File No: STD/1365

September 2010

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

# FULL PUBLIC REPORT

## Urea, *N*-(hydroxyethyl)- (INCI Name: Hydroxyethyl Urea)

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 the Environment, Water, Heritage and the Arts.

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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# FULL PUBLIC REPORT

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### 1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Akzo Nobel Pty Limited (ABN: 59 000 119 424)
8 Kellaway Place
Wetherill Park, NSW, 2164

Unilever Australia Limited (ABN: 66 004 050 828)
20 Cambridge Street
Epping, NSW, 2121

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: Degree of purity, spectral data and residual monomers/impurities.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Partition Coefficient, Adsorption/Desorption, Dissociation Constant, Particle Size, Flash Point, Flammability Limits, Autoignition Temperature and Explosive Properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada (NSN EAU-192)

### 2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Hydrovance (<50% notified chemical)

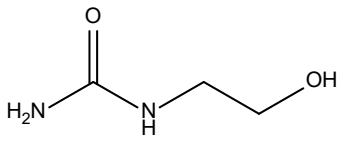
CAS NUMBER
1320-51-0

CHEMICAL NAME
Urea, *N*-(hydroxyethyl)-

OTHER NAME(S)
INCI name: Hydroxyethyl Urea
Urea, *N*-(2-hydroxyethyl)- (CAS: 2078-71-9)

MOLECULAR FORMULA
$C_3H_8N_2O_2$

STRUCTURAL FORMULA




MOLECULAR WEIGHT

104.11

ANALYTICAL DATA
IR and HPLC reference spectra were provided.

## 3. COMPOSITION

DEGREE OF PURITY <50% in Hydrovance, purity of the chemical is higher (likely >90%)

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
All hazardous impurities and residual monomers are present at levels under the concentration cut-offs.

## 4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Hydrovance (<50% notified chemical) is a light-yellow aqueous liquid. The notified chemical (white solid) is hygroscopic.

| Property | Value | Data Source/Justification |
|---|---|---|
| Melting Point | 56-88 °C | Measured |
| Boiling Point | Decomposed with boiling at *ca.* 150 °C at 102.94 kPa | Measured |
| Density | 1360 kg/m$^3$ at 22.3 °C | Measured |
| Vapour Pressure | 0.028 Pa at 25°C | Measured |
| Water Solubility | >699 g/L at 20°C | Measured |
| Hydrolysis as a Function of pH | $t_{½}$ >1 year at 25°C | Measured |
| Partition Coefficient (n-octanol/water) | log Pow = -2.06 | Calculated by KOWWIN (v1.67) (EPISuite (v4.00); US EPA, 2009) |
| Adsorption/Desorption | log $K_{oc}$ <0.0 | Calculated by KOCWIN (v2.00) (EPISuite (v4.00); US EPA, 2009) |
| Dissociation Constant | Not determined | The notified chemical does not contain any readily dissociable functionalities. |
| Particle Size | Not determined | The notified chemical is imported in aqueous solution |
| Flash Point | Not determined | The notified chemical is imported in aqueous solution |
| Autoignition Temperature | >100 °C | For Hydrovance; stated on MSDS |
| Explosive Properties | Not determined | Expected to be stable under normal conditions of use. |

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. Formulations utilizing Hydrovance (<50% notified chemical) have shown to drift to higher pH over time.

*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/polymer.

## 5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be introduced into Australia as a component of Hydrovance (<50% notified chemical) and as a component of finished products (≤8% notified chemical).

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
The notified chemical will be imported into Sydney, NSW.

IDENTITY OF MANUFACTURER/RECIPIENTS
The notified chemical will be imported as a component of Hydrovance (<50% notified chemical) and/or as a component of finished products (≤8% notified chemical) by Akzo Nobel Pty Ltd and Unilever Australia Ltd.

TRANSPORTATION AND PACKAGING
Hydrovance (<50% notified chemical) will be imported in 226.8 kg plastic drums on pallets. Finished products containing the notified chemical (≤8%) will be imported in ≤400 ml bottles/tubes suitable for retail sale. The bottles and tubes will be further packed in cardboard cartons (12 cartons/shipper). Transport within Australia to reformulation sites and/or retail stores will be by road.

USE
The notified chemical will be used as a component of cosmetic and personal care products at concentrations ≤8%. The notified chemical may be used in a variety of rinse-off and leave-on products.

OPERATION DESCRIPTION
The notified chemical may be imported in finished cosmetic products or as a component of Hydrovance (<50% notified chemical) and will undergo reformulation within Australia. The following refers to reformulation processes:

The operation description details will likely vary depending on the nature of the cosmetic and personal care products formulated, and may involve both automated and manual transfer steps. In general, it is expected that the notified chemical will undergo quality-assurance analysis before being manually weighed and added to mixing vessels (final concentration ≤8%), where it will be blended with other ingredients whilst closed. The resulting blend will then be filled into retail containers using automated filling machines. The finished products are then packed into pallets for distribution.

The finished products containing the notified chemical will be used by consumers and professionals such as hairdressers or workers in beauty salons. Depending on the nature of the product these could be applied in a number of ways such as by hand, using an applicator or sprayed.

## 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 | 10 | 4 | 12 |
| Compounder | 1 | 8 | 12 |
| Chemist | 1 | 3 | 12 |
| Packers | 2 | 8 | 12 |
| Store staff | 2 | 4 | 12 |
| Salon workers | unspecified | unspecified | unspecified |

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical [as a component of Hydrovance (<50%) or end-use products (≤8%)] only in the event of accidental rupture of containers.

