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July 2011

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Weston 705

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)
Chemtura Australia Pty Ltd (ABN: 18 005 225 507)
Level 7, 435 King William Street,
Adelaide, SA 5000

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, 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: Dissociation constant, Hydrolysis as a function of pH and Bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA, Philippines

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Weston 705

MOLECULAR WEIGHT
>500 Da.

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

3. COMPOSITION

DEGREE OF PURITY >87%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Clear colourless liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	~2°C	Measured
Boiling Point	>400°C at 101.3 kPa	Measured
Density	1.02 x 10^3 kg/m^3 at 20°C	Measured
Vapour Pressure	1.6 x 10^{-11} kPa at 25°C	Measured
Water Solubility	<1.0 × 10^{-4} g/L at 20°C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemical is essentially insoluble in water and is a mixture; therefore, the rate of hydrolysis as a function of pH could not be determined. However, the notified chemical contains functional groups that are expected to hydrolyse under environmental conditions
Partition Coefficient (n-octanol/water)	log P_{ow} = 6.58 at 25°C	Measured
Adsorption/Desorption	log K_{oc} >5.63 at 40°C	Measured
Dissociation Constant	Not determined	The notified chemical does not contain any dissociable functionality
Particle Size	Not determined	The test material is a liquid
Flash Point	228 ± 2°C at 101.325 kPa	Measured
Flammability	Not expected to be flammable	Based on measured flash point.
Autoignition Temperature	None below 400°C	Measured
Explosive Properties	Not expected to be explosive	Measured. Based on the notified chemical structure
Oxidizing Properties	Not an oxidant	Measured. Based on the notified chemical structure

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

Reactivity
The notified chemical is expected to hydrolyse under normal environmental conditions.

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 into Australia as a liquid in the neat form at a concentration >87%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 10	< 10	< 10	< 10	< 10

PORT OF ENTRY
Sydney, Melbourne

TRANSPORTATION AND PACKAGING

The notified chemical (at >87%) will be imported in drums, totes and isotainers and transported by road and rail to customer sites.

USE
The notified chemical will be used as an additive up to 0.5% in the production of rubber and plastics such as in polyvinylchloride (PVC) film**,** rubber, linear low density polyethylene (LLDPE)**,** and high density polyethylene (HDPE).

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. It will be imported into Australia at a concentration of >87% and transported to a polymer or rubber factory. At the factory the notified chemical will be pumped via an entirely closed automated system and dosed at up to 0.5% into the polymer before extruding and pelletising the polymer. The pellets containing the notified chemical at up to 0.5% will then be shipped to a film producer who converts the polymer into film using typical manufacturing process. e.g. blown film line or cast film line. The film is collected in rolls and then shipped to the end-user.

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	1-5	1-2	28
Operators	1-2	Up to 8	330
QC samplers	1-2	0.5	14
Maintenance	1-2	Up to 8	1
Manufacture workers	20	Up to 8	330

EXPOSURE DETAILS
Transport and warehousing
It is expected that transport and warehouse workers handling the imported notified chemical at >87% will only be exposed to the notified chemical in the event of spills due to an accident or as a result of container leakage. The main route of exposure in these situations will be dermal.

Reformulation/Pellets formulation
Dermal, ocular and inhalation exposure of operators and maintenance workers to the notified chemical (at >87%) may occur when opening storage tanks/drums containing the notified chemical, connecting and disconnecting automated pumps during transfer operations, when mixing and blending during extrusion and pelletising processes and during sampling for quality control purposes by the QC samplers. However, exposure of workers to the notified chemical will be limited by the use of personal protective equipment (PPE) such as safety glasses, gloves and overalls and engineering controls in place such as enclosed and fully automated systems and exhaust extraction systems.

Dermal or ocular exposure of workers to the pellets may occur during the automated packing operation of the pellets containing the notified chemical at up to 0.5%. As the pellets are expected to be non-dusting, inhalation exposure is not expected.

Manufacture of plastic/rubber products
In the injection process, the compound pellets containing up to 0.5% notified chemical will be either transferred by vacuum or manually tipped into the feeding hopper on the injection-moulding machine. Once heated, the melted pellets are moulded to form the shape of the plastic/rubber article, and then cooled within the closed mould, prior to ejection into a suitable receptacle. The compounded product will be removed from moulds either manually or automatically ejected.

Although dermal or ocular contact with the pellets may occur during their manual transfer, exposure to the notified chemical is not expected as it is not considered to be bioavailable. However, personal protective equipment is expected to be used including eye protection, chemical impermeable gloves and overalls.

Occupational exposure to the notified chemical is not expected to occur after the plastic or rubber articles are made since the notified chemical is encapsulated within the finished plastic/rubber articles. In this form, the notified chemical is not considered to be bioavailable.

6.1.2. Public exposure

The notified chemical will not be sold to the general public. Therefore, the general public will not be exposed to the notified chemical as such.

The notified chemical will be used in the production of many polymers including PVC, LLDPE, HDPE and rubber. As soon as the polymers have been formed and the final products/articles have been manufactured, the notified chemical will be bound into the polymer matrix and will remain within the plastic or rubber article for the duration of the product useful life. Therefore, the notified chemical will not be bioavailable.

