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October 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Troysol LAC

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

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

This 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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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1560	Sherwin-Williams Diversified Brands (Australia) Pty Ltd & Woolworths Limited	Chemical in Troysol LAC	Yes	≤ 8 tonnes per annum	Component of paints

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Skin irritation (Category 2)	H315: Causes skin irritation
Eye irritation (Category 1)	H318: Causes serious eye damage
Specific target organ toxicity - Repeated exposure (Category 2)	H373: May cause damage to organs through prolonged or repeated exposure if inhaled

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R38: Irritating to skin
R41: Risk of serious damage to eyes
R48/20: Danger of serious damage to health by prolonged exposure through inhalation

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 PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin irritation (Category 2) - H315: Causes skin irritation
  - Eye irritation (Category 1) - H318: Causes serious eye damage

- Specific target organ toxicity - Repeated exposure (Category 2) - H373: May cause damage to organs through prolonged or repeated exposure if inhaled

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

CONTROL MEASURES

Occupational Health and Safety

- Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.
- Spray applications should be carried out in accordance with the Safe Work Australia Code of Practice for *Spray Painting and Powder Coating* (SWA, 2012) or relevant State or Territory Code of Practice.
- A copy of the (M)SDS should be easily accessible to employees.
- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, physical 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 component of paints, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

No additional secondary notification conditions are stipulated.

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

(Material) Safety Data Sheet
The (M)SDS of the products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Sherwin-Williams Diversified Brands (Australia) Pty Ltd (ABN: 31 604 851 658)
Level 21, 300 Murray Street
Perth WA 6000

Woolworths Limited (ABN: 88 000 014 675)
3 City View Road
Pennant Hills NSW 2120

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, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, import volume, and identity of manufacturer.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: boiling point, density, dissociation constant, flash point, flammability, and explosive properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Italy (2006)
USA (1980)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Troysol LAC (contains the notified chemical at ~ 50% concentration)

MOLECULAR WEIGHT
< 500 Da

ANALYTICAL DATA
Reference LC/MS, NMR, FT-IR, and UV-VIS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: solid

Property	Value	Data Source/Justification
Melting Point	243 °C	Measured
Boiling Point	95 -115 °C at 101.3 kPa*	(M)SDS

Density	1,040 – 1,070 kg/m^3 at 25 °C*	(M)SDS
Vapour Pressure	1.124 kPa at 20 °C 1.484 kPa at 25 °C	Measured
Water Solubility	1.52 (± 0.117) × 10^{-4} g/L at 25 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains no hydrolysable functionalities and has limited solubility in water
Partition Coefficient (n-octanol/water)	log Pow = 8.2	Calculated from measured data. The notified chemical is surface active and estimations may not adequately characterise the partitioning behaviour. The notified chemical is expected to partition to phase boundaries
Adsorption/Desorption	log K_{oc} < 1.25	Estimated from measured data. The notified chemical is surface active and estimations may not adequately characterise the adsorption/desorption behaviour. The notified chemical is expected to adsorb strongly to soil and sediment
Dissociation Constant	Not determined	The notified chemical is a salt, and is expected to be ionised under environmental conditions (pH 4-9)
Flash Point	> 110 °C (closed cup)*	(M)SDS
Flammability	Not determined	Not expected to be highly flammable based on flash point
Autoignition Temperature	290 °C	Measured
Explosive Properties	Not determined	Not expected to be explosive based on chemical structure
Oxidising Properties	Not oxidising	Based on chemical structure

* For Troysol LAC containing the notified chemical at ~50% concentration in solvent solution

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

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical cannot be classified hazardous according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of finished paints at < 1% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 8	< 8	< 8	< 8	< 8

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The finished paints containing the notified chemical at < 1% concentration will be imported in 1 L, 4 L and 10 L metal containers and 15 L plastic containers, and transported by road within Australia.

USE

The notified chemical will be used as a component of finished paints at < 1% concentration.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of finished paints at < 1% concentration. There will be no reformulation or repackaging within Australia.

End-use
The finished paints containing the notified chemical at < 1% concentration will be used by professional painters and do-it-yourself (DIY) users. Professional painters will likely apply the paint by roller, brush and spray whereas the DIY users are expected to primarily use brush or roller.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Stevedores	4	20
Transport	4	20
Warehousing	6	260
Commercial printers	8	260

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical in finished paints (at < 1% concentration) only in the event of accidental rupture of containers.

End-use
Professional painters may be exposed (dermal, ocular and inhalation) to the notified chemical (at < 1% concentration) during application of finished paints by brush, roller or spray. Exposure should be minimised through the recommended use (on the paint SDS) of PPE including coveralls, gloves and goggles, as well as respiratory protection when applied by spray.

Once the paints are dried, the notified chemical will be bound within a polymer matrix and will not be available for exposure.

6.1.2. Public Exposure
The general public may be exposed (dermal and ocular) to the notified chemical in finished paints (at < 1% concentration) during application by brush or roller. Inhalation exposure may also occur where the paints are applied by spray although this is only expected to occur infrequently.