During formulation, exposure to the notified chemical (up to 50%) may occur during weighing and transfer stages, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of mechanical ventilation and personal protective equipment (MSDS of Hydrovance

recommends the wearing of safety glasses and gloves).

Exposure to the notified chemical in end-use products may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hair dressers, workers in beauty salons). Such professionals may use some personal protective equipment to minimise repeated exposure, and good hygiene practices are expected to be in place. Exposure of such workers is expected to be of either a similar or higher level than that experienced by consumers using products containing the notified chemical.

### 6.1.2. Public exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of the rinse-off and leave-on cosmetic and personal care products. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

Data on typical use patterns of the product categories in which the notified chemical may be used are shown below (SCCP, 2006). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. The default dermal absorption of 100% was assumed for calculation purposes (European Commission, 2003). The actual level of dermal absorption may be lower than 100%. An adult bodyweight of 60 kg has been used for calculation purposes.

| **Product type** | mg/event | events/day | **C** (%) | **RF** | **Daily exposure** (mg/day) | **Daily systemic exposure** (mg/kg bw/day) |
|---|---|---|---|---|---|---|
| ***Leave on*** | | | | | | |
| Body lotion | 7820 | 1 | 8 | 1 | 626 | 10.4 |
| Face cream | 1540 | 1 | 8 | 1 | 123.2 | 2.05 |
| General purpose cream | 1200 | 2 | 8 | 1 | 192 | 3.2 |
| ***Rinse off*** | | | | | | |
| Facial cleansers | 4060 | 1-2 (1 used for calcs) | 8 | 0.01 | 3.25 | 0.0541 |
| Make up remover | 2500 | 2 | 8 | 0.1 | 40 | 0.667 |
| Shower gel | 5000 | 2 | 8 | 0.01 | 8 | 0.133 |
| Shampoo | 10460 | 1 | 8 | 0.01 | 8.37 | 0.139 |
| Hair conditioner | 14000 | 0.28 | 8 | 0.01 | 3.14 | 0.0523 |
| Hair styling products | 5000 | 2 | 8 | 0.1 | 80 | 1.33 |
| Total | | | | | | 18.06 |

C = concentration; RF = retention factor; 100% dermal absorption assumed.
Daily exposure = mg/event x events/day x C(%/100) x RF; Daily systemic exposure = daily exposure x dermal absorption (%) /60 kg

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table. This would result in a combined internal dose from dermal exposure of 18.06 mg/kg bw/day.

## 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 >2,000 mg/kg bw; low toxicity |
| Rat, acute dermal toxicity | LD50 >2,000 mg/kg bw; low toxicity |
| Rat, acute inhalation toxicity | LC50 >5.152 mg/L/4 hour; low toxicity |
| Rabbit, skin irritation | slightly irritating |
| Skin irritation – in vitro EpiDerm | Expected to be very mild to skin |
| Rabbit, eye irritation | slightly irritating |

| | |
|---|---|
| Guinea pig, skin sensitisation – adjuvant test | no evidence of sensitisation |
| Rat, repeat dose dermal toxicity – 90 days. | NOAEL >1,000 mg/kg bw/day |
| Mutagenicity – bacterial reverse mutation | non mutagenic |
| Genotoxicity – in vivo mouse micronucleus assay | non genotoxic |
| Developmental effects | NOAEL >1,000 mg/kg bw/day |

*Toxicokinetics, metabolism and distribution.*
Given the notified chemical has a low molecular weight and high water solubility (>699 g/L) dermal absorption may occur. However, this is expected to be limited based on the partition coefficient (log $P_{ow}$ estimated to be -2.06). In the GI tract, the notified chemical may pass through aqueous pores or be carried through the epithelial barrier by the passage of water. The inhalation of aerosols containing the notified chemical may result in uptake to the respiratory tract.

*Acute toxicity.*
The notified chemical was predicted to be very mild to the skin based on an in vitro EpiDerm skin irritation study. The notified chemical was found to be of low acute oral toxicity (LD50 >2,000 mg/kg bw), low acute dermal toxicity (LD50 >2,000 mg/kg bw) and low acute inhalation toxicity (LC50 >5.152 mg/L/4 hour). Gross lesions noted at necropsy for animals treated via the inhalation route included lungs with foci. These were not considered by the study authors to be related to the test substance.

*Irritation and Sensitisation.*
The notified chemical (tested at <60% concentration) was a slight skin and eye irritant in rabbits. The notified chemical (tested at <60% concentration) was not a skin sensitiser in guinea pigs (Magnus-Kligman method).

*Repeated Dose Toxicity.*
A 90-Day repeat dose dermal toxicity study in rats established an NOAEL for the notified chemical of 1,000 mg/kg bw/day. Administration at this dosage level did not result in mortality or any toxicologically significant on behaviour or weight gain and did not induce histopathological changes in the organs/tissues.

*Mutagenicity.*
The notified chemical was not mutagenic in a bacterial reverse mutation study and was not clastogenic in an in vivo mouse micronucleus assay. There was no indication in the micronucleus test that the chemical had reached the bone marrow.

*Developmental toxicity.*
A dermal developmental toxicity study in rats established an NOAEL for the notified chemical of 1000 mg/kg bw/day for both maternal and developmental toxicity.

*Additional Information*
The specifications for the notified chemical indicate a maximum nitrosamines level of 50 µg/kg.