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
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days.	NOAEL: 1531 mg/kg bw
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro - Chromosome Aberration	non genotoxic
Genotoxicity – in vivo - Mouse Lymphoma	non genotoxic

Toxicokinetics, metabolism and distribution.

Low to moderate absorption of the notified chemical may occur following ingestion, inhalation, or dermal exposure considering its relatively high molecular weight, partition coefficient (log Pow =6.58), very low vapour pressure (1.6×10^{-11} kPa) and low water solubility (0.1 mg/L).

Acute toxicity.

The notified chemical was of low acute oral toxicity (LD_{50} >2 000 mg/kg) and low acute dermal toxicity (LD_{50} >2 000 mg/kg) in the rat. An acute inhalation study was not provided.

Irritation and Sensitisation.

The notified chemical was slightly irritating to rabbit skin and eye, and was non-sensitising in a local lymph node assay.

Repeated Dose Toxicity (sub acute, sub chronic, chronic).

In a repeat dose study, rats were administered the notified chemical daily in their diet at doses of 0, 70, 759 and 1531 mg/kg bw/day for 90 days followed by a recovery test where two groups were treated at 0 and 1531 mg/kg bw/day for 90 days and then maintained without treatment for a further 29 days on basal laboratory diet. There were no treatment-related changes in any of the measured parameters. Treatment related effects were observed in animals treated with 1531 and 759 mg/kg/day. Aspartate aminotransferase levels were higher than controls and the increase was still evident for recovery 1531 mg/kg bw/day males. The liver and spleen weights in females treated with 1531 mg/kg bw/day were higher than controls with no effects evident following the treatment free period. The minor treatment-related changes observed at 1531 and 759mg/kg/day were considered unlikely to be toxicologically significant. Given the changes observed were not considered to be adverse,, the NOAEL was established as 1531 mg/kg/day (highest dose tested).

Mutagenicity.

In genotoxicity studies, the notified chemical was not mutagenic in bacteria, nor did it induce an increased

incidence of chromosomal aberrations in human lymphocytes *in vitro*. The notified chemical was non-mutagenic in mouse lymphoma cell line.

Health hazard classification
Based on the data provided, the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Based on data provided, the notified chemical is a slight skin and eye irritant and not a sensitiser. The notified chemical is of low acute toxicity, non mutagenic and non genetoxic. No data are provided on inhalation toxicity of the notified chemical, however the risk of inhalation toxicity is expected to be low due to very low vapour pressure (1.6×10^{-11} kPa) of the notified chemical. The risk of systemic effects is expected to be low due to the relatively high NOAEL of 1531 mg/kg bw/day established in the 90 day repeat dose study.

Workers handling the notified chemical at a concentration of >87% will be most at risk of skin and eye irritation. However, given the proposed use of PPE, exhaust extraction systems in place and largely enclosed, automated processes used in reformulation facilities, exposure of these workers to the notified chemical is expected to be low.

Occupational exposure to the notified chemical at up to 0.5% cannot occur after the plastic and rubber articles are made since the notified chemical is encapsulated within the finished plastic or rubber articles. In this form, the notified chemical is not bioavailable, hence health risk to workers is expected to be negligible.

Overall, the risk to the occupational health and safety of workers is not considered unreasonable, due to the expected low exposure to the notified chemical from the use of PPE and the use of enclosed and automated processes.

6.3.2. Public health
The notified chemical is not available for sale to the general public but will be used in the production of plastics and rubber that may be publicly available as household products. The risk to public health from the notified chemical is likely to be low due to the notified chemical, which is physically contained within the plastics and rubber matrix, not being bioavailable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
Release to the environment during shipping, transport and warehousing will only occur in the unlikely event of accidental spills from the import containers and are expected to be handled by physical containment, collection and subsequent safe disposal. During reformulation in Australia, the notified chemical is transferred using closed systems from import containers into local storage containers and subsequently to mixing vessels. In the mixing vessel the notified chemical is incorporated directly into plastic or rubber polymers. Accidental spills and leaks during reformulation are expected to be physically contained and disposed of to landfill. Import container residues are estimated at 0.1% of the import volume and will be thermally decomposed on disposal of the containers. Washings from equipment cleaning will be treated using an on-site wastewater treatment plant prior to release to sewer.

RELEASE OF CHEMICAL FROM USE
The notified chemical is expected to be physically bound within the inert polymer matrix of plastic and rubber articles and is not expected to be released to the environment during use.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of the notified chemical will share the fate of plastic and rubber articles which are expected to be disposed of to landfill, thermally decomposed or recycled at the end of their useful life.

7.1.2 Environmental fate

The vast majority of the notified chemical will be incorporated into plastic and rubber articles. The notified chemical will be physically bound into the inert polymer matrix and in this form it is not expected to be mobile or bioavailable.

