File No: STD/1418

May 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Rewomid SPA

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

For the purposes of subsection 78(1) of the Act, this 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 SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1418	Salkat Australia Pty Ltd	Rewomid SPA	Yes	< 20 tonnes per annum	Component of personal care products

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the data provided, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)], with the following risk phrase:

R38 Irritating to skin

and

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

	Hazard category	*Hazard statement*
Skin Corrosion/Irritation	Category 2	Causes skin irritation
Aquatic Environment	Acute Category 2 Chronic Category 2	Toxic to aquatic life Toxic to aquatic life with long lasting effects

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

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia, should consider the following health hazard classification for the notified chemical:
  - Xi: R38 Irritating to skin

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc. ≥ 20%: Xi; R38

- Based on ecotoxicity data, the notifier should consider their obligations under the Australian Dangerous Goods Code.

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:
  - Enclosed, automated processes, where possible

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

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  – Coveralls, impervious gloves, 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 chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  – the concentration of the chemical exceeds or is intended to exceed 3% in rinse-off personal care products

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 personal care products, or is likely to change 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 provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Salkat Australia Pty Ltd (ABN: 30 318 540 786)
262 Highett Road
Highett VIC 3190

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Hydrolysis as a function of pH.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Europe (2010)
Korea (2011)
China (2010)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Rewomid SPA

MOLECULAR WEIGHT
< 500 Da

3. COMPOSITION

DEGREE OF PURITY > 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Slightly yellow, highly viscous liquid

Property	Value	Data Source/Justification
Freezing Point	8°C	Measured
Boiling Point	Decomposed at > 150°C	Measured
Density	895 kg/m^3 at 50°C	Measured
Vapour Pressure	4.13 x 10^{-8} kPa at 25°C	Measured
Water Solubility	~8.5 × 10^{-3} g/L	Measured (Critical micelle concentration)
Hydrolysis as a Function of pH	Not determined	Not required as the notified chemical is readily biodegradable.
Partition Coefficient	Log Pow = 3.3 to 7.0 at 20°C	Measured

Property	Value	Data Source/Justification
(n-octanol/water)		
Adsorption/Desorption	Not determined	Expected to adsorb to organic carbon, soil and sediment
Dissociation Constant	Not determined	Contains no dissociable functionality
Flash Point	178°C at 101.3 kPa	Measured
Flammability	Not expected to be flammable	Based on the flash point, not classified as flammable (NTC, 2007)
Autoignition Temperature	355°C	Measured
Explosive Properties	Not expected to be explosive	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Not expected to oxidise	Contains no functional groups that would imply oxidative properties.

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported neat (at > 90% purity) as a highly viscous liquid.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 5	< 5	< 15	< 20	< 20

PORT OF ENTRY
Sydney and Melbourne

TRANSPORTATION AND PACKAGING
The neat notified chemical (purity > 90%) will be imported in 180 kg steel drums and transported by road to the customer's site. The finished products containing the notified chemical at concentrations of 1- 3% will be packaged in various pack sizes up to 1 L.

USE
Component of rinse-off personal care products, such as shampoos, foam baths, shower gels and skin cleansers, at concentrations of 1-3%.

OPERATION DESCRIPTION
Formulation of the finished products from the notified chemical will be highly automated and occur in a fully enclosed environment, followed by automated filling of the reformulated products into containers of various sizes.

The finished products containing the notified chemical in concentrations of 1 – 3% may be used by consumers and professionals such as hairdressers. Given the nature of the products, these will be applied by hand.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

EXPOSURE DETAILS
Transport and storage workers may come into contact with the neat notified chemical (purity > 90%) only in the event of accidental rupture of containers.

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical may occur at concentrations of > 90% during transfer of the notified chemical to the mixing vessels and at concentrations of 1-3% during blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses and impervious gloves.

Exposure to the notified chemical at concentrations up to 3% in end-use products may occur in professions where the services provided involve the application of rinse-off personal care products to clients (e.g. hair dressers). Such professionals may use some personal protective equipment to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using rinse-off personal care products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical (at 1-3% concentration) through the use of the rinse-off personal care products. The principal route of exposure will be dermal. Accidental ocular exposure is also possible.