***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
Exposure of workers to the notified chemical at up to 50% may occur during formulation of cosmetics. Due to the low hazard associated with the chemical and control measures in place to reduce exposure (including mechanical ventilation and the use of PPE), the OHS risk presented by the notified chemical (during formulation) is not considered to be unacceptable.
The risk for beauty care professionals who regularly use products containing the notified chemical is expected to be of a similar or perhaps higher level than that experienced by members of the public who use such products on a regular basis. This is because the duration of exposure will be longer for workers applying products in many clients.

#### 6.3.2. Public health

At the proposed maximum use concentration of up to 8% notified chemical in rinse-off and leave-on cosmetic products, acute toxicity effects are not expected. In addition, as the 90-day dermal repeat dose toxicity study established an NOAEL of 1,000 mg/kg bw/day, indicating no adverse effects at this dose level, quantitative risk assessment is not required.

Therefore, when used in the proposed manner, the risk to the public associated with the use of the notified chemical at up to 8% concentration in rinse-off and leave-on cosmetic products is not considered to be unacceptable.

## 7. ENVIRONMENTAL IMPLICATIONS

### 7.1. Environmental Exposure & Fate Assessment

#### 7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported as a component of finished cosmetic products and will also be imported as a raw material (<50% aqueous solution) for blending. The notified chemical is expected to be released to landfill as residue in containers (estimated to be up to 1% of the annual import volume) and released to sewer from the cleaning of blending equipment (up to 3%).

Accidental spills during transport or reformulation are expected to be collected with inert material and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The notified chemical is a component in cosmetic products. Therefore, it is expected that the majority of the imported quantity of notified chemical will be released to sewer from removal from the skin during bathing.

RELEASE OF CHEMICAL FROM DISPOSAL

Expired cosmetic wastes and residue of the notified chemical in empty containers (1%) are likely either to share the fate of the container and be disposed of to landfill, or to be washed to the sewer when containers are rinsed before recycling.

#### 7.1.2 Environmental fate

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

The majority of the notified chemical will be disposed of to the sewer, with minor amounts disposed of to landfill. The notified chemical is not expected to adsorb to sludge, soils or sediments, due to its high water solubility and its calculated adsorption coefficient (log Koc <0 (KOCWIN (v2.00); EPISuite (v4.00) US EPA (2009)). However, it is readily biodegradable and is not expected to bioaccumulate, due to its high water solubility, low predicted octanol-water partition coefficient (log Pow = -2.06, (KOWWIN (v1.67); EPISuite (4.00)) and bioconcentration factor (log BCF ≤0.5, BCFBAF (v2.00); EPISuite (4.00)). In landfill, the notified chemical is likely to leach, due to its high water solubility and its calculated soil adsorption coefficient. It is expected to degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

### 7.1.3 Predicted Environmental Concentration (PEC)

Assuming that most of the notified chemical will be washed into the sewer, the following predicted environmental concentration (PEC) in sewage effluent on a nationwide basis was calculated.

| ***Predicted Environmental Concentration (PEC) for the Aquatic Compartment*** | | |
|---|---|---|
| Total Annual Import/Manufactured Volume | 10,000 | kg/year |
| Proportion expected to be released to sewer | 100% | |
| Annual quantity of chemical released to sewer | 10,000 | kg/year |
| Days per year where release occurs | 365 | days/year |
| Daily chemical release: | 27.40 | kg/day |
| Water use | 200.0 | L/person/day |
| Population of Australia (Millions) | 21.161 | million |
| Removal within STP | 0% | |
| Daily effluent production: | 4,232 | ML |
| Dilution Factor - River | 1.0 | |
| Dilution Factor - Ocean | 10.0 | |
| PEC - River: | 6.47 | µg/L |
| PEC - Ocean: | 0.65 | µg/L |

The notified chemical is readily biodegradable, hence the removal of the notified chemical from influent by sewage treatment plant (STP) processes is expected. However, in this worst case model the majority of the notified chemical is assumed to be released in effluent. STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/$m^2$/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/$m^3$). Using these assumptions, irrigation with a concentration of 6.474 µg/L may potentially result in a soil concentration of approximately 43.16 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 215.8 µg /kg and 431.6 µg /kg, respectively. However, given the expected degradation of the notified chemicals, these values should be considered as theoretical maximum concentrations only.

## 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 | LC50 (96 h) >1000 mg/L | Not harmful to fish |
| Daphnia Toxicity | EC50(48 h) >1000 mg/L | Not harmful to aquatic invertebrates |
| Algal Toxicity | $E_rC50$ (72 h) >1000 mg/L | Not harmful to algae |

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is not harmful to fish, aquatic invertebrates or algae.

### 7.2.1 Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) was calculated with the minimum toxicity for fish, daphnia and algae (>1000 mg/L), and an assessment factor of 100, as the endpoints for three trophic levels are available.

| ***Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment*** | | |
|---|---|---|
| $E_rC50$ (Alga). | 1,000 | mg/L |
| Assessment Factor | 100 | |
| PNEC: | 10,000 | µg/L |

## 7.3. Environmental risk assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

| *Risk Assessment* | *PEC µg/L* | *PNEC µg/L* | *Q* |
|---|---|---|---|
| Q - River: | 6.47 | 10000 | 0.001 |
| Q - Ocean: | 0.65 | 10000 | 0.000 |

The risk quotient is <1 and, therefore, the notified chemical is not expected to pose a risk to the environment based on the reported use in cosmetics and the maximum annual importation volume.