The notified chemical has low solubility in water ($<1\times10^{-4}$ g/L) and a high adsorption/desorption coefficient (log K_{oc} > 5.63) which indicate that the notified substance will partition to soil and sludge and have low mobility. Therefore, when wastewater generated during formulation containing the notified chemical is treated on-site, it is expected that the majority of the notified chemical will partition to sludge and be disposed to landfill. Notified chemical disposed of to landfill as wastes and residues from reformulation are expected to be immobile due to the strong sorption to soils.

The notified chemical is not readily biodegradable but is non-inhibitory to microbial respiration. The notified chemical may be susceptible to hydrolysis under environmental conditions in landfill. Therefore in landfill, the notified chemical in polymers and residues is expected to undergo biotic and abiotic degradation to form water and oxides of carbon and phosphorus.

The notified chemical has low water solubility, a high partition coefficient (Log P_{ow} = 6.58), and is not readily biodegradable, which indicate a potential for bioaccumulation. However, the notified chemical is not likely to persist in the environment due to the potential for biodegradation and hydrolysis, and bioaccumulation is not likely as there is limited environmental exposure expected from the reported use pattern.

For the details of the environmental fate studies, refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

The notified chemical is not expected to be discharged to the aquatic compartment in significant quantities based on the intended use and likely disposal pathway. Therefore, the predicted environmental concentration (PEC) was not calculated.

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	LL50 (96 h) > 100 mg/L	Not harmful to fish up to the limit of solubility
Daphnia Toxicity – Acute	EL50 (48 h) > 100 mg/L	Not harmful to aquatic invertebrates up to the limit of solubility
Daphnia Toxicity – Chronic	EL50 (21 d) > 100 mg/L NOELR (21 d) = 100 mg/L	Not harmful to aquatic invertebrates with long lasting effects up to the limit of solubility
Algal Toxicity	EL50 (72 h) > 100 mg/L NOELR (72 h) = 100 mg/L	Not harmful to algae up to the limit of solubility
Inhibition of Bacterial Respiration	IC50 (3 h) > 360 mg/L	Not inhibitory to bacterial respiration.

LL50 – Lethal loading rate resulting in 50% mortality
EL50 – Effective loading rate resulting in 50% effect
NOELR – No-observable-effect loading rate

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is not harmful to fish, aquatic invertebrates and algae. The reported endpoints are based on nominal loading rates of the water accommodated fraction (WAF) used for testing, consistent with international best practice (OECD, 2000), as the notified chemical is a multi-component substance with low aqueous solubility. The actual concentration of the notified chemical in the studies ranged from less than the limit of quantification (LOQ) to 0.419 mg/L (determined by HPLC), and therefore these values should be treated with caution. The notified chemical is not expected to inhibit microbial respiration.

7.2.1 Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) was not calculated as low potential for aquatic exposure is expected based on the reported use pattern.

7.3. Environmental risk assessment

The Risk Quotient, Q (= PEC/PNEC), has not been calculated since a PEC is not available.

The notified chemical will be used in the manufacture of plastic and rubber polymer articles. The majority of the notified chemical will be bound within the inert polymer matrix and will not be mobile or bioavailable. On the basis of the low toxicity to aquatic organisms and low potential for exposure to the aquatic environment, the notified chemical is not expected to pose an unreasonable risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the data provided the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

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*
Not classified for acute or long term hazard	

Human health risk assessment

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

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

Environmental risk assessment

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

Recommendations

CONTROL MEASURES

Occupational Health and Safety

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

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced for formulation:
  - *Impervious gloves*
  - *Safety glasses*
  - *Impervious clothing*

  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 plastic and rubber additive at up to 0.5% or is likely to change significantly;
  - the amount of chemical being introduced has increased from 10 tonnes 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 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 -1.15 - 4.85ºC

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Test Facility Harlan Laboratories Ltd (2010a)

Boiling Point >400ºC at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Directive 92/69/EEC A.2 Boiling Temperature.
Test Facility Harlan Laboratories Ltd (2010a)

Density 1.02 x 10^3 kg/m^3 at …ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Test Facility Harlan Laboratories Ltd (2010a)

Vapour Pressure 1.6 x 10^{-11} kPa at 25ºC

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Test Facility Harlan Laboratories Ltd (2010b)

Water Solubility <1.0 × 10^{-4} g/L at 20 ºC

Method OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Flask Method with HPLC/UV analysis. Three samples (containing approximately 100 mg of the test substance in 500 mL water) were stirred at 30ºC for 24, 48 and 72 hours. The samples were equilibrated at 20ºC for 24 hours and then centrifuged (15 minutes at 10 000 rpm) to remove the excess test material. The pH of the samples ranged 3.6-3.7. Duplicate aliquots were freeze-dried and the residue was quantified. The notified chemical was not detected in any of the samples. While it is expected that the water solubility of the notified chemical will be low, it is possible that the result is also attributable to hydrolysis of the notified chemical noting that several impurities (potentially hydrolysis products) are observed in the sample chromatogram.
Test Facility Harlan Laboratories Ltd (2010a)