The general public may also come into contact with the notified chemical in finished paints after application to surfaces. However, once the paints are dried, the notified chemical will be bound within a paint matrix and will not be available for exposure.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemical or the product Troysol LAC containing the notified chemical at ~50% concentration are summarised in the following table. Solvents including water that make up the balance of the product Troysol LAC are not expected to contribute to the toxicity. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 5000 mg/kg bw; low toxicity*
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity*
Rat, acute inhalation toxicity	LC50 > 2.12 mg/L/4 h*
Skin irritation (in vitro)	irritating*
Eye irritation (in vitro)	severely irritating*

Guinea pig, skin sensitisation – non-adjuvant test	no evidence of sensitisation*
Rat, repeat dose oral toxicity – 28 days.	NOAEL 100 mg/kg bw/day**
Rat, repeat dose inhalation toxicity – 14 days.	LOAEL 0.036 mg/L/6 h/day*
Rat, repeat dose inhalation toxicity – 28 days.	LOAEL 0.0038 mg/L/6 h/day*
Mutagenicity – bacterial reverse mutation	non mutagenic**
Genotoxicity – in vitro mammalian cell chromosomal aberration	non genotoxic*
Genotoxicity – in vitro cell mutation assay	non genotoxic**

*Troysol LAC containing the notified chemical at ~ 50% concentration
**The notified chemical at > 90% concentration

Toxicokinetics
The notified chemical is a salt, highly lipophilic and is of low water solubility; hence dermal absorption is not expected. Absorption across the gastrointestinal and respiratory tracts is also expected to be limited. However, the notified chemical is a skin irritant and surface active which may enhance absorption.

Acute toxicity
The product containing the notified chemical at ~50% concentration is of low acute oral, dermal and inhalation toxicity based on studies conducted in rats.

Irritation and sensitisation
The product containing the notified chemical at ~50% concentration was found to be irritating to skin and severely irritating to eyes based on studies conducted in rabbits. These observations are consistent with the structure of the notified chemical, which contain a structural alert for corrosion (Tsakovska *et al.,* 2007, Hulzebos *et al.,* 2005). Based on the results of these studies with the notified chemical at ~50% concentration, the notified chemical is at least a skin and eye irritant but may also present as a corrosive. Furthermore, the results from the acute and repeated dose inhalation toxicity studies conducted on the product containing the notified chemical at ~50% concentration indicate that the notified chemical may also present as a respiratory irritant.

The notified chemical was not found to be a sensitiser in a Guinea pig study using the Buehler Method.

Repeated dose toxicity
The notified chemical was tested in rats at doses of 100, 300 and 1,000 mg/kg bw/day in a 28-day repeated dose oral gavage toxicity study. A NO(A)EL was established as 100 mg/kg bw/day in this study, based on effects in the stomach at 300 mg/kg bw/day or 1000 mg/kg bw/day. Effects in the stomach included squamous cell hyperplasia and hyperkeratosis, parakeratosis, submucosal inflammation, submucosal edema and pustules in both sexes as well as forestomach ulceration and erosion in females only. The effects are consistent with the irritant nature of the notified chemical.

Two repeated dose inhalation toxicity studies have been conducted on the product containing the notified chemical at ~50% concentration. In the 14-day range finding study, animals were exposed to the test substance (nose only) at 0.036, 0.106 and 0.307 mg/L for 6 h/day/5 days/week. In the subsequent 28-day study, animals were exposed to the test substance (nose only) at 0.0038, 0.0113 and 0.037 mg/L for 6 h/day/5 days/week. In both studies, effects were observed at all doses in a dose dependent manner in the larynx, lungs and nasal turbinate. A NO(A)EL could not be established by the study authors in these studies.

At the end of the recovery period (additional 14 days) in the 28-day study, the changes in the lungs had resolved but treatment related significant changes remained in the larynx and nasal turbinates. Necrosis of the ventral cartilage was present in the larynx of all treated groups with ventral epithelial hyperplasia in some high dose animals and one mid dose female. Some animals from all groups showed eosinophilic inclusions in the olfactory epithelium in the nasal turbinates with an increased incidence and/or severity in treated and higher dose animals. Based on the marked effects seen in the respiratory tract of high dose treated animals after the recovery period, the notified chemical may cause damage to the respiratory tract by prolonged exposure through inhalation.

Mutagenicity/Genotoxicity
The notified chemical was negative in an *in vitro* bacterial reverse mutation study. The product containing the notified chemical at ~50% concentration was also found to be non genotoxic in an *in vitro* chromosome aberration test using Chinese hamster lung fibroblasts. The notified chemical was found to be non-genotoxic in an *in vitro* cell mutation assay.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Skin irritation (Category 2)	H315: Causes skin irritation
Eye irritation (Category 1)	H318: Causes serious eye damage
Specific target organ toxicity - Repeated exposure (Category 2)	H373: May cause damage to organs

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R38: Irritating to skin
R41: Risk of serious damage to eyes
R48/20: Danger of serious damage to health by prolonged exposure through inhalation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available toxicological data, the notified chemical is expected to be at least severely irritating to eyes and irritating to skin. The notified chemical may cause damage to the respiratory tract by repeated exposure if inhaled. The notified chemical may also present as a respiratory irritant.

The notified chemical will be imported as a component of finished paints at < 1% concentration. Given workers will only be exposed to the notified chemical at low concentrations, the abovementioned risks of irritation effects and damage to respiratory tract are not expected.

Therefore, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health
The finished paints containing the notified chemical at < 1% concentration will be made available to the general public. The paints are expected to be predominantly applied by brush or roller. The notified chemical is a skin, eye and a potential respiratory irritant. The notified chemical may also cause damage to the respiratory tract by repeated exposure if inhaled. Given the proposed low end-use concentration and infrequent use by the public the risks associated with the notified chemical are not expected.

Therefore, under the proposed use, the notified chemical is not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported as a component of finished paints and coating formulations, and will not be reformulated or repackaged in Australia. Therefore, no release of the notified chemical is expected from manufacturing or reformulation.