## 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)].

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

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

**Environmental risk assessment**
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not expected to pose a 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 (<50%) during formulation of products:
  - 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 (<50%) during formulation of products:
  - Gloves and goggles.

  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 polymer 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.

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

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 removal.

**Regulatory Obligations**

*Secondary Notification*
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the notified chemical is to be used in rinse-off or leave-on cosmetic products at concentrations >8%;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of rinse-off and leave-on cosmetic products, 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.

*Material Safety Data Sheet*
The MSDS of the notified chemical (and a product containing the notified chemical) provided by the notifier were 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** 56-88 ºC

Method OECD TG 102 Melting Point/Melting Range.
Remarks Determined by differential scanning calorimetry (DSC).
Test Facility Harlan (2010)

**Boiling Point** Decomposed with boiling at *ca.* 150 ºC at 102.94 kPa

Method OECD TG 103 Boiling Point.
Remarks Determined by differential scanning calorimetry (DSC).
Test Facility Harlan (2010)

**Density** 1360 kg/m$^3$ at 22.3ºC

Method OECD TG 109 Density of Liquids and Solids.
Remarks Determined using a gas comparison pycnometer.
Test Facility Harlan (2010)

**Vapour Pressure** 0.028 Pa at 25ºC

Method OECD TG 104 Vapour Pressure.
Remarks Determined using a vapour pressure balance, with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure.
Test Facility Harlan (2010)

**Water Solubility** >699 g/L at 20ºC

Method Modification of OECD TG 105 Water Solubility.
Flask Method. After a preliminary test, a series of test concentrations (50.2 to 74.9 % w/w) were prepared and shaken at 30°C for a period of 43 hours. The samples were equilibrated at 20.0°C ± 0.5°C for 24 hours before visual assessment.
Remarks The high solubility of the notified chemical precluded preparation of samples at five times the estimated saturation concentration as required by OECD TG 105. All concentrations in the definitive test (50.2% w/w – 69.9% w/w) were visually assessed to be soluble, except for the highest test concentration (74.9% w/w) which contained undissolved test material. The water solubility was thus determined to be in the range 69.9% w/w and 74.9% w/w.
The pH of each solution was measured and ranged between 9.7-9.8.
Test Facility Harlan (2010)

**Hydrolysis as a Function of pH** $t_{½}$ >1 year at 25°C

Method OECD TG 111 Hydrolysis as a Function of pH.
Test concentrations (523 mg/L) at pH 4, 7, and 9 were maintained at 50°C. After 5 days, samples were analysed by HPLC to determine concentrations of the test substance and hydrolysis products.

| *pH* | *T (°C)* | $t_{½}$ |
|---|---|---|
| 4 | 25 | >1 year |
| 7 | 25 | >1 year |
| 9 | 25 | >1 year |

Remarks Less than 2% hydrolysis was observed after 5 days at 50°C at pH 4, 7, and 9. Therefore, the test material is considered stable with a half life greater than 1 year at 25°C.
Test Facility Toxikon Corporation (2005)

# APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

## B.1. Acute toxicity – oral

| | |
|---|---|
| TEST SUBSTANCE | Hydrovance (<60% notified chemical) |
| METHOD | OECD TG 401 Acute Oral Toxicity – Limit Test. |
| Species/Strain | Rat/Sprague-Dawley, 5M/5F |
| Vehicle | None |
| Remarks - Method | No significant protocol deviations. Dosage was adjusted for the purity of the test substance. |
| RESULTS | |
| Remarks - Results | There were no mortalities observed |
| LD50 | >2000 mg/kg bw notified chemical |
| Signs of Toxicity | Clinical abnormalities included transient incidences of fecal stain, mucoid stools and dark material around the nose. An isolated incidence of foci on the thymus was not considered to be significant. |
| Effects in Organs | None |
| CONCLUSION | The notified chemical is of low toxicity via the oral route. |
| TEST FACILITY | SLI (2001a) |

## B.2. Acute toxicity – dermal

| | |
|---|---|
| TEST SUBSTANCE | Hydrovance (<60% notified chemical) |
| METHOD | OECD TG 402 Acute Dermal Toxicity – Limit Test. |
| Species/Strain | Rat/Sprague-Dawley, 5M/5F |
| Vehicle | None |
| Type of dressing | Occlusive |
| Remarks - Method | No significant protocol deviations. Dosage was adjusted for the purity of the test substance. |
| RESULTS | |
| Remarks - Results | There were no mortalities observed |
| LD50 | >2000 mg/kg bw notified chemical |
| Signs of Toxicity - Local | Dermal irritation was noted at the site of test article application |
| Signs of Toxicity - Systemic | Clinical abnormalities included few feces and dark material around the facial area. Slight body weight loss was recorded for 1 male and 1 female in the first week of observation. |
| Effects in Organs | None. |
| CONCLUSION | The notified chemical is of low toxicity via the dermal route. |
| TEST FACILITY | SLI (2001b) |

## B.3. Acute toxicity – inhalation

| | |
|---|---|
| TEST SUBSTANCE | Hydrovance (≤50% notified chemical) |
| METHOD | OPPTS 870.1300 Acute Inhalation Toxicity – Limit Test. |
| Species/Strain | Rat/Sprague-Dawley [(CRI:CD®(SD)IGS BR], 5M/5F |
| Vehicle | None (2/3 exposures) and Water (1/3 exposures; Hydrovance diluted 1:1) |
| Method of Exposure | Nose only |
| Exposure Period | 4 hours |
| Physical Form | liquid aerosol |
| Particle Size | Mean mass median aerodynamic diameter (MMAD)/geometric standard deviation: 1.06 μm / 1.80 (Exposure 1); 1.63 μm / 2.33 (Exposure 2); |

1.90 µm / 2.87 (Exposure 3)

Remarks - Method: No significant protocol deviations.