Partition Coefficient (n-octanol/water) log P_{ow} = 6.58 at 25ºC

Method U.S. EPA Product Properties Test Guidelines, OPPTS 830.7560 (Partition Coefficient (n-Octanol/Water), Generator Column Method.
Remarks Generator column method with HPLC/UV analysis. The column was packed with a nominal 1.0% (w/w) solution of the notified chemical in *n*-octanol. Column temperature was maintained at 25ºC ± 0.1ºC and the flow rate was 1.0 mL/minute. The concentrations in the aqueous phase (column effluent) were determined following solvent extraction, concentration and reconstitution in a medium suitable for analysis. A minimum of three samples were collected and analysed. The partition coefficient was determined from the ratios of the notified chemical in *n*-octanol (4848 mg/L) and water (0.00154 mg/L).
Test Facility Wildlife International, Ltd (2008)

Adsorption/Desorption – screening test log K_{oc} >5.63 at 40°C

Method OECD TG 121. Estimation of the Adsorption Coefficient on Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC).

EC Directive 92/69/EEC C.19.
Remarks A screening test only was conducted. The retention time for the notified chemical was not in the domain of the reference substances (log K_{oc} 1.25-5.63). Therefore, a lower limit is reported as the result.
Test Facility Harlan Laboratories Ltd (2010a)

Flash Point 228 ± 2ºC at 101.325 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
Remarks Equilibrium method: closed cup
Test Facility Harlan Laboratories Ltd (2010b)

Autoignition Temperature None below 400ºC

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility Harlan Laboratories Ltd (2010b)

Explosive Properties Not expected to be explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Test Facility Harlan Laboratories Ltd (2010b)

Oxidizing Properties Not an oxidant

Method EC Directive 92/69/EEC A.21 Oxidizing Properties (Liquids).
Test Facility Harlan Laboratories Ltd (2010b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (purity >87%)
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. EC Directive 92/69/EEC B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rat/Wistar
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	1 F	300	0
II	1 F	2000	0
III	4 F	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity	There were no deaths and no sign of systemic toxicity.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	All animals showed expected gains in bodyweight. Pale faeces were noted in one animal at the 4-hour, day 1 and day 2 observations.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan Laboratories Ltd (2010e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical (purity >87%)
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/Wistar
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	1 M	2000	0
II	1 F	2000	0
III	4 M	2000	0
IV	4 F	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Increased respiratory rate and/or hunched posture were noted in two animals.
Signs of Toxicity - Systemic	There were no deaths and no signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted in any animal at necropsy.
Remarks - Results	

CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Harlan Laboratories Ltd (2010f)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical (purity >87%)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	1, 24, 48 and 72 hours after removal of the dressing.
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	0.33	0.33	2	<72 hours	0
Oedema	0.33	0	0	1	<48 hours	0

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

Remarks - Results	<u>3-minutes exposure period:</u> Well defined erythma and very slight oedema were noted at the treated skin site 1-hour after patch removal and at the 24-hour observation with very slight erythma noted at the 48-hour observation. The treated skin site appeared normal at the 72-hour observation. <u>1-hour exposure period:</u> Very slight erythma was noted at the treated skin site 1hour after patch removal and at the 48-hour observation with well-defined erythma and very slight oedema noted at the 24-hour observation. <u>4-hour exposure period:</u> Well-defined erythma and very slight oedema was noted at one treated skin site immediately after patch removal and at the 1 and 24-hour observations with very slight erythma noted at the two remaining treated skin sites at the 24-hour observation. Very slight erythma was noted at one treated skin site at the 48-hour observation. Two treated skin sites appeared normal at the 48-hour observation and the remaining treated skin site appeared normal at the 72-hour observation. All animals showed expected gain in bodyweight during the study.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Harlan Laboratories Ltd (2010g)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical (purity >87%)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Observation Period	1, 24, 48 and 72 hours after the administration

Remarks - Method

Initially a single rabbit was treated with a 0.1 mL of the test material placed in the conjunctival sac of the right eye. The left eye remained untreated and was used for control purpose. Additional observation was made on day 7 to assess the reversibility of the ocular reactions.
After consideration of the ocular responses produced in the first treated animal, two additional animals were treated.

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	0.33	0.33	0.33	2 (1 hour)	<48 hours	0
Conjunctiva: chemosis	0.66	0.66	0.66	2 (1 hour)	<72 hours	0
Conjunctiva: discharge	1	0.66	0.66	2 (1 hour)	<7 days	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results

No corneal or iridial effects were noted during the study.
Moderate conjunctival irritation was noted in all treated eyes one hour after treatment with minimal conjunctival irritation noted at the 24 and 48-hour observations. Minimal conjunctival irritation was noted in one treated eye at the 72-hour observation. Two treated eyes appeared normal at the 72-hour observation and the remaining treated eye appeared normal at the 7-day observation.

All animals showed expected gain in bodyweight during the study.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Harlan Laboratories Ltd (2010h)

B.5. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical (purity >87%)

METHOD

OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain: Rat/Wistar Han
Route of Administration: Oral - diet
Exposure Information: Total exposure days: 90 days
Dose regimen: 7 days per week
Vehicle: None
Remarks - Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Mortality*
		Nominal (ppm)	*Actual (mg/kg bw/day)*	
control	20 M/ 20 F	10	0	0/40
low dose	20 M/ 20 F	1000	70	0/40
mid dose	20 M/ 20 F	10000	759	0/40
high dose	20 M/ 20 F	20000	1531	0/40
control recovery	20 M/ 20 F	0	0	0/40
high dose recovery	20 M/ 20 F	20000	1531	0/40

Mortality and Time to Death

There were no deaths during the treatment or recovery periods of the study.