Release of the notified chemical during transport and storage is expected to be limited to accidental spills or leaks. Spills or accidental release of the product containing the notified chemical are expected to be contained and collected using absorbents, and disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

Products containing the notified chemical will be used by both professional and Do-It-Yourself (DIY) users. During use, paints and coatings containing the notified chemical are expected to be applied by brush, roller and spray techniques. It is expected that some of the coating product will be in the form of overspray during spraying operations, and will typically entail disposal to landfill after being collected with absorbents.

During use, the notified chemical may also be released to the environment as accidental spills and container residues. These releases are expected to be collected and disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM DISPOSAL

The notified chemical in surface coatings are expected to share the fate of the substrate to which it has been applied, and are predominantly expected to be disposed to landfill, or thermally decomposed during substrate reclamation.

Residues containing the notified chemical on brushes and rollers are expected to be rinsed into containers, and then allowed to cure before disposal as solid wastes to landfill. As a worst case scenario, it is assumed that up to 5% of the notified chemical used by DIY users may be incorrectly disposed of to the sewer, drains, or ground from waste and washing of application equipment.

7.1.2. Environmental Fate

The majority of the notified chemical is expected to be cured within an inert coating matrix and is expected to share the fate of the articles to which it has been applied, which will involve eventual disposal to landfill or undergo thermal decomposition during substrate reclamation. The notified chemical is also expected to enter landfill as collected wastes and residues. Once cured, the notified chemical is not expected to be bioavailable nor biodegradable.

The notified chemical is not readily biodegradable (49-53% in 28 days), but has the potential to degrade in the aquatic environment. Bioaccumulation of uncured notified chemical is unlikely as it is expected to partition to phase boundaries based on its surfactant properties and dispersibility in water. The notified chemical is not likely to be mobile in the environment, due to its limited solubility in water and strong potential to adsorb to soil and sediment, based on its surfactant properties. Therefore, a significant portion of the notified chemical (> 85%) is expected to partition to sludge during wastewater treatment processes in sewage treatment plants (STPs). In surface waters and in landfill, the notified chemical is expected to eventually degrade via biotic and abiotic processes to form water and oxides of carbon and sulphur. Details of the environmental fate studies can be found in Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. Based on the reported use in paints and coatings for professional and DIY-users, a conservative release of 5% to sewers on a nationwide basis over 365 days per year is used for the notified chemical. It is also assumes a worst case scenario where none of the notified chemical is removed during Sewage Treatment Plant (STP) processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	8,000	kg/year
Proportion expected to be released to sewer	5%	
Annual quantity of chemical released to sewer	400	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	1.1	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.242	µg/L
PEC - Ocean:	0.024	µg/L

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 0.242 µg/L may potentially result in a soil concentration of approximately 1.615 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 8.077 µg/kg and 16.15 µg/kg, respectively.

7.2. Environmental Effects Assessment

Several study reports were submitted on ecotoxicological investigations conducted on the notified chemical or the product Troysol LAC containing the notified chemical at ~50% concentration. Only the results of the studies conducted on the notified chemical for fish, algae and daphnia are summarised in the table below. Details of all the submitted studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LL50 > 0.161 mg/L (WAF*)	Not harmful up to the limit of solubility
Daphnia Toxicity	EL50 > 0.67 mg/L (WAF*)	Not harmful up to the limit of solubility
Algal Toxicity	E_rL50 > 0.196 mg/L (WAF*)	Not harmful up to the limit of solubility
Inhibition of Bacterial Respiration	IC50 > 1000 mg/L**	Not inhibitory to bacterial respiration

* Water Accommodated Fraction

** For Troysol LAC containing the notified chemical at ~ 50% concentration

Based on the above acute ecotoxicological endpoints, the notified chemical is not expected to be harmful to aquatic organisms up to the limit of its solubility in water. Therefore, the notified chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for fish. A safety factor of 100 was used given acute endpoints for three tropic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LL50 (Fish, 96 h)	> 0.161	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	> 1.61	µg/L

7.3. Environmental Risk Assessment

The Risk Quotients (Q = PEC/PNEC) have been calculated based on the predicted PEC and PNEC.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.242	> 1.61	**< 0.151**
Q - Ocean	0.024	> 1.61	**< 0.015**

The Risk Quotients for a conservative discharge scenario have been calculated to be less than 1 for both the riverine and marine compartments. Based on the biodegradation study, the notified chemical is not readily biodegradable, however it is expected to ultimately biodegrade. Based on its surfactant properties, the notified chemical is not expected to be bioaccumulative, and is expected to partition to soil and sludge. The notified chemical is not harmful to aquatic organisms up to the limit of its water solubility. Therefore, on the basis of the PEC/PNEC ratio and assessed usage pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 243 °C

Method	Similar to OECD TG 102 Melting Point/Melting Range.
Remarks	Differential Scanning Calorimetry
Test Facility	Rutgers University (2006)

Vapour Pressure 1.124 kPa at 20 °C
1.484 kPa at 25 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Static method
Test Facility	Case Consulting Laboratories (2008)

Water Solubility $1.52 (\pm 0.117) \times 10^{-4}$ g/L at 25 °C

Method	OECD TG 105 Water Solubility.
Remarks	Generator Column Method
Test Facility	Wildlife International (2008a)