Data on typical use patterns of rinse-off personal care product categories in which the notified chemical may be used are shown in the following tables (SCCS, 2010). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, the default dermal absorption of 100% was assumed for calculation purposes (European Commission, 2003). An adult bodyweight of 60 kg has been used for calculation purposes.

- Cosmetic products:

Product type	Estimated Daily Amount Applied (A) (mg)	Concentration (C) (%)	Retention Factor (RF) (unitless)	Daily systemic exposure (D) (mg/kg)
Shampoo	10460	3	0.01	0.0523
Shower gel	18670	3	0.01	0.0933
Hand wash soap	20000	3	0.01	0.1000
Hair conditioner	3920	3	0.01	0.0196
Total				**0.265**

D* = A × C × RF / BW
BW = body weight (kg)
*Calculations assume 100% dermal absorption

As a conservative assumption, the exposure estimate from use of the notified chemical in rinse-off products such as hand soaps and hair conditioners was undertaken to cover products of limited expected exposure such as foam baths and skin cleansers.

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 that contain the notified chemical. This would result in a combined internal dose of 0.265 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 > 2006 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	irritating
Rabbit, eye irritation	non-irritating
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity 28 days.	NOAEL = 200 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non-mutagenic
Genotoxicity – *in vitro* Chromosome Aberration in Chinese Hamster V79 cells	genotoxic
Genotoxicity – *in vivo* Mammalian Erythrocyte Micronucleus test in mouse	non genotoxic

Toxicokinetics.
Based on the partition coefficient (log P_{ow} = 3.3 to 7.0 at 20ºC) and the low molecular weight (< 500 Da) of the notified chemical passive diffusion across the gastrointestinal (GI) tract is expected to occur. This is supported by the systemic toxicity observed in the 28-day repeated dose oral study in rats where effects in the liver were noted. Dermal absorption is also expected to occur but may be limited by the low water solubility (8.5 mg/L) and relatively high partition coefficient. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.
The notified chemical has low acute oral (LD50 > 2006 mg/kg bw) and dermal toxicity (LD50 > 2000 mg/kg bw) in rats. There is no data available on the acute inhalational toxicity of the notified chemical.

Irritation and Sensitisation.
The notified chemical was determined to be irritating to the skin of rabbits, with significant dermal reactions observed in three animals for at least 48 hours. No oedema was noted. On Day 5, the erythema started to subside and disappeared by Day 8. Skin dryness persisted up to the end of the observation period (i.e. Day 8).

The notified chemical is non-irritating to the eyes of rabbits and is not a skin sensitiser in guinea pigs.

Repeated Dose Toxicity.
In a 28-day repeat dose gavage study in rats the NOAEL was established as 200 mg/kg bw/day based on changes in the liver and clinical chemistry parameters in both sexes in the high dose group (1000 mg/kg bw/day).

Mutagenicity.
The notified chemical was found to be non-mutagenic in a bacterial reverse mutation assay but gave a positive response in an *in vitro* mammalian chromosome aberration test with metabolic activation at the highest dose tested only. However, it was non-genotoxic in an *in vivo* mammalian micronucleus test. The bone marrow cells are likely to have been exposed to the notified chemical given that weak cytotoxicity was observed at the highest dose tested. In addition, effects on the liver and clinical chemistry parameters observed in the 28-day repeated dose toxicity study provides further support that the notified chemical is systemically absorbed and reaches the general circulation. Therefore, based on the results from the *in vivo* micronucleus assay, the notified chemical is not likely to be an *in vivo* genotoxicant.

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

R38 Irritating to skin.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical is irritating to the skin but is not irritating to the eyes. It has low acute oral and dermal toxicity, is not a skin sensitiser and is not likely to be mutagenic.