Clinical Observations

No clinical signs of toxicity were detected during the study.
There were no treatment-related changes in sensory reactivity.
No adverse effect on bodyweight change was observed for treated animals compared to controls.
No intergroup differences in water consumption were detected.
Daily clinical observations detected the presence of an abdominal mass for one female (treated with 20000 ppm) from day 81 until the end of the treatment. This was accompanied by diuresis on day 89 and day 90.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

Aspartate aminotransferase levels for animals treated with 20000 and 10000 ppm were higher than controls during the treatment period (908.1 and 324.3 compared to 209.0 IU/L respectively for males and 386.4 and 263.2 against 125.6 IU/L respectively for females) and the increase was still evident for recovery 20000 ppm males (101.4 compared to 72.1 IU/L).
No such effects were detected for animals of either sex treated with 1000 ppm.

Haematology

No treatment-related effects were detected for treated animals in comparison to controls at the end of the treatment or recovery periods of the study.

During the week 2 assessments, males treated with 20000 and 10000 ppm showed statistically significant increases in leucocyte counts. An increase in lymphocyte counts was also detected for males treated with 10000 ppm. The significance in each case was minimal ($p<0.05$) as similar effects was not observed during the remainder of the study.

Increases in the platelet counts were detected for males treated with 20000 and 10000 ppm during the week 2 and week 7 assessments (738.0 $x10^9$ and 741.1 $x10^9$ respectively compared to the controls 642.8 $x10^9$ platelets/L). Although no such effect was detected during the final week of treatment, an increase in clotting times was detected for males treated with 20000 ppm during this period.

Mean cell haemoglobin concentration (MCHC) was reduced for males treated with 20000 ppm during week 7. Recovery 20000 ppm females showed a statistically significant increase in MCHC during the final week of the treatment period. Similar effects were not observed during the remainder of the study.

Urinalysis

There were no treatment-related effects detected for treated animals in comparison to controls at the end of the treatment or recovery periods of the study.
The finding of red coloured urine with the presence of protein, bilirubin and haemoglobin in a female treated with 20000 ppm, which displayed the abdominal mass and diuresis during the daily clinic observations, and a higher number of leucocytes and erythrocytes for this animal in sediment analysis, were considered to be attributed to the abdominal mass detected in this animal.

Effects in Organs

An increase in liver and spleen weights was detected in females treated with 20000 ppm compared to controls (3.477 compared to 3.310% and 0.563 compared to 0.449% respectively). No such effects were evident as a reduction in liver weights was detected for recovery 20000 ppm females compared to controls.

No such effects were detected for males treated with 20000 ppm or for animals of either sex treated with 10000 or 1000 ppm.

Remarks – Results

Treatment related effects were observed in animals treated with 20000 and 10000 ppm. Aspartate aminotransferase levels were higher than controls and the increase was still evident for recovery 20000 ppm males. In addition, the liver and spleen weights in females treated with 20000 ppm were higher than controls and a reduction in liver weights was detected for recovery 20000 ppm females compared to controls. These

changes were not considered by the study authors to be adverse.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 20000 ppm or 1531 mg/kg bw/day in this study, based on changes observed at this dose level which are not considered adverse.

TEST FACILITY Harlan Laboratories Ltd (2009a)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (purity >87%)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure/Pre incubation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA$^-$,
Metabolic Activation System	S9-mix from Phenobarbitone/β-naphthoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 50-5000 µg/plate b) Without metabolic activation: 50-5000 µg/plate
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	> 5000 µg/plate	> 5000 µg/plate	≥ 1500	Negative
Test 2	> 5000 µg/plate	> 5000 µg/plate	≥ 1500	Negative
Present				
Test 1	> 5000 µg/plate	> 5000 µg/plate	≥ 1500	Negative
Test 2	> 5000 µg/plate	> 5000 µg/plate	≥1500	Negative

Remarks - Results

All positive controls induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level and was tested up to the maximum recommended dose level of 5000 µg/plate. An oily precipitate was noted at and above 1500 µg/plate, this observation did not prevent the scoring of revertant colonies.

No toxicologically significant increases in the frequency of revertant colonies were recorded for any bacterial strains with or without S9. A small but statistically significant increase in TA100 revertant colony was observed in the presence of S9 at 500 µg/plate in the preliminary test only. However, this increase exhibited no dose-response relationship, exhibited counts within the in-house historical range for the strain and was non-reproducible in two separate tests. The increase was, therefore considered to be biologically irrelevant.

