to be high based on flammability study and low vapour pressure
Flammability	Not determined	Not highly flammable	Expected to be similar to STD/1663	Measured
Autoignition Temperature	Not determined	> 400 °C	Expected to be similar to STD/1663	Measured
Explosive Properties	Not explosive	Not determined	Measured	Contains no functional groups that imply explosive properties
Oxidising Properties	Not oxidising	Not determined	Measured	Contains no functional groups that imply oxidising properties

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 chemicals are not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemicals (as the neat raw materials) will be imported into Australia as solid granules.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 30	< 30	< 30	< 30	< 30

PORT OF ENTRY
Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
BASF Australia Ltd
Level 12, 28 Freshwater Place
Southbank VIC 3006

TRANSPORTATION AND PACKAGING
The notified chemicals will be introduced in 40-kg fibreboard cartons and transported by road.

USE
The notified chemicals (at a concentration of < 1%) will be used as light stabilizer for plastic films in agricultural applications (e.g. greenhouse film covers, mulch films and non-woven films).

OPERATION DESCRIPTION
The notified chemicals (at 100% concentration and in granular form) will be reformulated with other ingredients and pelletized. The notified chemicals will be manually weighed, transferred and loaded into a hopper where they will be mixed with other ingredients. After blending, the mixture (containing the notified chemicals at < 1%) will be extruded into masterbatch pellets.

At the injection moulding site, workers will remove the masterbatch pellets from packaging and add them into the hopper of an injection moulding machine. The pellets will be heated to about 250°C in the machine and injected as a liquid, under pressure into moulds to form articles.

The plastic articles or films containing the notified chemicals will be used in various agricultural applications such as greenhouse film covers, mulch films and non-woven films.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	1 - 2	30 – 50
Pellet formulation	2 - 4	30 – 50
Injection moulding	6 - 8	80 – 100

EXPOSURE DETAILS

Transport and Storage
Worker exposure to the notified chemicals in neat form during the importation, transport and storage is not expected, except in the unlikely event of an accident where the packaging may be breached. .

Pellet formulation
Dermal and ocular exposure to the notified chemicals at concentrations up to 100% may occur during weighing, transferring and loading of the chemicals into to the mixing vessel and hoppers, quality control, and equipment cleaning and maintenance processes. Exposure is expected to be minimised through the use of automated blending and feeding systems and the use of personal protective equipment (PPE) such as gloves, eye protection and protective clothing. The vapour pressure of the chemicals is low and inhalation exposure will be further minimised through the use of general and local ventilation.

Injection moulding
At the injection mould sites, dermal and ocular exposures of workers to the notified chemicals will be minimal as the notified chemicals are present at low concentrations (between 0.1-1%) and will be further minimised by the expected use of PPE by workers. Inhalation exposure will be reduced due to the chemicals encapsulation in the masterbatch pellets and the use of local exhaust ventilation.

6.1.2. Public Exposure
The notified chemicals will not be made available to the general public. Plastics containing the notified chemicals (at 0.1-1% concentration) will be only used in agricultural applications. Moreover, the notified chemicals will be encapsulated within the articles plastic matrix and hence exposure to the chemicals from contact with the articles is expected to be negligible.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemicals 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 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity*	LD50 > 5,000 mg/kg bw; low toxicity
Skin irritation (*in vitro*)**	non-irritating
Rabbit, skin irritation*	slightly irritating
Eye irritation**	non-irritating
Rabbit, eye irritation*	slightly irritating
Mouse, skin sensitisation – Local lymph node assay**	no evidence of sensitisation
In vitro Sensitisation (DPRA)**	evidence of sensitisation
In Vitro Sensitization - Dendritic Cell Line Activation Assay**	no evidence of sensitisation
Rat, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	NOAEL > 840 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* – Chromosome Aberration Test	non genotoxic
Genotoxicity – *In vitro* - Mammalian Cell Gene Mutation Test	non clastogenic

*Chemical 1 in GSID 3056-2 FF
**Chemical 2 in GSID 3056-2 FF

Toxicokinetics, metabolism and distribution
No data on toxicokinetics for the notified chemical was provided. Liquids and substances in solution are taken up more readily than dry particulates (ECHA, 2017). For dermal absorption, molecular weights below 100 g/mol. are favourable for absorption and molecular weights above 500 g/mol. do not favour absorption (ECHA, 2017). Dermal uptake is likely to be low to moderate if water solubility is below 100 mg/L and the log P values are above 4 (ECHA, 2017). Given the high molecular weight (> 500 g/mol) and low water solubility (< 11 mg/L at 20 °C) and high log P values (> 5 at 23 °C) of the notified chemicals, absorption across biological membranes is expected to be limited.

Acute toxicity

The notified chemicals were found to be of low acute oral and dermal toxicity in studies conducted in rats.

Irritation and sensitisation
Based on a studies conducted in rabbits, the notified chemicals were slightly irritating to skin and to eyes.

Based on the *in vitro* skin corrosion in reconstructed human epidermis test and eye irritation Bovine corneal opacity and permeability test, the notified chemicals were considered not irritating to skin and to eyes respectively.

In a mouse LLNA study, the notified chemicals were determined to be a non-sensitising at 2% concentration, which was the maximum concentration that did not produce skin irritation in the preliminary study.

A battery of tests consisting of one *in chemico* and one *in vitro* cell based assay were conducted on each of the two notified chemicals to evaluate their skin sensitisation potential. The notified chemicals showed moderate chemical reactivity in the *in chemico* Direct Peptide Reactivity Assay (DPRA). The notified chemicals did not meet the criteria under the criteria in OECD TG 442e to be considered a positive indication of skin sensitisation in the in vitro dendritic cell line Myeloid U937 activation assay.

On the weight of evidence of all the conducted sensitisation studies the notified chemicals are not considered to be skin sensitisers.