Reformulation

Reformulation workers are most at risk of irritation effects when handling the neat notified chemical (purity > 90%) as introduced. However, given these workers are expected to wear PPE and the reformulation process is largely enclosed and automated, exposure to the notified chemical, and hence the risk of irritation effects, should be minimised. Therefore provided the stated control measures are in place, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use

Workers involved in professions where the services provided involve the application of rinse-off personal care products containing the notified chemical (≤ 3%) to clients (e.g. hairdressers and beauty salon workers), may be exposed to the notified chemical. The risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using rinse-off personal care products containing the notified chemical (for details of the public health risk assessment, see Section 6.3.2.).

6.3.2. Public Health

Repeated dose toxicity

Members of the public may experience repeated exposure to the notified chemical through the use of rinse-off personal care products containing the notified chemical at the concentration of 1 - 3%.

The repeated dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products of 0.265 mg/kg bw/day and the NOAEL of 200 mg/kg bw/day (see Section 6.1.2). A MoE value greater than 100 is considered acceptable to account for intra- and inter-species differences. Based on the estimated exposure of 0.265 mg/kg bw/day and a NOAEL of 200 mg/kg bw/day, a MOE of 755 is estimated.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 3% concentration in rinse-off personal care products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

During formulation and mixing, release of notified chemical to environment is expected to be negligible as these processes occur in a closed system in industrial settings. 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 expected to be released to the sewer in domestic situations across Australia as a result of its use in personal care products, such as shampoos and body washes, which are either washed off the hair and skin of consumers, or disposed of following cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in end-use containers are expected to share the fate of the container and be disposed of to landfill, or to be washed to sewer when containers are rinsed before recycling.

7.1.2. Environmental Fate

The notified chemical is expected to be largely degraded during sewage treatment as it is readily biodegradable. A small proportion may be discharged to receiving waters in treated effluent where the notified chemical is expected to disperse and degrade. Under Australian PBT criteria, it has low potential to bioaccumulate based on its bioconcentration factor (BCF = 874 ± 340). A proportion of the notified chemical may be applied to land when effluent is used for irrigation, and residues in empty containers are expected to be disposed of to landfill. The notified chemical in landfill, soil and sludge is expected to be immobile and degrade through biotic or abiotic processes to form water and oxides of carbon and nitrogen. For the details of the environmental fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

Since most of the notified chemical will be washed into the sewer, under a worst case scenario, with no removal of the notified chemical in the sewage treatment plant (STP), the resultant Predicted Environmental Concentration (PEC) in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	20,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	20,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	54.79	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.61	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	12.12	µg/L
PEC - Oce□n:	1.2□	µg/L

The notified chemical that is not removed from waste water during STP processes may be released to the environment in STP 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 12.11 µg/L may potentially result in a soil concentration of approximately 80.77 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 403.9 µg/kg and 807.7 µg/kg, respectively. However, due to the ready biodegradability of the notified chemical, these calculated values represent theoretical maximum concentrations only.

7.2. Environmental Effects Assessment

The results from an ecotoxicological investigation 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*
Acute Toxicity		
Fish	96 h LC50 = 75 mg/L	Not expected to be harmful to fish up to limit of solubility
Daphnia	48 h EC50 = 1.8 mg/L	Toxic to aquatic invertebrates
Algae	72 h ErC50 = 126 mg/L	Not expected to be harmful to algae up to limit of solubility

Classification should be based only on toxic responses observed in the soluble range, and the critical micelle concentration is considered as the appropriate parameter for solubility to be used for toxicity classification for surface active substances. Toxicity to Daphnia with 48 h EC50 of 1.8 mg/L is below the solubility limit (CMC = 8.5 mg/L). Therefore, this toxicity value is considered appropriate for the GHS classification. The notified chemical is classified as 'Acute Category 2: toxic to aquatic life' under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009). Further, on the basis of the GHS bioaccumulation threshold of BCF > 500 and acute toxicity, the notified polymer is classified as 'Chronic Category 2: toxic to aquatic life with long lasting effects'.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from acute daphnia toxicity of the notified chemical. An assessment factor of 100 was used as acute toxicity endpoints are available for the effects of the notified chemical on aquatic species from three trophic levels.