Partition Coefficient (n-octanol/water) log Pow = 8.2

Method	OECD TG 107 Partition Coefficient (n-octanol/water); Shake Flask Method. OECD TG 117 Partition Coefficient (n-octanol/water); HPLC Method. EC Directive 92/69/EEC Method A.8 Partition Coefficient.
Remarks	Calculated based on the individual solubilities in water and n-octanol.
Test Facility	RCC (2000a)

Adsorption/Desorption $\log K_{oc} < 1.25$

Method	OECD TG 121 Adsorption HPLC Screening Method. EC Directive 2001/59/EEC Method C.19 Estimation of the Adsorption Coefficient (K_{OC}).
Remarks	HPLC method. The notified chemical eluted before the reference chemical thiourea.
Test Facility	Wildlife International (2008b)

Autoignition Temperature 290 °C

Method	ASTM method E 659-78 (reapproved in 2000) *Standard Test method for Autoignition temperature of Liquid Chemicals*
Remarks	Measurement was made on 50 mg sample size rather than 100 mg prescribed in the test method due to the nature of the test substance (an extremely viscous, almost gelatinous liquid). There was a sharp delineation for autoignition temperature with autoignition observed at 290 °C and no visible ignition observed at 287 °C. Cool-flame autoignition was not observed.
Test Facility	Case Consulting Laboratories (2008)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Troysol LAC (containing notified chemical at ~50% concentration)
METHOD	Similar to OECD TG 401 Acute Oral Toxicity – Limit Test.
Species/Strain	Rat/Sprague-Dawley
Vehicle	None
Remarks - Method	No significant deviations from protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	10/sex	5000	0

LD50	> 5000 mg/kg bw
Signs of Toxicity	Within 30 minutes of administration all animals developed clinical signs including hunched posture, piloerection, irregular respiration, hypoactivity, diarrhoea and ano-genital staining which were cleared by day 4.
Effects in Organs	There were no remarkable necropsy findings.
Remarks - Results	None
CONCLUSION	The test substance is of low toxicity *via* the oral route.
TEST FACILITY	Products Safety Labs (1995a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Troysol LAC (containing notified chemical at ~50% concentration)
METHOD	Similar to OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Sprague-Dawley
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	No significant deviations from protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5/sex	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Dermal irritation was observed at the application site of all animals.
Signs of Toxicity - Systemic	There were no deaths or test-substance related clinical signs.
Effects in Organs	There were no remarkable necropsy findings.
Remarks - Results	None
CONCLUSION	The test substance is of low toxicity *via* the dermal route.
TEST FACILITY	Products Safety Labs (1995b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Troysol LAC (containing notified chemical at ~50% concentration)

METHOD	Similar to OECD TG 403 Acute Inhalation Toxicity – Limit Test.
Species/Strain	Rat/Sprague-Dawley
Vehicle	None
Method of Exposure	Whole-body exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	MMAD = 2.15 µm
Remarks - Method	No deviation from protocol

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
1	5/sex	2.00	2.12	1/10

LC50	> 2.12 mg/L/4 hours
Signs of Toxicity	One animal died on day 11 of the study. During exposure clinical signs of toxicity included ocular and nasal discharge, facial staining, irregular respiration, dyspnea, gasping, hunched posture and hypoactivity which persisted in all animals upon chamber removal. Animals also developed piloerection, rales, emaciation, ano-genital staining and an unthrifty appearance. Transient signs of abnormal respiration (rales and irregular respiration) were noted in several animals for an extended period of time. Transient bodyweight losses were also noted.
Effects in Organs	There were no remarkable necropsy findings in survivors. Gross necropsy of the descendent showed discoloration of the lungs, GI-tract and liver, gaseous distention of the GI-tract, oedema of the lungs and retraction of the testes into the abdominal cavity. Gross necroscopy at terminal sacrifice of the surviving animals revealed red lung discolouration which is consistent with euthanasia by CO_2.
Remarks - Results	Histological evaluation of the lung tissues of the survivors revealed pulmonary congestion: the presence of alveolar macrophages; the accumulation of mucus in the bronchi and bronchioles; and chronic interstitial inflammation. Mucus accumulation and the presence of alveolar macrophages may represent a pulmonary response to a very mild irritant. The possibility of a very mild irritant causing pulmonary congestion along with interstitial chronic inflammation cannot be ruled out.

CONCLUSION	The test substance is of low toxicity *via* inhalation.
TEST FACILITY	Products Safety Labs (1995c)

B.4. Irritation – skin

Test Substance	Troysol LAC (containing notified chemical at ~50% concentration)
Method	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	Rabbit/New Zealand White
Vehicle	6 (3M, 3F)
Observation Period	None
Type of Dressing	14 days
Remarks - Method	No significant deviations from OECD guideline

Results

Lesion	*Mean Score* Animal No.*						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Erythema/Eschar	2.7	2	2	2.3	2.3	2	3	14 days	1
Oedema	3	3	2.7	2.7	2	2.3	3	< 14 days	0

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

Remarks - Results: Well-defined erythema to moderate erythema and oedema were seen in all animals in all animals at 60 minutes. Desquamation appeared in all animals at 7 days and persisted to day 14. Erythema, oedema and desquamation resolved completely in all animals except one by day 14. Barely perceptible erythema remained in one animal. There were no deaths or test substance-related clinical signs or remarkable body weight changes during the study period.

Conclusion: The notified chemical is irritating to the skin.