Repeated dose toxicity
In a repeated dose oral (gavage) toxicity study combined with the reproduction/developmental toxicity screening test, the notified chemical was administered to rats at the nominal doses 0, 100, 300 and 1000 mg/kg bw/day (actual doses were 0, 84, 252, and 840 mg/kg bw/day respectively).

No test substance-related, adverse findings were observed in all test and recovery groups. No treatment related adverse effects or signs of toxicity on any reproductive or developmental parameters at any dose level and no significant abnormal findings of pups or fertility and implantation effects were noted. The No Observed Adverse Effect Level (NOAEL) for systemic and reproduction/developmental toxicity was considered to be 840 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemicals tested negative in a bacterial reverse mutation assay, an *in vitro* mammalian cell chromosome aberration test and in an *in vitro* mammalian cell gene mutation test. Based on these results, the notified chemicals are not considered to be genotoxic.

Health hazard classification
Based on the available information, the notified chemicals are not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Dermal and ocular exposure to the notified chemicals, at concentrations up to 100%, by workers may occur during transport, storage and reformulation of the notified chemicals, and at concentrations up to 1% during injection mould operation / production process of plastic articles.

Toxicological studies on the notified chemicals indicate that they are expected to be of low toxicity. Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemicals is not considered to be unreasonable.

6.3.2. Public Health
The notified chemicals will not be made available to the general public and will be only used in agricultural applications. The notified chemicals at < 1% concentration will be encapsulated within the articles plastic matrix. Exposure of the general public to the notified chemicals will be negligible. Therefore, the notified chemicals are not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemicals will be imported into Australia in sealed fibreboard cartons. The most likely source of release during importation, storage, and transport to the environment will be from an accident during transport. Any release that does occur as a result of an accident is expected to be physically contained. Spilt granules can be reused to the extent practicable or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

Plastic films containing the notified chemicals will be used as light stabilisers in agriculture as greenhouse film covers, mulch films and non-woven films. Exposure to the notified chemicals in these films will be minimal as it will be present as an intimate mixture with other components of the plastic film. There should be no disposal to drains, surface waters and groundwater.

RELEASE OF CHEMICAL FROM DISPOSAL

The notified chemicals in plastic films are expected to share the same fate as these products, which are likely to be disposed of to landfill at the end of their useful lives. Similarly, the notified chemicals from factory spills will be disposed of to landfill. No significant aquatic release of the notified chemicals is expected from such disposal.

7.1.2. Environmental Fate

The notified chemicals are not biodegradable (Appendix C). Based on their low water solubility and high adsorption coefficient values, the notified chemicals are expected to bind strongly to soil and sediment, and are therefore not likely to be mobile. In landfill the notified chemicals are expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated for the notified chemicals, as no significant release to the aquatic compartment is expected from the proposed use pattern.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity – Zebrafish (96 h)	EC50 > 100 mg/L	Not toxic
Fish Toxicity – Chinese minnow (96 h)	EC50 > 100 mg/L	Not toxic
Daphnia Toxicity (48 h)	EC50 > 100 mg/L	Not toxic
Algal Toxicity (72 h)	EC50 > 1000 mg/L	Not toxic

Based on the above ecotoxicological endpoints for the notified chemicals, they are not expected to be harmful to aquatic life. Therefore, the notified chemicals are not formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has not been calculated, as the submitted ecotoxicological studies indicate that the notified chemicals are not expected to be harmful to aquatic life, and no significant release to the aquatic compartment is expected from the proposed use pattern.

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) for the aquatic compartment has not been calculated, since as discussed above, release to the aquatic compartment is not expected, and neither a PEC nor PNEC were calculated. The notified chemicals are not considered readily biodegradable, and due to their high molecular weight are expected to have a low potential for bioaccumulation.

Therefore, on the basis of the low expected release to the aquatic compartment, submitted ecotoxicological studies that indicate low toxicity to aquatic life, and the assessed use pattern as an inert component of plastics, the notified chemicals are not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

STD/1662:

Melting Point/Freezing Point Not determined

Method OECD TG 102 Melting Point/Melting Range
Remarks The melting temperature was measured by Differential Scanning Calorimetry. The test item (solid) had no melting temperature between 0 °C and 250 °C. However, The visual inspection after determination in duplicate A and B (run up to 170 °C) showed that the test item at this temperature of 170 °C has been liquid. A glass transition was found at 105 °C.
Test Facility BASF (2015a)

Relative Density 1.169

Method OECD TG 109 Density of Liquids and Solids
Remarks The density was measured by the gas pycnometer method.
Test Facility BASF (2015a)

Water Solubility < 11 mg/L at 20 °C

Method OECD TG 105 Water Solubility
EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks Flask Method
Test Facility BASF (2015b)

Adsorption/Desorption log K_{oc} = > 5

Remarks Application for new chemical notification indicates that this value is sourced from 'expert statement'.

Particle Size < 100 µm: 29.35%
< 10 µm 2.85%

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions

Range (µm)	*Mass (%)*
< 4	0.35
< 10	2.85
< 100	29.35

Remarks Laser diffraction method
Test Facility BASF (2016b)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Test for Explosive Properties after UN has not been carried out because the exothermic decomposition energy, determined by a DSC, is less than 500 J/g
Test Facility BASF (2016a)

Oxidizing Properties Not oxidising

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids)

Remarks The test substance was combusted with cellulose and compared to a potassium bromate reference.
Test Facility BASF (2016a)

STD/1663:

Melting Point/Freezing Point Not determined

Method	OECD TG 102 Melting Point/Melting Range
Remarks	The melting temperature was measured by Differential Scanning Calorimetry. The test item (solid) had no melting temperature between 20 °C and 400 °C. A glass transition was found at 87 °C (A continuing weight loss starting at about 250 °C up to 500 °C shows the decomposition / vaporisation of the test item).
Test Facility	BASF (2012a)