RESULTS

| *Group* | *Number and Sex of Animals* | *Concentration mg/L* | | *Mortality* |
|---|---|---|---|---|
| | | *Nominal* | *Actual* | |
| Exposure 1 | 5 per sex | 39.5 | 0.59 | 0 |
| Exposure 2 | 5 per sex | 52.4 | 5.152 | 0 |
| Exposure 3 | 5 per sex | 1.48 | 0.132 | 0 |

LC50: >5.152 mg/L/4 hours

Signs of Toxicity: With the exception of the observation of redness/red material around the nose, observations were determined not to be attributable to the chemical.

Effects in Organs: For Exposure 1, gross lesions noted at necropsy included lungs with foci in three females. For Exposure 2, gross lesions noted at necropsy included lungs with foci on four males and one female, one male with a lesion on the small intestine, and one female with a white opaque lesion on the left kidney. For Exposure 3, gross lesions noted at necropsy included lungs with foci in two males. Histopathologic evaluation of the lungs from two animals from the third exposure having lung foci showed no hemosiderophages in the lymph node of either animal. The lung foci found in animals from this study were not considered, by the study authors, to be related to treatment with the test substance.

Remarks - Results: All exposed rats gained weight during the study period.

CONCLUSION: The notified chemical is of low toxicity via inhalation.

TEST FACILITY: IITRI (2008)

### B.4. Irritation – skin

TEST SUBSTANCE: Hydrovance (<60% notified chemical)

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain: Rabbit/New Zealand White

Number of Animals: 6 male

Vehicle: i) None, ii) deionized water

Observation Period: ≤10 days

Type of Dressing: Occlusive

Remarks - Method: Each rabbit received a 0.5 mL dose of each test article (100% and 52%) to one intact and one abraded site (i.e. total of four test sites).

RESULTS

100% test substance:

| *Lesion* | *Mean Score** | *Maximum Value* | *Maximum Duration of Any Effect* | *Maximum Value at End of Observation Period* |
|---|---|---|---|---|
| *Intact Skin:* | | | | |
| *Erythema/Eschar* | 0.5 | 1 | <7 days | 0 |
| *Oedema* | 0 | 0 | <24 hrs | 0 |
| *Abraded Skin:* | | | | |
| *Erythema/Eschar* | 0.8 | 2 | <10 days | 0 |
| *Oedema* | 0.2 | 1 | <72 hrs | 0 |

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

52% test substance:

| *Lesion* | *Mean Score** | *Maximum Value* | *Maximum Duration of Any Effect* | *Maximum Value at End of Observation Period* |
|---|---|---|---|---|
| *Intact Skin:* | | | | |
| *Erythema/Eschar* | 0.5 | 1 | <10 days | 0 |
| *Oedema* | 0 | 0 | <24 hrs | 0 |
| *Abraded Skin:* | | | | |
| *Erythema/Eschar* | 0.3 | 1 | <7 days | 0 |
| *Oedema* | 0 | 0 | <1 hr | 0 |

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

Remarks - Results: Slight erythema and oedema was reported. Desquamation was also noted in 2/6 animals treated with 100% test substance at abraded sites.

CONCLUSION: The test substance is slightly irritating to the skin.

TEST FACILITY: SLI (2001c)

**B.5. Irritation – skin irritancy potential by In vitro MatTek EpiDerm skin model**

TEST SUBSTANCE: Hydrovance (expected ≤50% notified chemical)

METHOD: The MatTek Corporation EpiDerm™ Skin Model *In vitro* Toxicity Test.

Remarks – Method: MatTek Epiderm tissue samples were treated with the test substance (100 µL) for 1, 4 and 24 h exposure times. Each treatment was conducted in duplicate. Following treatment, the viability of the tissues was determined using MTT uptake and conversion and the absorbance of each sample was measured at 540 nm using a reference wavelength of 690 nm. The mean percent viability was used to calculate the $ET_{50}$ (the time at which the EpiDerm tissue viability was reduced 50% compared to control tissues).

A negative control was performed at the 4 hour time point and a positive control (1% Triton X-100) was performed in duplicate for the 4 and 24 hour exposure times.

RESULTS

| *Exposure Time (hrs)* | *Mean Viability Score* |
|---|---|
| 1 | 98.4 |
| 4 | 94.3 |
| 24 | 22.5 |

Remarks - Results: The $ET_{50}$ was determined to be 12.1 h

CONCLUSION: The test substance is expected to be very mild to the skin.

TEST FACILITY: MB (2005)

**B.6. Irritation – eye**

TEST SUBSTANCE: Hydrovance (<60% notified chemical)

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.

Species/Strain: Rabbit/New Zealand White

Number of Animals: 6 (3M/3F)

Observation Period: ≤7 days

Remarks - Method: No significant protocol deviations.