Test Facility: Products Safety Labs (1995d)

B.5. Irritation – eye

TEST SUBSTANCE: Troysol LAC (containing notified chemical at ~50% concentration)

METHOD: Similar to OECD TG 405 Acute Eye Irritation/Corrosion.
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 9 (5 M, 4 F)
- Observation Period: 21 days
- Remarks - Method: The test substance was instilled into one eye of nine healthy rabbits. The other eye remained untreated with the test substance and served as a control. The treated eyes of three rabbits were washed after instillation of the test substance. The eyes of the remaining six rabbits were not washed. A fluorescein dye (2% ophthalmic fluorescein sodium solution) was used to facilitate corneal observations.

RESULTS

Washed

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.7	0.7	2.3	3	< 7 days	0
Conjunctiva: chemosis	2	1	2.7	3	< 7 days	0
Conjunctiva: discharge	1.3	0.3	2.3	3	< 7 days	0
Corneal opacity	0.7	0	1	1	< 4 days	0
Iridial inflammation	0	0	0.7	1	< 3 days	0

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

Unwashed

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Conjunctiva: redness	3	3	2.7	2.7	3	2.3	3	< 21 days	0
Conjunctiva: chemosis	3	3	3	2.7	3	2.3	4	21 days	2
Conjunctiva: discharge	1.7	2.7	2.3	2	2.3	2	3	< 21 days	0
Corneal opacity	1	1	1	1	1	1	2	21 days	2
Iridial inflammation	0.3	0	1	0	1	1	1	< 17 days	0

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

Remarks - Results: All washed eyes exhibited conjunctival irritation and iridial inflammation

and corneal opacity were noted in one and two animals, respectively. The incidence and severity of irritation decreased with time with all animals free of irritation by day 7.

All unwashed eyes exhibited conjunctival irritation, corneal opacity and iridial inflammation. The overall incidence and severity of irritation decreased with time, however conjunctival irritation and corneal opacity persisted in 2 animals through day 21.

CONCLUSION The test substance is severely irritating to the eye.

TEST FACILITY Products Safety Labs (1995e)

B.6. Skin sensitisation

TEST SUBSTANCE Troysol LAC (containing notified chemical at ~50% concentration)

METHOD Similar to OECD TG 406 Skin Sensitisation – Buehler method.

Species/Strain Guinea pig/Hartley albino

PRELIMINARY STUDY Maximum Non-irritating Concentration:
Topical: 20% w/w

MAIN STUDY

Number of Animals Test Group: 20 (11 M, 9 F) Control Group: 10 (3 M, 7 F)

Positive control 0.08% 1-Chloro-2,4-dinitrobenzene (DNCB) in aqueous ethanol and 0.04% DNCB in acetone were used in the induction and challenge phase of the study, respectively.
Test group: 20 (11M, 9F) Control Group: 10 (3 M, 7 F)

INDUCTION PHASE Induction Concentration:
Topical: 75% (w/w) for induction 1 & 2, 50% (w/w) for the remaining inductions.

Signs of Irritation Very faint to severe erythema (0.5-3) was noted at all test sites during the induction phase. Irritation was most severe following the 2nd, 3rd and 5th inductions. Eschar and/or desquamation were also evident at several sites following these doses. A reduction in the severity of irritation was noted shortly after the induction concentration was reduced (75% to 50%) or after the dose sites were relocated to an adjacent area.

CHALLENGE PHASE

1st challenge topical: 20% (w/w)

2nd challenge topical: 20% (w/w)

Remarks - Method No significant deviation from the protocol

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	20% (w/w)	1	0	0	0
Control Group	20% (w/w)	0	0	0	0

Remarks - Results No signs of ill health or toxicity were observed. Very faint erythema was observed in both test and control animals during challenge, but this did not interfere with scoring. Faint erythema was noted in one animal after the challenge dose which turned into very faint erythema at 48 hours. Due to the presence of this faint erythema in one animal, a rechallenge was conducted. Very faint erythema was observed in 5 animals after rechallenge which was cleared by 48 hours.

The positive control study validated the test system used in the study.

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the

test substance under the conditions of the test.

TEST FACILITY Products Safety Labs (1995f)

B.7. Repeat dose toxicity (Oral)

TEST SUBSTANCE Notified chemical (98.3%)

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain	Rat/HanRCC:WIST(SPF)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	PEG 300
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	5/sex	0	0
low dose	5/sex	100	0
mid dose	5/sex	300	0
high dose	5/sex	1000	0

Mortality and Time to Death
No mortality was observed during the treatment period.

Clinical Observations
During daily observations, soft faeces were noted in all animals at all dose levels. This finding was considered by the study authors to be likely due to the vehicle rather than the test substance. A test substance related reduction in mean absolute body weight and mean body weight gain were noted in males treated with 1000 mg/kg bw/day from day 8 onwards.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Several changes were noted in clinical biochemistry parameters in males of mid dose animals and both sexes of high dose animals. In high dose animals changes included significant elevations in leucocytes, aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, creatinine kinase, creatinine, phosphorus, and potassium levels. Alanine aminotransferase and potassium levels were also significantly elevated in males of mid dose animals. Protein was significantly reduced in both sexes in high dose animals with a concomitant increase in albumin/globulin ratios (not significant for females).

Effects in Organs
Crateriform retractions were reported in high dose animals (both sexes). Test substance related liver enlargement was observed in both mid dose and high dose animals (males only). Both absolute and relative weights were increased significantly in the heart, liver, thymus, adrenal and spleen of both sexes in high dose animals. Similar changes in the liver was also noted in males of mid dose animals. Hepatocellular hypertrophy was noted in both sexes of high dose animals and males only of mid dose animals. In the stomach squamous cell hyperplasia and hyperkeratosis, parakeratosis, submucosal inflammation, submucosal edema and pustules were observed in both sexes of high dose animals, with forestomach ulceration and erosion in females only. Squamous cell hyperplasia and hyperkeratosis was also observed in females in mid dose animals. Thymus atrophy was noted with increased incidence in both sexes of high dose animals.