Boiling Point Not determined

Method	OECD TG 103 Boiling Point
Remarks	The melting temperature was measured by Differential Scanning Calorimetry (DSC). The DSC measurements show that at about 250 °C the test item starts decomposition.
Test Facility	BASF (2012a)

Relative Density 0.926

Method	OECD TG 109 Density of Liquids and Solids
Remarks	The density was measured by the pycnometer method.
Test Facility	BASF (2012a)

Vapour Pressure $< 1 \times \cdot 10^{-6}$ hPa at 20, 25 or 50 °C

Method	OECD TG 104 Vapour Pressure
Remarks	The vapour pressure was determined by effusion method.
Test Facility	BASF (2012a)

Partition Coefficient (n-octanol/water) (Estimate value) log Pow = > 5 at 23 °C

Method	Single solubilities in n-octanol and in water.
Remarks	Determination by either OECD TG 107 and OECD TG 117 was not feasible
Test Facility	BASF (2012a)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Remarks	The method consists of the measurement of the burning time after ignition of the test item under defined conditions (conditions were not stated).
Test Facility	BASF (2012b)

Autoignition Temperature > 400 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Remarks	The test item was placed in an oven at room temperature and then increased to 400 °C at a rate of 0.5 °C/min. There was no self-heating detected
Test Facility	BASF (2012b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Chemical 2 in GSID 3056-2 FF
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar/Crl:WI (Han)
Vehicle	Olive oil Ph.Eur
Remarks - Method	No significant protocol deviations GLP compliant

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	2,000	0/3
2	3 F	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	There were no deaths observed during the study period. No signs of toxicity were observed
Effects in Organs	There were no macroscopic pathological findings in the animals sacrificed at the end of the observation period.
Remarks - Results	The mean body weight increased within the normal range throughout the study period.

CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Bioassay (2012)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Chemical 1 in GSID 3056-2 FF
METHOD	OECD TG 402 Acute Dermal Toxicity EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal)
Species/Strain	Rat/ Wistar / Crl:WI (Han) SPF
Vehicle	Corn oil Ph.Eur
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 5,000 mg/kg bw
Signs of Toxicity - Local	There were no deaths observed during the study period. However, the following local effect findings were observed: very slight to severe erythema (grade 1 to 4), very slight to slight oedema (grade 1 to 2), incrustations, scaling, test item residues, and erythema and oedema were noted beyond the application area.
Signs of Toxicity - Systemic	No sign of systemic toxicity effects were observed.
Effects in Organs	The body weight of the female and male animals increased within the normal range throughout the study period with two exceptions (in both females the body weights nearly stagnated during the first week which persisted in one of these animals during the second week, while the other

	animal showed a normal weight increase during the second week).
Remarks - Results	No macroscopic pathologic abnormalities were noted in all animals examined at the end of the study.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Bioassay (2016a)

B.3. Irritation – skin

TEST SUBSTANCE	Chemical 2 in GSID 3056-2 FF
METHOD	OECD TG 431 *In vitro* Skin Corrosion - Human Skin Model Test OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method EC Council Regulation No 440/2008 B.40. *In vitro* Skin Corrosion – Transcutaneous Electrical Resistance Test EC Council Regulation No 761/2009. *In vitro* Skin Corrosion - Human Skin Model Test
Vehicle	The test substance administered as supplied
Remarks - Method	No significant protocol deviations. For the corrosion test, two EpiDerm™ tissue samples were incubated with the test substance for 3 minutes and 1 hour, respectively. The irritation test was performed with three EpiDerm™ tissue samples, which were incubated with the test substance for 1 hour followed by a 42-hours post-incubation period. Negative control (NC): Corrosion test: De-ionized water Irritation test: PBS sterile Positive control (PC): Corrosion test: 8-n potassium hydroxide solution Irritation test: 5% (w/v) sodium dodecyl sulfate in sterile deionized water

RESULTS

Corrosion test

Test material	*Mean OD_{570} of duplicate tissues – Exposure 3 min*	*Relative mean Viability (%)*	*Mean OD_{570} of duplicate tissues – Exposure 1 hour*	*Relative mean Viability (%)*
Negative control	1.906	100	1.664	100
Test substance	1.848	97	1.780	107
Positive control	0.494	26	0.137	8

OD = optical density

Irritation test

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.870	100	9.67
Test substance	1.859	99	7.04
Positive control	0.168	9	0.17

OD = optical density; SD = standard deviation

Remarks - Results	The positive controls returned the expected results confirming the validity of the tests.
CONCLUSION	The notified chemical was non-corrosive and non-irritating to the skin under the conditions of the tests.
TEST FACILITY	BASF (2012c)

B.4. Irritation – skin

TEST SUBSTANCE	Chemical 1 in GSID 3056-2 FF
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)
Species/Strain	Rabbit/New Zealand White :Hsdlf:NZW-(SPF)
Number of Animals	3 F
Vehicle	Test substance administered as supplied
Observation Period	7 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.0	0.3	0.7	1	< 7 days	0
Oedema	0.0	0.0	0.0	0	-	0

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

Remarks - Results	Very slight erythema (grade 1) was observed and was reversible in two animals within 72 hours and in one animal within 7 days after removal of the patch.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Bioassay (2017)

B.5. Irritation – eye

TEST SUBSTANCE	Chemical 2 in GSID 3056-2 FF
METHOD	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants
Vehicle	De-ionized water
Remarks - Method	No significant protocol deviations

RESULTS

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Vehicle control	5.5(2.1)	-0.001(0.002)	5.5(2.1)
*Test substance**	-2.0(3.6)	0.007(0.002)	-1.9(3.6)
*Positive control**	80.2(15.1)	2.804(0.408)	122.2(14.5)

SD = Standard deviation; IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results	As the *in vitro* irritancy score (IVIS) was ≤ 55 the chemical does not pose a risk of serious damage to the eyes.
CONCLUSION	The notified chemical was not corrosive or a severe eye irritant under the conditions of the test.
TEST FACILITY	BASF (2012d)