Describe how the PNEC was calculated, if possible.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment	
EC50 (*Daphnia magna*)	1.80 mg/L
Assessment Factor	100
PNEC:	18.0 µg/L
EC50 (*Daphnia magna*)	1.80 mg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC, the following Risk Quotient has been calculated.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	12.12	18	**0.673**
Q - Ocean	1.21	18	**0.067**

The risk quotient for discharge of effluents containing the notified chemical to the aquatic environment, assuming a worst case with no removal during sewage treatment plant (STP) processes, indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual use quantity. The notified chemical has a low potential for bioaccumulation and is unlikely to be persistent in the environment. On the basis of the PEC/PNEC ratio, maximum annual use volume and assessed use pattern in personal care products, such as shampoos and body washes, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 8ºC

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks The test item was cooled slowly in a glass tube immersed in a cooling bath, while the consistency of the sample was judged visually.
Test Facility RCC (1999a)

Boiling Point Decomposed without boiling at > 150ºC

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Using the capillary test, degradation was observed to occur at or above 150 ºC where gaseous degradation products are formed.
Test Facility RCC (1999b)

Density 895 kg/m^3 at 50ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Oscillating Densitometer Method
Test Facility RCC (1999c)

Vapour Pressure 4.13 x 10^{-8} kPa at 25ºC

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Gas Saturation Method. The determination of the gas saturation method was performed at 70ºC and 80ºC, as the evaporation of test item could not be conducted at lower temperatures. The vapour pressure at 25 ºC was extrapolated from the measured values.
Test Facility RCC (1999d)

Water Solubility 8.5 × 10^{-3} g/L

Method OECD TG 105 Water Solubility.
EC Directive 92/69, A.6 Water Solubility.
Remarks In a shake flask method, about 160 mg of the notified chemical was weighed in 6 flasks and 50 mL of water were added to each flask in accordance with the above guidelines. The tightly closed flasks were shaken at 30 ºC for 24, 48 and 72 hours. The supernatant solution were centrifuged, filtrated and measured without dilution. The quantification was performed using HPLC method and water solubility was determined to be below the limit of quantification (<3.46 × 10^{-3} g/L). However, the critical micelle concentration (CMC) is considered more appropriate for the notified chemical with surface-active properties.

The notified chemical has surface activity properties with complex solubility behaviour due to aggregation. The critical micelle concentration (CMC) is therefore the appropriate parameter for a solubility value. In a preliminary study by inverse CMC measurement (plate method), the CMC of 8.5 × 10^{-3} g/L was calculated by plotting surface tension against concentration.

Test Facility RCC (1999e)

Partition Coefficient (n-octanol/water) log Pow = 3.3 to 7.0.at 20 ºC

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks The partition coefficient was estimated to be > 5.1 in a flask shaking preliminary test. The

	model calculation indicated a log Pow of 6.6. Therefore the HPLC method was used during the main study. The chromatography of the test substance resulted in several peaks reported to be due to the test substance consisting of a mixture of isomers and impurities. Therefore, the log Pow was derived from three main compounds in the chromatogram. The individual partition coefficients for the three main compounds were determined to be 3.3, 6.0 and 7.0.
Test Facility	RCC (1999f)

Flash Point 178ºC at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Pensky-Martens flash point apparatus
Test Facility	RCC (1999g)

Autoignition Temperature 355ºC

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Apparatus according to DIN 51794 with improved temperature measurement.
Test Facility	ISS (1999)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity.
Species/Strain	Rat/ Sprague-Dawley
Vehicle	None
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M	2006	0
2	5F	2006	0