Remarks – Results
The microscopical changes in the stomachs of the rats in both mid and high dose animals are considered adverse by the study authors. These effects are consistent with the irritant nature of the notified chemical.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 100 mg/kg bw/day in this study, based on effects in the stomach at 300 mg/kg bw/day and 1000 mg/kg bw/day.

TEST FACILITY RCC (2008)

B.8. Repeat dose toxicity (Inhalation; 14 days-range finding)

TEST SUBSTANCE Troysol LAC (containing the notified chemical at ~50% concentration)

METHOD OECD TG 412 Repeated Dose Inhalation Toxicity: 14-day (range finding)

Species/Strain	Rat/Crl:CD BR
Route of Administration	Inhalation –snout only
Exposure Information	Total exposure days: 14 days Dose regimen: 5 days per week Duration of exposure (inhalation): 6 hours/day Post-exposure observation period: None
Vehicle	None
Physical Form	liquid aerosol
Particle Size	MMD 2.8, 2.7 and 2.3 μm for low, mid and high dose, respectively.
Remarks - Method	No deviation from the protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
control	5/sex	0	0	0
low dose	5/sex	0.030	0.036	0
mid dose	5/sex	0.095	0.106	0
high dose	5/sex	0.300	0.307	0

Mortality and Time to Death

None

Clinical Observations

During exposure, exaggerated respiration was observed for the high dose animals from Day 5 of the exposure period, which lasted till the end of the study. Immediately post-exposure, noisy and exaggerated respiration was observed in a small proportion of mid dose animals and a large proportion of high dose animals. Hunched posture was also observed in high dose animals.

Body weight gain and food consumption were reduced in all groups exposed to the test subsance, however, the effects were marked in males and minimal in females. Water consumption was reduced in mid and high dose animals for the first 4 days of treatment.

Effects in Organs

There was a dose related increase in lung weight in treated animals. Dark areas on the lungs were observed in 3/5 male rats were observed in the high dose group compared to 1/5 males in the control group.

In the larynx, epithelial hyperplasia and squamous metaplasia, epithelial keratinisation, inflammation in lamina propria, necrosis of ventral cartilage, and regenerative hyperplasia of epithelium over the ventral cartilage were reported in all treated animals.

In the lungs, fibrosis of alveolar ducts, bronchiolar goblet cell hyperplasia and mucous with inflammatory cells in the bronchiolar lumen are reported in all treated groups. Terminal bronchiolar epithelial hyperplasia was seen in mid and high dose animals. There was a marginally increased incidence and degree of alveolar macrophage aggregations in high dose males.

Epithelial hyperplasia at the point of bifurcation was reported in some males and females of the mid and high dose animals. There was also epithelial hyperplasia at the first bronchiolar bifurcation and goblet cell hyperplasia in the bronchus in occasional animals from the treated groups.

Remarks – Results
The clinical signs in this study were consistent with exposure to an irritant.

CONCLUSION
The Low Observed (Adverse) Effect Level (LO(A)EL) was established as 0.036 mg/L/6 h/day in this study, based on evidence of respiratory tract irritation at all doses.

TEST FACILITY Huntingdon Life Sciences Ltd (2000)

B.9. Repeat dose toxicity (Inhalation)

TEST SUBSTANCE Troysol LAC (containing the notified chemical at ~50% concentration)

METHOD OECD TG 412 Repeated Dose Inhalation Toxicity: 28-day
Species/Strain Rat/Crl:CD (SD)IGS BR
Route of Administration Inhalation – oro-nasal exposure
Exposure Information Total exposure days: 28 days
Dose regimen: 5 days per week
Duration of exposure (inhalation): 6 hours/day
Post-exposure observation period: 14 days
Vehicle None
Physical Form Aerosol
Particle Size MMD 1.4, 1.2 and 1.3 µm for low, mid and high dose, respectively.
Remarks - Method No deviation from the protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
control	10/sex	0	0	0
low dose	10/sex	0.005	0.0038	0
mid dose	10/sex	0.01	0.0113	0
high dose	10/sex	0.04	0.037	0
recovery*	5/sex	-	-	0

*5 males and 5 females from each group (control, low, mid and high dose)

Mortality and Time to Death
None

Clinical Observations
There were no treatment related clinical signs noted throughout the study.

Body weight gain and food consumption were reduced in high dose male animals over the 4 weeks of exposure, and remained lower during the 2-week recovery period.

Effects in Organs
Dose related changes were reported in the larynx, lungs and nasal turbinates.

Epithelial hyperplasia and necrosis of ventral cartilage in the larynx of all treated animals and squamous metaplasia in mid and high dose animals were reported following the 4 weeks exposure. Alveolar duct thickening was reported in the lungs of mid and high dose animals. Eosinophilic inclusions in the olfactory epithelium inside the nasal turbinates were reported in all groups with an increased incidence and/or severity in the treated and high dose animals.

At the end of the recovery period the changes in the lungs had resolved but treatment related changes remained in the larynx and nasal turbinates. Necrosis of the ventral cartilage was present in the larynx of all treated groups with ventral epithelial hyperplasia in some high dose animals and one mid dose female. Some animals from all groups showed eosinophilic inclusions in the olfactory epithelium in the nasal turbinates with an

increased incidence and/or severity in treated and higher dose animals.