B.6. Irritation – eye

TEST SUBSTANCE Chemical 1 in GSID 3056-2 FF

METHOD OECD TG 405 Acute Eye Irritation/Corrosion
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation)

Species/Strain	Rabbit/New Zealand White :Hsdlf:NZW-(SPF)
Number of Animals	3 F
Observation Period	7 days
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.7	1.0	1.0	1	< 7 days	0
Conjunctiva: chemosis	0.0	0.0	0.3	1	< 48 hours	0
Conjunctiva: discharge	0.0	0.0	0.3	1	< 7 days	0
Corneal opacity	0.0	0.0	0.0	0	-	0
Iridial inflammation	0.0	0.0	0.0	0	-	0

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

Remarks - Results Slight conjunctival irritation was seen in all animals but had resolved by the day 7 observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Bioassay (2016b)

B.7. *In Chemico* Skin Sensitisation (DPRA Test)

TEST SUBSTANCE Chemical 2 in GSID 3056-2 FF

METHOD Similar to OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA; 2015)

Remarks - Method No significant deviations from the OECD test guideline.

The test substance was dissolved at 100 mM concentration in propanol. Propanol was used as the vehicle control. p-Benzoquinone (prepared as a 100 mM in propanol) was used as positive control. The test substance was incubated in dark with the peptide solutions for 24 h at room temperature for the reaction to take place. The ratios of test substance: peptides were 1:10 cysteine peptides and 1:50 lysine peptides. After incubation, peptide depletion was monitored by HPLC coupled with a UV detector at wavelength of 220 nm using a reverse-phase HPLC column.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle	0.0 ± 1.3	0.0 ± 0.2
Test Substance	61.8 ± 1.8	-3.6 ± 0.5
Positive Control	98.3 ± 0.1	97.5 ± 0.6

Remarks - Results No co-elution of the test substance and peptides occurred.

Negative depletions were considered to be “zero” for calculation of the mean peptide depletion, which was thus calculated to be 30.9% (positive prediction for skin sensitisation). Based on the test results the test substance showed moderate chemical reactivity in the DPRA under the test conditions.

The positive controls and references fulfilled all quality criteria confirming the validity of the test.

CONCLUSION The notified chemical showed moderate chemical reactivity in the DPRA under the test conditions.

TEST FACILITY BASF (2012f)

B.8. *In Vitro* Skin Sensitisation (MUSST)

TEST SUBSTANCE Chemical 2 in GSID 3056-2 FF

METHOD In vitro sensitisation: Dendritic Cell line Activation Assay - Myeloid U937 Skin Sensitisation Test (MUSST) - similar to draft OECD TG 442e *In Vitro* Skin Sensitisation: human Cell Line Activation Test (h-CLAT; 2015)

Vehicle 0.25% Ethanol

Remarks - Method The potential of test substance to induce the cell membrane markers CD86 expression was evaluated in the Human Cell Line Activation Test. For this purpose the test substance was incubated with human cell line Myeloid U937 for approximately 48 hours and membrane markers expression were measured by flow cytometry. Stimuli mediated increase in expression of the cell surface markers CD86 was measured using fluorescence.

A pre-test was performed in order to determine the concentrations suitable for the two main experiments up to 500 µg/mL. The main tests were conducted to evaluate the ability of the test substance to induce expression of CD86. The following concentrations were used: 2.74, 5.48, 10.95, 21.90 and 43.80 µg/mL

Positive Control: Ethylene diamine (EDA, 70 µg/ml)
Negative Control: Lactic acid (LA), 200 µg/mL
Test acceptance criteria: Cell Viability ≥ 75% non-cytotoxic for test substance.

The study authors used a stimulation index (SI) for the CD86 treated cells of 120% and viability of ≥ 70% as the cut off for a positive response (BASF, 2013), whereas the OECD TG 442e protocol sets the SI cut off at 150% with the relative viability also at ≥ 70%.

RESULTS

Sample	*Concentration (µg/mL)*	*MFI* CD86 Mean (%) experiment 1/experiment 2*	*Relative Viability (%) Experiment 1/experiment 2*
Vehicle Control		1.00/1.00	100.0/100.0
Test substance			
	2.74	0.94/1.22	99.9/100.0
	5.48	1.03/1.57	99.9/99.9
	10.95	1.40/1.29	99.8/99.9
	21.90	1.04/0.6	99.5/99.7
	43.80	0.18/0.18	86.1/96.3
Controls			
LA	200	0.9/1.0	99.9/100.1
EDA	70	2.7/2.0	93.3/93.3

*Relative fluorescence intensity

Remarks - Results The test substance produced a SI >120% at 2.74, 5.48 and 10.95 µg/mL and the study authors considered it to be sensitising based on their criteria. However, if

using the criteria set out in OECD TG 442e where the SI needs to be 150%, only at a concentration of 5.48 µg/mL was this met, and only then in 1/2 experiments. Therefore, under the criteria set out in OECD TG 442e the test substance would not be considered to show a positive indication for sensitisation based on the results.

No decrease in cell viability below 70% was observed. In experiments 1 and 2 an induction of the expression of CD 86 was observed at sufficiently non-cytotoxic (cell viability~ 70%) concentration.

Precipitates were observed at ≥ 50 µg/mL concentration of the preliminary test and 43.80 µg/mL of the main experiments after 48 hours incubation / exposure of the test substance.

CONCLUSION The test substance did not meet the criteria under the criteria in OECD TG 442e to be considered a positive indication of skin sensitisation in the MUSST assay.

TEST FACILITY BASF (2012g)

B.9. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Chemical 2 in GSID 3056-2 FF

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain	Mouse/BALB
Vehicle	Methyl ethyl ketone 99%
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance.
Remarks - Method	No significant protocol deviations.