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

TEST FACILITY Harlan Laboratories Ltd (2009b)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (purity >87%)
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Human lymphocytes
Metabolic Activation System	S9-fraction from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	Acetone
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 156.25, 312.5, 625*, 1250*, 2500*, 5000 and MMC 0.4*	4 hours	24 hours
Test 2	0*, 156.25, 312.5, 625*, 1250*, 2500*, 5000 and MMC 0.2*	24 hours	24 hours
Present			
Test 1	0*, 156.25, 312.5, 625*, 1250*, 2500*, 5000 and CP 5*	4 hours	24 hours
Test 2	0*, 156.25, 312.5, 625*, 1250*, 2500*, 5000 and CP 5*	4 hours	24 hours

*Cultures selected for metaphase analysis.

RESULTS

All vehicle controls (acetone) had frequencies of cells with aberrations within the range expected for normal human lymphocytes.

All the positive control materials (Mitomycin C (MMC) at 0.4 and 0.2 μg/mL used without S9 and Cyclophosphamide (CP) at 5 μg/mLwith S9) induced statistically significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and the activity of the metabolising system.

The test material did not induce any statistically significant increases in the frequency of cells with aberrations, in either of two separate tests, using a dose range that induced some level of mitotic inhibition.

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥625	≥1250	≥156.25	Negative
Test 2	≥625	≥625	≥156.25	Negative
Present				
Test 1	≥625	≥1250	≥156.25	Negative
Test 2	-	≥1250	≥156.25	Negative

Remarks - Results

CONCLUSION

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

TEST FACILITY

Harlan Laboratories Ltd (2009c)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical (purity >87%)

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test. EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.

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

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/ml	4 hours	48 hours	14 days
Test 2	0, 39.06, 78.13, 156.25, 312.5, 625, 1250, 1875 and 2500 µg/ml	24 hours	48 hours	14 days
Present				
Test 1	0, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 937.5 and 1250 µg/ml	4 hours	48 hours	14 days
Test 2	0, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 937.5 and 1250 µg/ml	4 hours	48 hours	14 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥39.0 µg/mL	≥1250 µg/mL	≥78.13 µg/ml	Negative
Test 2	≥19.53 µg/mL	≥39.0 µg/mL	≥78.13 µg/ml	Negative
Present				
Test 1	≥78.13 µg/mL	≥156.25 µg/mL	≥78.13 µg/ml	Negative
Test 2	-	≥312.5 µg/mL	≥39.06 µg/m l	Negative

Remarks - Results

The maximum dose level used was limited by the test material induced toxicity in all but the 4-hour exposure group in the absence of metabolic activation of test 1 where the maximum recommended dose level (5000 µg/mL) was used. Precipitate of test material was observed at and above 78.13 µg/mL. In test 2, a precipitate of test material was observed at and above 78.13 µg/mL in the absence of metabolic activation and at and above 39.06 µg/mL in the presence of metabolic activation.
The vehicle controls (Acetone) had acceptable mutant frequency values that were within the normal range for the L5178Y cell line at the TK +/- locus.
The positive controls (Ethylmethanesulphonate in the absence of S9 and Cyclophophamide in the presence of S9) induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.
The test material did not induce any toxicologically significant dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in both tests.

CONCLUSION The notified chemical was not clastogenic to Mouse Lymphoma L5178Y cell line treated in vitro under the conditions of the test.

TEST FACILITY Harlan Laboratories Ltd (2010d)

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

TEST SUBSTANCE	Notified chemical (purity >87%)
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	Acetone/Olive oil 4:1
Remarks - Method	No significant protocol deviations

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	2109.44	1.00
10	3309.32	1.57
25	1659.66	0.79
50	2201.86	1.04
Positive Control		
15% (positive control)		3.12

Remarks - Results	There were no deaths and no signs of systemic toxicity were noted. The positive control α-Hexylcinnamaldehyde was considered to be a sensitiser under the conditions of the test.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Harlan Laboratories Ltd (2010c)

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. Method C.4-C of Commission Regulation (EC) No. 440/2008. US EPA Fate, Transport, and Transformation Test Guidelines OPPTS 835.3110 (Paragraph (M)).
Inoculum	Activated sewage sludge
Exposure Period	28 days
Auxiliary Solvent	Due to poor water solubility, the test substance was adsorbed onto granular silica gel to aid dispersion into the test medium.
Analytical Monitoring	HPLC/UV
Remarks - Method	The test substance was added at a concentration of 10 mg carbon/L. A reference (sodium benzoate, 10 mg carbon/L) and toxicity control were run in parallel.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	2	2	45
8	0	8	75
14	0	14	71
28	20	28	87

Remarks - Results	The test material attained 20% degradation after 28 days and therefore is not considered to be readily biodegradable under the conditions of OECD Guideline 301B. The toxicity control test attained 33% degradation by day 14 and the notified chemical is thus considered to be non-inhibitory to microbial respiration. Sodium benzoate attained 71% degradation after 14 days thereby confirming the suitability of the inoculum and test conditions. All validation criteria were satisfied.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Harlan Laboratories Ltd (2010i)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Semi-static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Semi-static.
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	Not applicable. Water accommodated fraction (WAF) is used as the notified chemical is a multi-component substance with low aqueous solubility.
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Based on the results of the range-finding test (WAF: 10 and 100 mg/L

loading), a limit test was conducted (WAF: 100 mg/L loading). The notified chemical (2100 g) was added to the surface of dechlorinated tap water (21 L) to achieve the loading rate of 100 mg/L. The test medium was stirred for 23 h and allowed to stand for 1 h. The WAF was removed by mid-depth siphoning (discarding the first 75–100 mL). Microscopic inspection showed dispersed test substance in the water column; therefore, for the definitive test the WAF was filtered through a glass wool plug. The fish, introduced to the WAF and maintained at 13.5°C under semi-static conditions for 96 h (light/dark cycle of 16/8 h; pH 7.9–8.5; 9.3–10.3 mg O_2/L), were observed for mortality and sub-lethal effects.