Remarks – Results
The clinical signs in this study were consistent with exposure to an irritant atmosphere. However, the test substance may cause serious damage to respiratory tract by prolonged exposure through inhalation.

CONCLUSION
The Low Observed (Adverse) Effect Level (LO(A)EL) was established as 0.0038 mg/L/6 h/day in this study, based on evidence of respiratory tract irritation at all doses.

TEST FACILITY Huntingdon Life Sciences Ltd (2001)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (92.8%)

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1997).
EC Directive 92/69/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria (1992).
Plate incorporation (Test 1)/Pre incubation (Test 2)

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	S9 microsomal fraction from β-naphthoflavone/phenobarbital-induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 33-5000 µg/plate b) Without metabolic activation: 33-5000 µg/plate
Vehicle	DMSO
Remarks - Method	No deviation from standard protocol

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	> 5000	> 5000	Negative
Test 2		> 5000	> 5000	Negative
Present				
Test 1	> 5000	> 5000	> 5000	Negative
Test 2		> 5000	> 5000	Negative

Remarks - Results No significant increase in revertant colony numbers of any of the five tester strains was observed at any dose level in the presence or absence of metabolic activation.

Negative controls were within the historical limits and positive controls demonstrated the sensitivity of the study.

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

TEST FACILITY RCC (1998a)

B.11. Genotoxicity – in vitro Mammalian Chromosome Aberration Test

TEST SUBSTANCE Troysol LAC (containing the notified chemical at ~50% concentration)

METHOD OECD TG 473 In vitro Mammalian Cytogenetic Test (1998).
EC Directive 92/69/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test (1992).

Cell Type/Cell Line	Chinese Hamster V79 cells

Metabolic Activation System	S9 microsomal fraction from β-naphthoflavone/phenobarbital-induced rat liver
Vehicle	Ethylene glycol
Remarks - Method	No deviation from the protocol

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.625, 1.25, 2.5, 5, 10*, 20*, 40*	4 h	18 h
Test 2	5, 10, 20*, 40*, 80*, 160	18 h	28 h
Test 2	20, 40, 80*, 160	28 h	28 h
Present			
Test 1	0.625, 1.25, 2.5, 5, 10*, 20*, 40*	4 h	18 h
Test 2	5, 10, 20*, 40*, 80*, 160	4 h	28 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 39.06	≥ 20	> 40	Negative
Test 2		≥ 10	> 80	Negative
Test 2		> 80	> 80	Negative
Present				
Test 1	≥ 39.06	≥ 40	> 40	Negative
Test 2		≥ 80	> 80	Negative

Remarks - Results	There were no statistically significant increases in the frequency of cells with chromosomal aberrations in either test, with or without metabolic activation. The negative controls were within the historical range and the positive controls demonstrated the sensitivity of the test.
CONCLUSION	The test substance was not clastogenic to Chinese Hamster V79 cells treated in vitro under the conditions of the test.
TEST FACILITY	RCC(1999a)

B.12. Genotoxicity – in vitro Mammalian Cell Gene Mutation Test

TEST SUBSTANCE	Notified chemical (92.8%)
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test (1997). EC Directive 87/302/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.
Cell Type/Cell Line	Mouse lymphoma L5178Y
Metabolic Activation System	S9 microsomal fraction from β-naphthoflavone/phenobarbital-induced rat liver
Vehicle	Ethylene glycol
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	3.8, 7.5*, 15*, 30*, 40*, 60*	4 h	72 h	10-15 days
Test 2	3.8, 7.5*, 15*, 30*, 40*, 60*	24 h	72 h	10-15 days
Present				

Test 1	3.8, 7.5*, 15*, 30*, 40*, 60*	4 h	72 h	10-15 days
Test 2	70*, 80*	4 h	72 h	10-15 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	≥ 39.1	60	> 60	Negative
Test 2	> 40	> 60	> 60	Negative
Present				
Test 1	≥ 78.2	> 60	> 60	Negative
Test 2	> 40	≥ 70	> 80	Negative

Remarks - Results

No significant increases in mutation frequency were observed in the absence or presence of metabolic activation.

The results from the negative and positive controls demonstrated the sensitivity of the test.

CONCLUSION

The test substance was not clastogenic to mouse lymphoma L5178Y cells treated *in vitro* under the conditions of the test.

TEST FACILITY

RCC(1998b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Troysol LAC (containing the notified chemical at ~50% concentration)
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge from a domestic wastewater treatment plant (Füllinsdorf, Switzerland).
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Oxygen Demand (ThOD)
Remarks - Method	No significant deviation in protocol.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
7	33-37	7	83
14	41-43	14	92
21	45-48	21	93
28	49-53	28	95

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium benzoate (83%), surpassed the threshold level of 60% by 7 days. Therefore, the tests indicate the suitability of the inoculums. The percentage degradation of the toxicity control (69-71%) surpassed the threshold level of 25% by 14 days, showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days was 49-53%. Therefore, the test substance cannot be classified as readily biodegradable according to the OECD (301 F) guideline.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	RCC (1999b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Troysol LAC (containing the notified chemical at ~50% concentration)
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static.
Species	*Brachydanio rerio* (zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS
Remarks – Method	No significant deviation in protocol.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
0.45	Not determined	7	0	0	0	0	0
1	Not determined	7	0	0	0	0	0
2.2	Not determined	7	0	0	0	0	0
4.5	0.84 (a.i.)	7	0	0	0	0	0
10	1.8 (a.i.)	7	14.3	100	100	100	100