Vehicle: acetone/olive oil (4+1, v/v)
Positive control: α-hexylcinnamaldehyde dissolved in acetone/olive oil (4+1, v/v)

The test substance concentration was based on excessive skin irritation seen in preliminary studies at 5% concentration and above.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	603.5/2	-
0.5	5 F	900.7/2	1.49
1	5 F	1128.3/2	1.87
2	5 F	1170.7/2	1.94
Positive Control			
0	5 F	272.6	1.0
5	5 F	437.3	1.6
10	5 F	653.9	2.4
25	5 F	1611.9	5.9

EC3	Not determined
Remarks - Results	Although a statistically significant increase in DPM value was observed in the mid and high dose groups in comparison to the vehicle control group, this was not considered by the study author biologically relevant since the S.I. determined for this concentration did not exceed the threshold value of 3. A statistically significant or biologically relevant increase in lymph node weights and lymph node cell counts was not observed in any treated group

in comparison to the vehicle control group.

Slight skin irritation was seen in all animals in the 2% concentration test group, mean ear weights also showed a statistically significant increase (25.5%) in mean ear weight.

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

TEST FACILITY Harlan (2013)

B.10. Repeat dose toxicity

TEST SUBSTANCE Chemical 1 in GSID 3056-2 FF

METHOD OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Species/Strain	Rats / Wistar Crl:WI(Han)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days to Male rats; 14 days to female rats prior to pairing and then until entire gestation and lactation period in females. Dose regimen: 7 days per week Post-exposure observation period: Two additional groups of 5 male and 5 female animals at nominal doses of 0 and 1000 mg/kg bw/d were maintained for a subsequent period of at least 14 days of no test substance administration in order to observe reversibility of the findings.
Vehicle	0.5% sodium carboxymethyl cellulose suspension in water.
Remarks - Method	No deviations from protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration (units)*		*Mortality*
		Nominal	*Actual*	
control	10 M, 10 F	0	0	0/20
low dose	10 M, 10 F	100	84	0/20
mid dose	10 M, 10 F	300	252	0/20
high dose	10 M, 10 F	1000	840	0/20
control recovery	5 M, 5 F	0	0	0/10
high dose recovery	5 M, 5 F	1000	840	0/10

Mortality and Time to Death

All animals survived until scheduled necropsy.

Clinical Observations

F0 Parental animals: No test substance-related, adverse findings were observed in all test group and recovery test group animals (including for females during gestation and lactation) except for one male animal in the 100 mg/kg bw/day test group which showed an injury during post mating and considered by the study author as incidental and not related to treatment. A statistically significant decrease in food consumption was detected in males (↓9.6%) dosed at 100 mg/kg bw/day on day 14 during premating and in females (↓24.2%) on postnatal day (PND) 4. On PND 1-13 in females, food consumption was significantly decreased in test groups dosed at 100 mg/kg bw/day (↓9.3%) and at 300 mg/kg bw/day (↓9.5%), but not in test group at 1000 mg/kg bw/day. These changes in food consumption showed no dose-dependency and were assessed as incidental and not-related to treatment by the study authors.

No test substance-related findings in all treated animals from water consumption were observed.

No test substance-related changes in mean body weights were observed for all animals of test groups and recovery groups compared to the control group. Temporarily, mean body weight gain was significantly increased in female animals of test group at 300 mg/kg bw/day during the premating phase between study days 7 to 14 and during the gestation day 0 to 7. Body weight gain was significantly decreased (↓10%) in females of

test group at 300 mg/kg bw/day between lactation days 10 and 13. As the findings were transient and no dose-response relationship was determined, they were considered by the study authors to be not related to treatment.

Locomotor activity was not affected by the treatment with the test item at any dose level.

Oestrous cycle data showed regular cycles in the rearing F1 females of all test groups including the control. The mean oestrous cycle duration in the different test groups ranged from 3.87 to 4.00 days.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were no treatment related adverse effects on clinical chemistry, or haematology noted.

Effects in Organs
There were a statistically significant increase prostrate weights in the 300 mg/kg bw/day (↑17%) and 1000 mg/kg bw/day (↑17%) dose groups. Also in male animals in the same dose groups liver weights were significantly decreased (↓10%). There were no histopathological changes in the liver or prostrate that correlated with the slight changes seen and subsequently the study authors regarded the changes as spontaneous and not treatment related.

There were not treatment related gross lesions or histopathological changes noted in the examined organs from any of the treatment groups.

Reproductive and developmental effects
There were no treatment related adverse effects or signs of toxicity on any reproductive or developmental parameters at any dose level:
- Male and female fertility indices were 90% in test groups treated at 0, 100 or 1000 mg/kg bw/day and 100% in test group treated at 300 mg/kg bw/day. These values reflected the normal range of biological variation inherent in the strain of rats used for this study as all respective values were within the range of the historical control data.
- Mean duration of gestation was similar in all test groups and gestation index was 100% in all test groups.
- Viability and live birth indices reflected the normal range of biological variation inherent in the strain of rats used for this study as all respective values were within the range of the historical control data.
- Mean pup body weights of all pups in all test groups were comparable to the control group.
- No macroscopic abnormalities (No gross lesions in the main or recovery test groups) were observed

Remarks – Results
There were no treatment related adverse effects observed during the study.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 840 mg/kg bw/day in this study for systemic toxicity and reproduction /developmental toxicity, based on no test substance related adverse finding at the highest dose level of the test substance.