RESULTS

Loading Rate filtered WAF (mg/L)	*Number of Fish*	*Mortality*				
		1 h	*24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	0	0	0
100	14	0	0	0	0	0

LL50	> 100 mg/L at 96 hours (loading rate, filtered WAF)
NOELR	100 mg/L at 96 hours (loading rate, filtered WAF)
Remarks – Results	There were no sub-lethal effects of exposure observed in 14 fish exposed to a 100 mg/L loading rate WAF for a period of 96 h. All validity criteria were satisfied. Therefore, the test is considered reliable. Given that toxicity cannot be attributed to a single component or a mixture of components but to the test material as a whole, endpoints are reported of the nominal loading rates for the filtered WAF. The actual concentrations of the notified chemical were determined by HPLC to range 0.0054–0.01926 mg/L in fresh media and <0.00044 (LOQ) –0.00981 mg/L in old (24 h) media, indicative of a slight decline in concentration over the 24 h dosing period. This observation was contrary to the stability analysis, but was considered to be due to possible hydrolysis of the test substance. It is noted that method validation demonstrated poor recovery of the test substance from the test media. However, due to differences in the peak profile between the validation and test samples, the results were not corrected for recovery. Therefore, the concentration values should be treated with caution.
CONCLUSION	The notified chemical is not harmful to fish up to the limit of its solubility in water
TEST FACILITY	Harlan Laboratories Ltd (2010j)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not applicable. Water accommodated fraction (WAF) is used as the notified chemical is a multi-component substance with low aqueous solubility.
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC/UV, TOC
Remarks - Method	After a range-finding test (WAF: 1, 10 and 100 mg/L loading), a definitive test was conducted in accordance with the guidelines above.

The notified chemical (10, 18, 32, 56, 100, 180, 320, 560, 1000 mg) was added to the surface of reconstituted water (10 L) to achieve the reported loading rates. The test medium was stirred for 23 h and allowed to stand for 1 h. The WAF was removed by mid-depth siphoning (discarding the first 75–100 mL). Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. The daphnia were observed for immobilisation over two days (test conditions: artificial light dark cycle of 16 to 8 hours, 20–22°C, pH 7.7-8.0, 8.2-9.0 mg O_2/L). Daphnia unable to swim within 15 seconds of gentle agitation were considered to be immobile. The probit method was used to analyse the positive control (potassium dichromate) data.

RESULTS

Nominal Loading Rate WAF (mg/L)	*Concentration Measured HPLC[1] (mg/L)*		*TOC[2] (mg carbon/L)*		*Number of D. magna*	*Number Immobilised*	
	0 h	*48 h*	*0 h*	*48 h*		*24 h*	*48 h*
Control[3]	0.293	0.218	<LOQ	1.40	20	0	0
1	<LOQ	0.209	<LOQ	0.33	20	0	0
1.8	-	-	-	-	20	0	0
3.2	<LOQ	<LOQ	<LOQ	0.46	20	0	0
5.6	-	-	-	-	20	0	0
10	<LOQ	0.194	1.02	0.48	20	0	0
18	-	-	-	-	20	0	1[4]
32	<LOQ	0.160	1.28	1.01	20	0	0
56					20	0	0
100	0.419	0.421	1.63	1.24	20	1	4

[1] Chemical analysis using HPLC, limit of quantitation (LOQ) 0.0024 mg/L
[2] Total organic carbon, limit of quantitation (LOQ) 1.0 mg carbon/L
[3] Nominal concentration of the control is 0 mg/L, background media interference subtracted from all test samples
[4] Immobilisation not considered significant as none observed at 32 and 56 mg/L loading rate WAF

EL50	>100 mg/L at 48 hours (loading rate, WAF)
NOELR	56 mg/L at 48 hours (loading rate, WAF)
Remarks - Results	There was no observed immobility in the negative control and all method acceptability criteria were met. Therefore, the test is considered reliable. The actual concentrations of the notified chemical in the test vessels were determined by HPLC, and confirmed by TOC analysis, as reported above. Media interference was observed in the control for both methods. Given that toxicity cannot be attributed to a single component or a mixture of components but to the test material as a whole, endpoints are reported of the nominal loading rates for the WAF.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates up to the limit of its solubility in water

TEST FACILITY Harlan Laboratories Ltd (2010k)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna* Reproduction Test.
Method C.20 of Commission Regulation (EC) No. 440/2008.