RESULTS

| *Lesion* | *Mean Score** | *Maximum Value* | *Maximum Duration of Any Effect* | *Maximum Value at End of Observation Period* |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| *Conjunctiva: redness* | 0.6 | 1 | <7 days | 0 |
| *Conjunctiva: chemosis* | 0.2 | 1 | <48 h | 0 |
| *Conjunctiva: discharge* | 0 | 2 | <24 h | 0 |
| *Corneal opacity* | 0 | 0 | <1 h | 0 |
| *Iridial inflammation* | 0.06 | 1 | <48 h | 0 |

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

Remarks - Results: Iritis was noted in 3/6 animals at the 1 hour scoring interval, which resolved completely in all test eyes by the 48 hour scoring interval. Conjunctivitis (redness, swelling and/or discharge) was noted in 6/6 animals at the 1 hour scoring interval. The conjunctival irritation was resolved completely in all test eyes by study day 7.

CONCLUSION: The test substance is slightly irritating to the eye.

TEST FACILITY: SLI (2001d)

**B.7. Skin sensitisation**

TEST SUBSTANCE: Hydrovance (<60% notified chemical)

METHOD: OECD TG 406 Skin Sensitisation - Magnusson and Kligman guinea pig maximisation test.

Species/Strain: Guinea pig/Hartley

PRELIMINARY STUDY: Maximum Non-irritating Concentration:
intradermal: 5%
topical: 100%

MAIN STUDY

Number of Animals: Test Group: 10M/10F Control Group: 5M/5F

INDUCTION PHASE: Induction Concentration:
intradermal: 5%
topical: 100%

Signs of Irritation: None recorded.

CHALLENGE PHASE

1st challenge: topical: 100%

Remarks - Method: No significant protocol deviations. Test sites were pre-treated with sodium lauryl sulfate.
Vehicle: deionized water

RESULTS

| *Animal* | *Challenge Concentration* | *Number of Animals Showing Skin Reactions after: 1st challenge* | |
|---|---|---|---|
| | | *24 h* | *48 h* |
| *Test Group* | 100% | 0 | 0 |
| *Control Group* | 100% | 0 | 0 |

Remarks - Results: Dermal scores were limited to 0 in the test and challenge control animals at the 24 and 48 h scoring intervals. A positive control study using α-hexylcinnamaldehyde (completed during the preceding 6 months) is noted to demonstrate the susceptibility of the test system to sensitizing agents.

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

TEST FACILITY: SLI (2001e)

**B.8. Repeat dose toxicity**

TEST SUBSTANCE

| | |
|---|---|
| METHOD | OPPTS 870.3250 90-Day Dermal Toxicity |
| Species/Strain | Rat/Sprague-Dawley |
| Route of Administration | Dermal – semi-occluded |
| Exposure Information | Total exposure days: 90 days<br>Dose regimen: 7 days per week<br>Duration of exposure (dermal): 6 hours/day<br>Post-exposure observation period: 0.5-1.5 h |
| Vehicle | Reverse osmosis deionized water |
| Remarks - Method | Dose levels refer to the concentration of notified chemical. |

RESULTS

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| control | 10M/10F | 0 | 0 |
| low dose | 10M/10F | 100 | 0 |
| mid dose | 10M/10F | 330 | 0 |
| high dose | 10M/10F | 1000 | 0 |

*Clinical Observations*

Minor treatment-related dermal effects were observed during the study, including a dose-related increase in the incidence of focal/pinpoint eschar, desquamation and red pinpoint areas (a slightly higher incidence is noted in females). These were deemed to be superficial in nature.

*Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis*

Significant findings were not noted with regard to urinalysis and hematology. Statistically increased phosphorus (100, 330 and 1000 mg/kg bw/day groups) and calcium (1000 mg/kg bw/day) levels were noted on Day 90 in males. These finding were deemed to be related to the test article but not of biological significance.

*Effects in Organs*

Significant changes in gross necropsy or organ weight data were not noted. No test-article related microscopic lesions were noted following histopathological evaluation of the organs/tissues.

Remarks – Results

Significant changes in body weight gain and food consumption were not noted.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on the absence of any toxicologically significant effects at this dosage level.

TEST FACILITY SLI (2002a)

**B.9. Genotoxicity – bacteria**

| | |
|---|---|
| TEST SUBSTANCE | Hydrovance (<60% notified chemical) |
| METHOD | OECD TG 471 Bacterial Reverse Mutation Test.<br>Plate incorporation procedure |
| Species/Strain | *S. typhimurium*: TA1535, TA1537, TA98, TA100<br>*E. coli*: WP2uvrA |
| Metabolic Activation System | Aroclor 1254-induced rat liver (S9 homogenate) |
| Concentration Range in Main Test | a) With metabolic activation: 75, 200, 600, 1800, 5000 µg/plate<br>b) Without metabolic activation: 75, 200, 600, 1800, 5000 µg/plate |
| Vehicle | water |
| Remarks - Method | A preliminary toxicity test (2.5-5000 µg/plate) was performed to define the dose levels for the main test. Dosage was adjusted for the purity of the |

test substance.

All dose levels of test article, vehicle controls and positive controls were plated in triplicate.

Positive controls: i) without S9: 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537) and methyl methanesulfonate (WP2uvrA); with S9: 2-aminoanthracene

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 | >5,000 | >5,000 | Negative |
| *Present* | | | | |
| Test 1 | >5,000 | >5,000 | >5,000 | Negative |

Remarks - Results: No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains up to and including the maximum dose of 5000 µg/plate, either with or without metabolic activation.