TEST FACILITY BASF (2017)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE	Chemical 2 in GSID 3056-2 FF
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Commission Regulation (EC) No 440/2008 Mutagenicity – Reverse Mutation Test using Bacteria Standard Plate test and Pre incubation test
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100, *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0 – 5,000 µg/plate (Standard plate test) 0 - 2,500 µg/plate (preincubation test) b) Without metabolic activation: 0 – 5,000 µg/plate (Standard plate test) 0 – 2,500 µg/plate (preincubation test)
Vehicle	Acetone

Remarks - Method No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1		≥ 5,000	≥ 333	Negative
Test 2		≥ 2,500	≥ 1,000 (	Negative
Present				
Test 1		≥ 2,500	≥ 1,000	Negative
Test 2		≥ 2,500	≥ 1,000	Negative

Remarks - Results A bacteriotoxic effect was observed depending on the strain and test conditions from about 1,000 µg/plate onward.

A biologically relevant increase in the number of his+ or trp+ revertants was not observed in the standard plate test or in the preincubation test either with or without metabolic activation S9 mix.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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

TEST FACILITY BASF (2012e)

B.12. Genotoxicity – *in vitro* -

TEST SUBSTANCE Chemical 1 in GSID 3056-2 FF

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
Commission Regulation (EC) No 440/2008 In vitro Mammalian Chromosome Aberration Test

Species/Strain *Chinese hamster*
Cell Type/Cell Line V79 cells
Metabolic Activation System S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle DMSO
Remarks - Method No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 6.25, 12.5, 25, 50*, 100*, 200*	4 h	18 h
Test 2	0*, 6.25, 12.5, 25, 50*, 100*, 200*	18 h	18 h
Present			
Test 1	0*, 6.25, 12.5*, 25*, 50*, 100, 200	4 h	18 h
Test 2	0*, 6.25, 12.5, 25, 50*, 100*, 200*	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				
Test 1	> 200	> 200	≥ 200	negative
Test 2	> 200	> 200	≥ 200	negative

Present				
Test 1	> 200	> 50	≥ 50	negative
Test 2	> 200	> 200	≥ 200	negative

Remarks - Results

No cytotoxicity indicated by reduced relative population doubling (RPD) or mitotic rates was observed up to the highest applied test substance concentration.

No biologically relevant increase in the frequency of cells containing numerical chromosome aberrations was demonstrated either.

Both positive control substances, ethyl methanesulfonate (EMS) and cyclophosphamide (CPA), and vehicle controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to V79 cells treated *in vitro* under the conditions of the test.

TEST FACILITY

BASF (2016c)

B.13. Genotoxicity – *in vitro* (HPRT Locus Assay)

TEST SUBSTANCE

Chemical 1 in GSID 3056-2 FF

METHOD

OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test
EC No 440/2008; B.17, In vitro Mammalian Cell Gene Mutation Test

Species/Strain: Chinese hamster
Cell Type/Cell Line: CHO Cells
Metabolic Activation System: S9 mix from phenobarbital- and β-naphthoflavone induced rats liver (exogenous metabolic activation).
Vehicle: DMSO
Remarks - Method: No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*; 46.9*; 93.8*; 187.5*; 375.0*; 750.0*; 1500.0*	4 h	20-24 h
Test 2	0*; 1.6; 3.1; 6.3*; 12.5*; 25.0*; 50.0*; 100.0*	4 h	20-24 h
Present			
Test 1	0*; 46.9*; 93.8*; 187.5*; 375.0*; 750.0*; 1500.0*	4 h	20-24 h
Test 2	0*; 1.6; 3.1; 6.3*; 12.5*; 25.0*; 50.0*; 100.0*	4 h	20-24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 2700	> 1,500	≥ 187.5	negative
Test 2		> 100	≥ 25.0	negative
Present				
Test 1	> 2700	> 1,500	≥ 187.5	negative
Test 2		> 100	≥ 25.0	negative

Remarks - Results

The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolising system in two experiments performed independently of each other.

No cytotoxicity was observed up to the highest applied concentration

	evaluated for gene mutations in the absence and the presence of metabolic activation. Both positive control substances, ethyl methanesulfonate (EMS) and cyclophosphamide (CPA), and vehicle controls gave satisfactory responses, confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to CHO cells treated *in vitro* under the conditions of the test.
TEST FACILITY	BASF (2016d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Chemical 2 in GSID 3056-2 FF
METHOD	OECD TG 301B Closed bottle test
Inoculum	Activated sludge from municipal sewage plant
Exposure Period	28 d
Concentration Range	Nominal: 20 mg/L total organic carbon
Remarks – Method	The test item was added at a concentration of 20 mg/L total organic carbon, this being equivalent to approximately 29 mg/L. Aniline at 20 mg/L was used as a reference control. Four test assays were prepared: (i) blank control; (ii) test item (29 mg/L); (iii) reference substance (aniline 20 mg/L); (iv) inhibition control (29 mg/L of test item and 20 mg/L of reference substance). The extent of degradation was determined by comparing the measured amount of carbon dioxide at the end of the test with the calculated maximal theoretical production ($ThCO_2$), and then indicated as biodegradation degree in percent.

RESULTS

Test substance (CO_2/$ThCO_2$)		*Aniline (CO_2/$ThCO_2$)*	
Day	*% Degradation*	*Day*	*% Degradation*
28	< 10	14	67

Remarks – Results	The validity criteria for the test were met. The inhibition control showed 33% degradation in 14 days and therefore the notified chemical is not regarded as inhibitory to the inoculum.
CONCLUSION	The test item is not readily biodegradable.
TEST FACILITY	BASF (2012 h),

C.1.2. Inherent biodegradability

TEST SUBSTANCE	Chemical 1 in GSID 3056-2 FF
METHOD	OECD 302C
Inoculum	Activated sludge from municipal sewage plant
Exposure Period	28 d
Concentration Range	Nominal: 30 mg/L Actual: -
Remarks – Method	The test item was added at a nominal concentration of approximately 30 mg/L. Sodium benzoate at 100 mg/L was used as a reference control. Four test assays were prepared: (i) blank control; (ii) test item (30 mg/L); (iii) reference (procedural) control (100 mg/L of reference substance); (iv) toxicity (inhibition) control (30 mg/L of test item and 100 mg/L of reference substance) (v) abiotic control (30 mg/L of test item). The extent of degradation was determined by comparing the difference of the measured biological oxygen demand and oxygen consumption at the end of the test with the calculated theoretical oxygen demand, and then expressing this as a percentage.