Species	*Daphnia magna*
Exposure Period	21 days

Auxiliary Solvent	Not applicable. Water accommodated fraction (WAF) is used as the notified chemical is a multi-component substance with low aqueous solubility.
Water Hardness	118-158 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	*Daphnia magna* (10 replicates of a single daphnid per group) were exposed to the test substance over a range of nominal loading rates of 1.0, 3.2, 10, 32, and 100 mg/L (WAF) for a period of 21 days, under semi-static conditions (test conditions: artificial light dark cycle of 16 to 8 hours, 19–22°C, pH 7.4-8.8, 7.1-10.1 mg O_2/L). The WAFs were renewed three times per week. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. The daphnia were fed with algal suspension and numbers of live and dead (adult and young) were monitored daily. Observed mortalities were compared to the control group using the corrected chi-square statistic. The EL50 (reproduction) and EL50 (immobilisation) values were estimated by inspection of the data. The estimation for LOEL and NOEL values were compared to the control values by Bartlett's and Dunnett's test.

	Day 21		
Loading rate WAF (mg/L)	*Mean Percent % Adult Survival*	*Mean Number of Living Offspring Produced per female – cumulative (Standard deviation)*	*Mean Total Body Length in mm (Standard deviation)*
Control	90	74 (13)	4.6 (0.2)
1.0	100	65 (21)	4.5 (0.2)
3.2	90	71 (15)	4.6 (0.2)
10	90	61 (14)	4.5 (0.2)
32	90	63 (7)	4.4 (0.3)
100	100	70 (13)	4.4 (0.3)

EL50 (immobilisation)	>100 mg/L at 21 days (loading rate, WAF)
EL50 (reproduction)	>100 mg/L at 21 days (loading rate, WAF)
LOELR	>100 mg/L at 21 days (loading rate, WAF)
NOELR	100 mg/L at 21 days (loading rate, WAF)
Remarks - Results	In the control, the mortality of the parent animals was 10% and the mean number of live offspring produced per surviving adult was 74, thus validating the test. The actual concentrations of the notified chemical were determined by HPLC to range from the limit of quantification (LOQ = 0.00044 mg/L)–0.0225 mg/L. Given that toxicity cannot be attributed to a single component or a mixture of components but to the test material as a whole, endpoints are reported of the nominal loading rates for the WAF.
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates with long lasting effects up to the limit of its solubility in water
TEST FACILITY	Harlan Laboratories Ltd (2011)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours

Concentration Range	Nominal (Limit test): 100 mg/L (loading rate, WAF) Measured 0 h: 0.13-0.15 mg/L 72 h: 0.067-0.12 mg/L
Auxiliary Solvent	Not applicable. Water accommodated fraction (WAF) is used as the notified chemical is a multi-component substance with low aqueous solubility.
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	Based on the results of the range-finding test (WAF: 10 and 100 mg/L loading), a limit test was conducted (WAF: 100 mg/L loading). The notified chemical (250 mg) was added to the surface of culture medium (2.5 L) to achieve the loading rate of 100 mg/L. The test medium was stirred for 23 h and allowed to stand for 1 h. The WAF was removed by mid-depth siphoning (discarding the first 75–100 mL). Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. The test mixtures with an initial algae density of 4.47×10^3 cells per mL were irradiated 24 h/day at pH 7.3–7.7 and 24 ± 1°C for a period of 72 hours. The positive control was provided by potassium dichromate (0.0625–1.0 mg/L). A student's t-test incorporating Bartlett's test for homogeneity of variance was carried out on the data to determine any statistically significant differences between test and control groups.

RESULTS

Biomass		*Growth*	
E_bL_{50} mg/L at 72 h	*NOELR mg/L*	*E_rL_{50} mg/L at 72 h*	*NOELR mg/L*
> 100	100	> 100	100

Remarks - Results	All validity criteria were satisfied. Therefore, the test is considered reliable. The actual concentrations of the notified chemical in the test medium was determined by HPLC and a decrease was observed. This is consistent with preliminary stability analysis that indicated that the test substance was unstable in the culture medium at 0.1 mg/L. Given that toxicity cannot be attributed to a single component or a mixture of components but to the test material as a whole, endpoints are reported of the nominal loading rates for the WAF.
CONCLUSION	The notified chemical is not harmful to algae up to the limit of its solubility in water
TEST FACILITY	Harlan Laboratories Ltd (2010l)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 320 and 1000 mg/L
Remarks – Method	No deviations to the test protocol were reported.
RESULTS	
IC50	> 320 mg/L
NOEC	320 mg/L

Remarks – Results	The test substance was present as visible small to large globules or oily layer on the surface. The validation criteria for the control respiration rates and reference material, (3,5-dichlorophenol) EC_{50} were met. The initial and final dissolved oxygen concentrations were below the limits recommended in the test guideline. As the oxygen consumption rate was determined over the linear portion of the trace this did not affect the results, except for the 1000 mg/L test sample where the readings were too low to allow for the calculation the oxygen consumption rate.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	Harlan Laboratories Ltd (2010m)

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