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

TEST FACILITY: BioReliance (2001)

**B.10. Genotoxicity – in vivo**

TEST SUBSTANCE: Hydrovance (<60% notified chemical)

METHOD: OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse, Crl:CD-1®(ICR) BR

Route of Administration: Oral – gavage

Vehicle: Deionized water

Remarks - Method: A range-finding study was conducted using 3 male and 3 female mice at 3 dosage levels (500, 1000 and 2000 mg/kg bw). Dose levels refer to the concentration of notified chemical. The same concentrations were then selected for the main study. Only male mice were treated in the main study.

| *Group* | *Number and Sex of Animals* | *Dose mg/kg bw* | *Sacrifice Time hours* |
|---|---|---|---|
| I (vehicle control) | 6 male/6 male | - | 24/48 |
| II (low dose) | 6 male | 500 | 24 |
| III (mid dose) | 6 male | 1000 | 24 |
| IV (high dose) | 6 male/6 male | 2000 | 24/48 |
| V (positive control, CP) | 6 male | - | 24 |

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity: No signs of clinical toxicity are noted at any dose level.

Genotoxic Effects: A statistically significant increase in micronucleated PCEs was not observed at any dose level. The positive control induced statistically significant increases in micronucleated PCEs.

Remarks - Results: 5 animals from each treatment and control group were selected for bone marrow analysis. It was not possible to confirm that the notified chemical reached the bone marrow.

| | |
|---|---|
| CONCLUSION | The notified chemical was not clastogenic under the conditions of this in vivo Mammalian Erythrocyte Micronucleus Test. |
| TEST FACILITY | Covance (2001) |

**B.11. Developmental toxicity**

| | |
|---|---|
| TEST SUBSTANCE | Hydrovance (<60% notified chemical) |
| METHOD | OPPTS 870.3700 – Prenatal Developmental Toxicity Study |
| Species/Strain | Rat/Sprague-Dawley |
| Route of Administration | Dermal – non-occluded |
| Exposure Information | Exposure days: days 6 through 19 of gestation<br>Duration of exposure: 6 hours/day |
| Vehicle | Reverse osmosis deionized water |
| Remarks - Method | Elizabethan collars were placed around the neck of each animal during the exposure period. Dose levels refer to the concentration of notified chemical. |

RESULTS

| *Group* | *Number of Animals* | *Dose mg/kg bw/day* | *Mortality* |
|---|---|---|---|
| 1 | 25 | 0 | 0 |
| 2 | 25 | 100 | 0 |
| 3 | 25 | 330 | 0 |
| 4 | 25 | 1000 | 0 |

*Mortality and Time to Death*
No mortalities were recorded.

*Effects on Dams*
Mean food consumption of females in the 1000 mg/kg bw/day group was statistically lower than controls during the treatment period. However, there were no statistically significant differences in mean body weights/body weight gain between the control and test groups. In addition no significant findings were noted in the caesarean section parameters (mean number of corpora lutea, implantation sites, viable foetuses, early and late resorptions, pre- and post-implantation loss, fetal sex ratios and mean fetal body weights).

*Effects on Foetus*
No statistically significant or toxicologically meaningful differences in the incidence of fetal malformations or developmental variations are noted between test and control groups.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study for both maternal and developmental toxicity.

TEST FACILITY SLI (2002b)

# APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

## C.1. Environmental Fate

### C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical (~60% in aqueous solution)

METHOD: OECD TG 301 B Ready Biodegradability: $CO_2$ Evolution Test.

| | |
|---|---|
| Inoculum | Activated sludge from Newton Abbot sewage treatment works |
| Exposure Period | 28 days |
| Auxiliary Solvent | None |
| Analytical Monitoring | Total organic carbon (TOC) and inorganic carbon (IC) analyses were conducted by a Dohrman DC 190 Analyser. |
| Remarks - Method | The test was conducted in accordance with the guidelines above at a nominal concentration (10 mg C/L) of test material. A reference (sodium acetate) control and toxicity control (test substance and sodium acetate) were run in parallel. The percentage biodegradation is expressed as a ratio of evolved carbon dioxide to the initial theoretical carbon added as test substance. |

RESULTS

| *Test substance* | | *Sodium Acetate* | |
|---|---|---|---|
| *Day* | *% Degradation* | *Day* | *% Degradation* |
| 6 | 3 | 6 | 57 |
| 10 | 3 | 10 | 72 |
| 21 | 61 | 21 | 84 |
| 28 | 88 | 28 | 89 |

Remarks - Results: The test substance achieved the pass level of at lest 60% biodegradation within the 10 day window. The reference material biodegraded by 89% over the duration of the test, and achieved the pass level in the ten day window, thus validating the test.
The toxicity control biodegraded by 76% over 28 days, indicating that the test substance is not toxic to the test inoculum.