LD50	> 2006 mg /kg bw
Signs of Toxicity	No clinical signs of toxicity were observed in any of the animals and there were no mortalities.
Effects in Organs	Nil in any animal
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Pharmakon (1993)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) Test.
Species/Strain	Rat/ HanIbm: WIST (SPF)
Vehicle	Polyethylene glycol PEG 300
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M	2000	0
2	5F	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No local signs of toxicity were observed.
Signs of Toxicity - Systemic	No systemic signs of toxicity were observed.
Effects in Organs	No macroscopic signs.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	RCC (1999h)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 84/449/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	None
Observation Period	8 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.7	2.0	2.0	2.0	7 days	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	Well defined erythema was observed for all 3 animals for at least 48 hours. On Day 5, the erythema starts to subside and disappears by Day 8. No oedema was observed. Skin dryness persists up to Day 8.
CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	CIT (1992a)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 84/449 Appendix V B.5.
Species/Strain	Rabbit/New Zealand White.
Number of Animals	3 males
Observation Period	72 hours
Remarks – Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0	0	2.0	< 24 hours	0
Conjunctiva: chemosis	0	0	0	1.0	< 24 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	Slight conjunctival reactions were observed in the 3 animals one hour after installation of the product. No irritation of the iris or corneal opacity was noted.
CONCLUSION	The notified chemical is non-irritating to the eye.
TEST FACILITY	CIT (1992b)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation - Maximization Test. EC Directive 96/54/EC B.6 Skin Sensitisation - Maximization Test.
Species/Strain	Guinea pig
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 1%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
INDUCTION PHASE	Induction Concentration: intradermal: 5% topical: 100%
Signs of Irritation	Slight erythema was observed for all treated animals.
CHALLENGE PHASE	
1st challenge	topical: 1%
Remarks-Method	No significant protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions After challenge*	
		24 h	*48 h*
Test Group	1%	0/9*	0/9
Control Group	0%	0/5	0/5

*One animal of the test group was found dead on test day 10. At necropsy, no findings were noted. The cause of death could not be established. The death was considered to be treatment-unrelated.

Remarks - Method	No skin irritation was observed in either the control group (bidistilled water) or test group (1% notified chemical in bidistilled water).
CONCLUSION	There was no evidence indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	RCC (1999i)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
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/HanIbm:WIST (SPF)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	PEG 300
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10/sex	0	0
low dose	5/sex	50	0
mid dose	5/sex	200	0
high dose	5/sex	1000	0

Mortality and Time to Death
There was no mortality in the test group during the course of the study.

Clinical Observations
There were not treatment related clinical signs of toxicity. However, a transient slight reduction of food consumption was noted in the mid and high dose groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were no significant treatment related changes in the haemological parameters.

Elevated levels of alanine aminotransferase, alkaline phosphatase, phosphorous, albumin levels, albumin/globulin ratios were observed in the high dose group only. These were considered to be metabolic changes caused by the introduction of a xenobiotic.

Effects in Organs
Gross Pathology:
The absolute and relative liver weights of male and female rats treated with high dose rate were markedly higher than those of the controls after 4 weeks. After the recovery period, the differences in liver weights were largely reversed.

The slightly lower thymus weights were observed in males only and were considered to be incidental.

Histopathology:
The changes observed to the livers of animals included hepatocellular hypertrophy at minor degrees and hepatocellular cytoplasmic eosinophilia in both sexes at the high dose group. These findings were considered to be caused by enzymatic changes and due to an adaptive response to a xenobiotic.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 200 mg/kg bw/day in this study, based on changes in the liver and clinical chemistry parameter in the high dose group.

TEST FACILITY RCC (1999j)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure/Pre incubation procedure
Species/Strain	*S. typhimurium*:, TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbitone/β-naphthoflavone
Concentration Range in Main Test	Test 1 (Plate incorporation procedure) a) With metabolic activation: 33, 100, 333, 1000, 2500 and 5000 µg/plate b) Without metabolic activation: 33, 100, 333, 1000, 2500 and 5000 µg/plate Test 2 (Pre-incubation procedure) a) With metabolic activation: 42, 125, 417, 1250, 2500 and 5000 µg/plate b) Without metabolic activation: 42, 125, 417, 1250, 2500

	and 5000 µg/plate
Vehicle	DMSO
Remarks - Method	No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	Negative
Test 2		5000	> 5000	Negative
Present				
Test 1	> 5000	> 5000	> 5000	Negative
Test 2		5000	> 5000	Negative