RESULTS

Biodegradation (%)		
Day	*Test item*	*Reference control*
28	- 1	74

Remarks – Results: The validity criteria for the test were met. The residual rates for the test item and abiotic control were 91 and 86%, respectively. The toxicity (inhibition) control showed 49% degradation in 28 days and therefore the notified chemical is not regarded as inhibitory to the inoculum.

CONCLUSION: The test item is not readily biodegradable.

TEST FACILITY: Bioassay (2016c)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Chemical 2 in GSID 3056-2 FF

METHOD: OECD 203 / GLP

Species: *Brachydanio rerio* (zebrafish)

Exposure Period: 96 h static test

Temperature: 22.6 – 23.7 ºC

pH: 7.67 – 7.83

Remarks - Method:

A preliminary range finding test was used to estimate the concentration to use in the definitive test. For each concentration, five fish were exposed for 96 h. Test samples were prepared by weighing of the ground test item, sonication and shaking, after which non-dissolved material was separated by filtration . The concentrations of the filtrates – water accommodated fractions (WAFs) of 1.0, 10, and 100 mg/L – were measured in triplicate by evaluation of the levels of total organic carbon. No mortality was observed for any of these concentrations.

Based on the results of this preliminary range test, a concentration of 100 mg/L WAF (prepared by the method described above) was used in the definitive limit test, together with a negative control. Seven fish were used for each of the test item and the control (one replicate each).

A positive control test with a reference substance, 3,4-dichloroaniline, was performed under similar conditions, using concentrations of 0 and 8.0 mg/L.

RESULTS

Definitive limit test	
mg/L	*Mortality (96 h)*
0	0/7
100	0/7

LC50: > 100 mg/L WAF

NOEC: ≥ 100 mg/L WAF

Remarks – Results: No mortality was observed using the concentration of 100 mg/L in the definitive limit test. For this test, mortality and oxygen concentration validity criteria were satisfied. The concentration of oxygen in the control and test item over 96 h were in the range 91 – 99%.

With respect to solubility, results from total organic carbon analysis indicates that only a small quantity of the test item may be dissolved in the test medium (0.83 mg C/L). However, since preparation of the 100 mg/L WAF involved prolonged mixing, it was assumed that the solubility limit

under the test conditions was reached.

For the positive control, the fish exposed to 8 mg/L of the reference substance recorded a range of adverse responses, but no mortality. LC 0 (96 h) ≥ 8 mg/L, LC 50 (96 h) > 8 mg/L. No indication was given to whether this was within the expected range.

CONCLUSION The test item is not toxic to fish.

TEST FACILITY Institute of Industrial Organic Chemistry (2012a)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE Chemical 1 in GSID 3056-2 FF

METHOD OECD 203
Species *Gobiocypris rarus* (Chinese rare minnow)
Exposure Period 96 h (semi-static)
Temperature 22.1 – 22.8 ºC
pH 8.07 – 8.44
Remarks - Method Due to poor solubility of the test item, concentrations were prepared as water-soluble fractions. The test item was stirred and filtered (0.45 µm) with, the third filtrate fraction being used in the experiments.

A preliminary range finding test, using nominal concentrations of the test item of 0 (negative control) and 100 mg/L, was used to estimate the concentration to use in the definitive test. No mortalities or abnormalities were observed.

Based on the results of this preliminary range test, a definitive (limit) test was conducted. Ten fish were used for each concentration. For both the preliminary and definitive tests, solutions were renewed every 24 h.

A positive control test with a reference substance, potassium dichromate, was performed under similar conditions, using six concentrations: 60, 90, 135, 200, 300 and 400 mg/L.

RESULTS

Definitive limit test	
mg/L	*Mortality (96 h)*
0	0/10
100	0/10

LL50 > 100 mg/L (Water Soluble Fraction)

Remarks – Results No mortalities or abnormalities were observed at a concentration of 100 mg/L in the limit test. For this test, all validity criteria were satisfied. The dissolved oxygen concentrations were in the range 60.3 – 91.1%. The HPLC analysis of the test item indicated that the concentration was below the LOD (0.422 mg/L).

For the positive control, the LC50 (96 h) was 243 mg/L (95% confidence interval: 194-297 mg/L), which was within the expected range 81.6 - 307 mg/L (±2 SD). .

CONCLUSION The test item is not toxic to fish.

TEST FACILITY Bioassay (2016d)

C.2.3. Acute toxicity to aquatic invertebrates

STUDY	*Daphnia magna* acute immobilisation test
TEST SUBSTANCE	Chemical 2 in GSID 3056-2 FF
METHOD	OECD 202
Species	*Daphnia magna*
Exposure Period	48 h static test
Temperature	20.0 – 21.2ºC
pH	7.79 – 7.77
Remarks – Method	Three preliminary tests were conducted. These differed in the way the test item was dissolved: (i) in acetone, (ii) by elevated temperature, and (iii) via sonication at an elevated temperature and then shaking at room temperature. Immobilisation was only recorded for a nominal concentration of 20 mg/L of the acetone dissolved compound (80% immobilisation). For both the second and third tests, no immobilisation was recorded for the highest nominal concentration used (100 mg/L (water accommodated function)). The concentration of the test item in the definitive limit test was nominally 100 mg/L (water accommodated function), prepared by sonication and shaking. This concentration, and a negative control, were tested in four replicates, each replicate having five *Daphnia*. A positive control test with a reference substance, potassium dichromate, was performed less than one month prior to the study on the test substance, using five concentrations: 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L.