CONCLUSION: The test substance and, by inference, the notified chemical are readily biodegradable

TEST FACILITY: Brixam Environmental Laboratory (2001)

## C.2. Ecotoxicological Investigations

### C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical (<60% in aqueous solution)

METHOD: OECD TG 203 Fish, Acute Toxicity Test – static.

| | |
|---|---|
| Species | Rainbow trout (*Onchorhynchus mykiss*) |
| Exposure Period | 96 hours |
| Auxiliary Solvent | None |
| Water Hardness | 140 mg $CaCO_3$/L |
| Analytical Monitoring | None |
| Remarks – Method | After a range finding test, a definitive test at concentrations 65, 130, 250, 500 and 1000 mg notified chemical/L (in duplicate) was conducted according to the guidelines above. The fish, 5 per test solution, were observed for mortality and sublethal responses every 24 hours. Test conditions were: 15.2-15.9°C, pH 7.40-8.03, and 6.92-8.27 mg $O_2$/L. |

RESULTS

| *Concentration mg/L* | | *Number of Fish* | *Mortality* | | | | |
|---|---|---|---|---|---|---|---|
| *Nominal* | *Actual* | | *1 h* | *24 h* | *48 h* | *72 h* | *96 h* |
| 0 | Not reported | 2 × 5 | 0 | 0 | 0 | 0 | 0 |
| 65 | Not reported | 2 × 5 | 0 | 0 | 0 | 0 | 0 |
| 130 | Not reported | 2 × 5 | 0 | 0 | 0 | 0 | 0 |
| 250 | Not reported | 2 × 5 | 0 | 0 | 0 | 0 | 0 |
| 500 | Not reported | 2 × 5 | 0 | 0 | 0 | 0 | 0 |
| 1000 | Not reported | 2 × 5 | 0 | 0 | 0 | 0 | 0 |

| | |
|---|---|
| LC50 | >1000 mg/L at 96 hours. |
| NOEC | 1000 mg/L at 96 hours. |
| Remarks – Results | All test solutions appeared clear and colourless throughout the test with no visible particulates, surface film, undissolved test substance or precipitate.<br>After 96 hours of exposure, there was no mortality in the test concentrations or control, thereby validating the test. |
| CONCLUSION | The test substance and, by inference, the notified chemical are not harmful to fish |
| TEST FACILITY | ABC Laboratories, Inc (2001a) |

**C.2.2. Acute toxicity to aquatic invertebrates**

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical (<60% in aqueous solution) |
| METHOD | OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – static. |
| Species | *Daphnia magna* |
| Exposure Period | 48 hours |
| Auxiliary Solvent | None |
| Water Hardness | 148 mg $CaCO_3$/L |
| Analytical Monitoring | None |
| Remarks - Method | After a range finding test, a definitive test at concentrations 65, 130, 250, 500 and 1000 mg notified chemical/L was conducted according to the guidelines above. Four replicates per concentration each had 5 daphnia added. The daphnia were observed for immobilisation every 24 hours over the course of the test. Test conditions were: 20.0-20.7°C, 16 h/8 h light dark cycle, 8.1-8.7 mg $O_2$/L, pH 7.82-8.29. |

RESULTS

| *Concentration mg/L* | | *Number of D. magna* | *Number Immobilised* | |
|---|---|---|---|---|
| *Nominal* | *Actual* | | *24 h* | *48 h* |
| 0 | | 4 × 5 | 0 | 0 |
| 65 | | 4 × 5 | 0 | 0 |
| 130 | | 4 × 5 | 0 | 0 |
| 250 | | 4 × 5 | 0 | 0 |
| 500 | | 4 × 5 | 0 | 0 |
| 1000 | | 4 × 5 | 0 | 0 |

| | |
|---|---|
| EC50 | >1000 mg/L at 48 hours |
| NOEC | 1000 mg/L at 48 hours |
| Remarks - Results | All test solutions appeared clear and colourless throughout the test with no visible particulates, surface film, undissolved test substance or precipitate.<br>After 48 hours of exposure, there was no immobility observed in the test |

concentrations or control, thereby validating the test.

CONCLUSION The test substance and, by inference, the notified chemical are not harmful to aquatic invertebrates

TEST FACILITY ABC Laboratories, Inc (2001b)

### C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical (<60% in aqueous solution)

METHOD OECD TG 201 Alga, Growth Inhibition Test.

| | |
|---|---|
| Species | *Selenastrum capricornutum* |
| Exposure Period | 72 hours |
| Concentration Range | Nominal: 0, 65, 130, 250, 500 and 1000 mg notified chemical/L |
| Auxiliary Solvent | None |
| Water Hardness | 0.15 mmol $Ca^{2+}$ and $Mg^{2+}$ |
| Analytical Monitoring | Cell counts were performed using a light microscope and a hemacytometer. |
| Remarks - Method | After a range finding test, a definitive test at concentrations 65, 130, 250, 500 and 1000 mg notified chemical/L (in triplicate) was conducted according to the guidelines above. Test conditions were: 23.8 - 25.0°C, continuous illumination, pH 7.67-8.25. Shapiro-Wilk's test, Levene's test, one way analysis of variance and dunnetts comparison were used for statistical analysis. |

RESULTS

| *Biomass* | | *Growth* | |
|---|---|---|---|
| $E_bC_{50}$ *mg/L at 72 h* | *NOEC mg/L* | $E_rC_{50}$ *mg/L at 72 h* | *NOEC mg/L* |
| >1000 | 1000 | >1000 | 1000 |

Remarks - Results After 72 hours the inhibition of cell growth was 0% in all treatments with the exception of the 500 mg/L concentration (84% inhibition). Since all other treatments showed no inhibition this effect is not believed to be test substance related, thus this value was not used in calculating the effective median concentration.
The biomass in the control increased by 139-fold, thereby validating the test.
The pH deviation of more than 1 pH unit did not affect the integrity of the test since acceptable growth (>16-fold increase) was observed in the controls.

CONCLUSION The test substance and, by inference, the notified chemical are not harmful to algae.

TEST FACILITY ABC Laboratories, Inc (2001c)

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