LL50	6.7 mg/L (WAF; 95% CL 4.5-10 mg/L) at 96 hours.
NOEL	4.5 mg/L (WAF) at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. The test solutions were not renewed during the 96 h test period. The actual concentrations of the test substance were measured at 0 and 96 hours during the 96 h test period. All fish were dead at 24 h at the 10 mg/L loading rate. The results were based on measured concentrations. Based on the measured concentrations of the active ingredient in the test substance (~ 18%), the actual toxicity of the notified chemical is therefore expected to be ~ 5.5 times greater than the reported LL50 concentration (~ 1.2 mg/L active ingredient).
CONCLUSION	Under the study conditions, the notified chemical is considered to be toxic to zebra fish.
TEST FACILITY	RCC (2000b)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Continuous Flow-through.
Species	*Pimephales promelas* (Fathead minnow)
Exposure Period	96 hours
Auxiliary Solvent	Dimethyl formamide
Water Hardness	144 mg $CaCO_3$/L
Analytical Monitoring	LC-MS/MS
Remarks – Method	The definitive test was conducted at nominal concentrations of 0.013, 0.025, 0.05, 0.1, and 0.2 mg/L (active ingredient, a.i.) of the notified chemical (measured concentrations of 0.01, 0.018, 0.04, 0.08, and 0.161 mg/L (a.i.) of the notified chemical, respectively). No significant deviation in protocol.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	20	0	0	0	0	0
Solvent control	Solvent control	20	0	0	0	0	0
0.2	0.161	20	0	0	0	0	0

LL50	> 0.161 mg/L (a.i.; WAF) at 96 hours.
NOEL	0.161 mg/L (a.i.; WAF) at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. No abnormalities in behaviour or appearance were observed. The results were based on measured concentrations.
CONCLUSION	Under the study conditions, the notified chemical is not harmful to the fathead minnow up to the limit of its water solubility.
TEST FACILITY	Wildlife International (2008c)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Troysol LAC (containing the notified chemical at ~50% concentration)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Test condition not reported.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS
Remarks - Method	No significant deviation in protocol.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
1	Not determined	20	0	0
3.2	Not determined	20	0	0
10	Not determined	20	0	0
32	0.55 (a.i.)	20	0	0
100	0.67 (a.i.)	20	0	15

EL50	> 0.67 mg/L (a.i.; WAF) at 48 hours
NOEL	0.67 mg/L (a.i.; WAF) at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The renewal of test solutions was not reported. The actual concentrations of the test substance were measured at 0 and 48 hours within the 48 h test period. The results were based on measured concentrations.
CONCLUSION	Under the study conditions, the notified chemical is not harmful to daphnids up to the limit of its water solubility.
TEST FACILITY	RCC (2000c)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Troysol LAC (containing the notified chemical at ~50% concentration)
METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
Species	*Scenedesmus subspicatus* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 0.31-100 mg/L Actual: 0.145-92.1 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	HPLC-MS
Remarks - Method	No significant deviation in protocol.

RESULTS

Biomass		*Growth*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
19 (95% CL 9.2-42.8)	3.1	58.7 (95% CL 45.1-81.8)	3.1

Remarks - Results	All validity criteria for the test were satisfied. The actual concentrations of the test substance were measured at 0 and 72 hours within the 72 h test period. No effects were observed. The results were based on measured concentrations.
CONCLUSION	Under the study conditions, the notified chemical is considered to be harmful to *Scenedesmus subspicatus*.
TEST FACILITY	RCC (2000d)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test. EC Directive 92/69/EEC Method C.3 Algal Inhibition Test.
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.013-0.2 mg/L (active ingredient) Actual: 0.01-0.196 mg/L (active ingredient)
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	LC-MS/MS
Remarks - Method	The definitive test was conducted at nominal concentrations of 0.013, 0.025, 0.05, 0.1, and 0.2 mg/L (active ingredient, a.i.) of the notified chemical (measured concentrations of 0.011, 0.024, 0.053, 0.083, and 0.196 mg/L (a.i.) of the notified chemical, respectively). No significant deviation in protocol.

RESULTS

Biomass		*Growth*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 0.196 (a.i.)	0.196 (a.i.)	> 0.196 (a.i.)	0.196 (a.i.)

Remarks - Results	All validity criteria for the test were satisfied. The actual concentrations of

	the test substance were measured at 0 and 72 hours within the 72 h test period. No effects were observed. The results were based on measured concentrations.
CONCLUSION	Under the study conditions, the notified chemical is not harmful to *Desmodesmus subspicatus* up to the limit of its water solubility.
TEST FACILITY	Wildlife International (2008d)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Troysol LAC (containing the notified chemical at ~50% concentration)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10-1000 mg/L Actual: Not determined
Remarks – Method	The test was conducted at nominal concentrations of 10, 32, 100, 320, and 1000 mg/L of the notified chemical. No significant deviation in protocol.
RESULTS	
IC50	> 1000 mg/L at 3 hours
NOEC	1000 mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. The respiration inhibition rate was 15.5% at 1000 mg/L, and was determined to be within the normal variability range. Consequently, the IC50 was determined to be > 1000 mg/L, the highest concentration in the study. The NOEC was determined to be 1000 mg/L.
CONCLUSION	Under the study conditions, the notified chemical is no inhibitory to microbial activity.
TEST FACILITY	RCC (1999c)

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