RESULTS

Definitive test	
Concentration test item mg/L	*Number immobilised (48 h)*
0	0/20
100	0/20

EC50NOEL	> 100 mg/L ≥ 100 mg/L
Remarks – Results	For the test, the validity criteria were satisfied. The dissolved oxygen concentration was in the range 8.0 – 8.1 mg/L. For the positive control, the EC50 (48 h) was 0.53 mg/L, which was within the expected range. No immobilisation of *Daphnia* was observed for 100 mg/L.
CONCLUSION	The test item is not toxic to invertebrates.
TEST FACILITY	Institute of Industrial Organic Chemistry (2012b)

C.2.4. Chronic toxicity to aquatic invertebrates

STUDY	*Daphnia magna* reproduction
TEST SUBSTANCE	Chemical 1 in GSID 3056-2 FF
METHOD	OECD 211
Species	*Daphnia magna*
Exposure Period	21 d
Temperature	20 ºC
pH	7.5 – 7.5
Water hardness	~ 2.4 mg/L

Remarks – Method

A preliminary test showed no significant effect on reproduction after 14 d of exposure 10 mg/L. On the basis of this result, a definitive limit test was conducted with a negative control and test item nominally 10 mg/L. The test solution was prepared by adding 10 mg of the test item to 1 L of a test medium, stirring for 2 days, and removing undissolved solid by filtration (0.2 µm). No reliable analytical method could be developed for the quantitative determination of the level of the test item in solution. Fresh solutions were prepared for each day of the test. Ten replicate test vessels, each with one *Daphnia*, were used for both the control and test.

RESULTS

Definitive test				
Nominal loading rate (mg/L)	Reproduction (mean living young per adult)	Mortality of parent	Mean growth (length mm)	% immobile young
0	123.7	1/10	4.21	0.0
10	128	0/10	4.26	0.1

NOEC ≥ 10 mg/L

Remarks – Results For the test, the validity criteria were satisfied.

CONCLUSION The test item is not toxic to invertebrates.

TEST FACILITY BASF (2017b)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE Chemical 2 in GSID 3056-2 FF

METHOD OECD TG 201 Alga, Growth Inhibition Test

Species *Pseudokirchneriella subcapitata*

Exposure Period 72 h static test

Temperature 22.2 – 22.6°C

pH 7.31 – 9.00

Remarks - Method

A preliminary range finding test, using concentrations of the test item of 0 (negative control), 0.1, 1.0, 10, 100 and 1000 mg/L, was used to determine the concentrations to be used in the definitive test. It was determined that the two higher concentrations gave greater growth inhibition.

Six nominal test concentrations of the test substance were used in the definitive limit test: 3.2, 10, 32, 100, 320 and 1000 mg/L. Each test concentration was prepared in three replicates, while a negative control had six replicates. Algal biomass was determined by measuring the absorbance of the algal suspension at 670 nm daily and cell morphology was observed at 72 h.

A positive control test with a reference substance, 3,5 dichlorophenol, was performed under similar conditions, using a negative control and six concentrations in the range 0.56 - 10 mg/L. The recorded temperature did not vary more than 0.4 °C, but the pH values varied up to approximately 1.5 units.

RESULTS

Definitive test	
Concentration test item (GSID 3056-1) mg/L	*% inhibition yield (compared to control)*

3.2	17.9
10	7.1
32	10.3
100	1.7
320	4.7
1000	20.1

EC50	> 1000 mg/L
NOEC	≥ 1000 mg/L

REMARKS – RESULTS

Validity criteria were met. The increase in biomass over 72 h was 252 fold, the mean coefficient of variation for section-by-section growth rates was 27.1%, and for the control the coefficient of variation of average specific growth rates was 0.2%.

For the positive control, the ErC50 (72 h) was 2.09 mg/L (95% confidence interval: 1.89-2.33), which was within the expected range.

No differences could be observed between the size or shape of algal cells for any concentration in the limit test. It was not possible to deduce any relationship between the nominal concentrations of the test item and the inhibition of growth rate.

The median concentration that causes greater than 50% inhibition must be greater than 1000 mg/L

CONCLUSION

The test item is not toxic to alga.

TEST FACILITY

Institute of Industrial Organic Chemistry Branch Pszczyna (2012c)

C.2.6. Acute toxicity to Earthworm (*Eisenia fetida*)

TEST SUBSTANCE

Chemical 1 in GSID 3056-2 FF

METHOD

OECD 207 / GLP

Species	*Eisenia fetida* (earthworm)
Exposure Period	14 d
Temperature	20.0 – 20.3 ºC
Remarks - Method	A preliminary range finding test, using nominal concentrations of the test item of 0 (negative control), 10, 100, and 1000 mg/kg dry soil weight (made up in dichloromethane) and a solvent control (dichloromethane) was used to estimate the concentration to use in the definitive test. Each group consisted of one replicate of 10 worms. Except for one mortality in the negative control, no mortalities were observed over 14 d. Based on the results of this preliminary range test, a definitive (limit) test was conducted with test concentration of 1000 mg/kg, a negative control and a solvent control, made up as for the range finding test. Each group consisted of four replicates of 10 worms per replicate. Mortalities were determined at 7 and 14 d. A positive control test with a reference substance, chloroacetamide, was performed under similar conditions, using the five concentrations 3.75, 7.50, 15, 30, and 60 mg/L.

RESULTS

Definitive limit test	
Concentration test item mg/kg dry soil	*Mortality after 14 d*

0	0/40
1000	1/40
Solvent control	1/40

LC50	> 1000 mg/kg dry soil
Remarks – Results	A single mortality was recorded (2.5 %) for test item at 1000 mg/kg in the limit test, and one mortality in the solvent control. For the LD50 (14 d) was 21.4 mg/L (95% confidence interval: 18.3-24.9), which was within the expected range.
CONCLUSION	The test item is not toxic to *Eisenia fetida.*
TEST FACILITY	Bioassay (2016e)

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