Remarks - Results	In Test 2, toxic effects were observed in strain TA 1537 at 5000 µg/plate with and without metabolic activation. In strain TA 100 slight toxicity was observed at 5000 µg/plate without metabolic activation. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the notified chemical at any dose level, neither in the presence nor absence of metabolic activation. All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	RCC (1999k)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Chinese Hamster
Cell Type/Cell Line	V79 cell line
Metabolic Activation System	S9 fraction from rat liver induced with phenobarbitone/β-naphthoflavone
Vehicle	DMSO
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	5.0*, 10.0*, 20.0*, 40.0*, 60.0, 80.0	4h	18h
Test 2	1.3, 2.5, 5.0*, 10.0*, 20.0*, 40.0	18h	18h
Test 3	5.0, 10.0, 20.0*, 40.0	28h	28h
Present			
Test 1	12.5*, 25.0*, 50.0*, 100.0, 200.0, 400.0	4h	18h
Test 2	12.5, 25.0*, 50.0*, 60.0*, 70.0, 80.0	4h	28h

*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	≥ 40.6[1]	≥ 60	> 80	Negative
Test 2	≥ 20.6[2]	≥ 40	> 40	Negative
Test 3		≥ 40	> 40	Negative
Present				
Test 1	≥ 40.6[1]	≥ 100	> 400	Positive
Test 2		≥ 70	> 80	Positive

[1]Harvest time 24h (concentrations 20.3-3400 µg/mL)
[2]24 h continuous exposure (concentrations 20.3-3400 µg/mL)

Remarks - Results

Clear toxic effects as evidenced by reduced mitotic indices were observed in the absence and the presence of S9 mix in both experiments at all preparation intervals treatment with the highest evaluated concentrations.

In the absence of S9 mix no significant increase was observed in the aberration rates at any of the experimental time points. In the presence of S9 mix the aberration rates (5.0% and 7.0%, respectively) were significantly increased after treatment with 50 µg/mL (Test 1) and with 60 µg/mL (Test 2) as compared to the corresponding controls (1.5% and 2.0%, respectively).

In all experiments, no biologically relevant increase was observed in the frequencies of polyploid metaphases.

CONCLUSION

The notified chemical was clastogenic to Chinese hamster V79 cell line treated in vitro under the conditions of the test.

TEST FACILITY

RCC (1999l)

B.9. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical.

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/NMRI
Route of Administration: Oral – gavage
Vehicle: Corn oil
Remarks - Method: No significant protocol deviations.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5/sex	-	24
II (low dose)	5/sex	200	24
III (mid dose)	5/sex	670	24
IV (high dose)	5/sex	2000	24
V (high dose)	5/sex	2000	48
VI (positive control, CP)	5/sex	40	24

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity: Weak cytotoxicity was observed at 2000 mg/kg bw/day with a preparation interval of 48 hours i.e. dose group V.

Genotoxic Effects: In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval after

	administration of the test item and with any dose level used.
Remarks - Results	The notified chemical did not induce micronuclei as determined by the micronucleus test in the bone marrow cells of the mouse.
CONCLUSION	The notified chemical was not clastogenic under the conditions of the micronucleus test.
TEST FACILITY	RCC (1999m)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified Chemical

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

Inoculum	Activated sludge from a domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	
Remarks - Method	The test was conducted for 28 days in accordance with the above guidelines. The test substance was added to a liquid medium inoculated with sewage microorganisms and aerated with CO_2-free air at 18.5 to 22.5 °C. CO_2 production was analysed. Degradation was calculated as a percentage of the theoretical CO_2 ("%TCO_2") that was produced from the organic matter of the test substance by complete combustion.

RESULTS

Test Solution 1		*Test Solution 2*	
Day	*% Degradation*	*Day*	*% Degradation*
9	49.4	9	50.6
13	58.3	13	58.9
28	69.0	28	70.5
29	74.2	29	75.2

Remarks - Results: After the test period of 28 days, mean degradation value of two parallel test solutions was 70%. The toxicity control was degraded 79% within 28 days. The control substance sodium benzoate was degraded 90% within 28 days. The threshold of readily biodegradability of ≥ 60% for the control substance was met within 6 days. All validity criteria for the test were satisfied.

CONCLUSION: The notified chemical is classified as readily biodegradable.

TEST FACILITY: Institut Fresenius (1998)

C.1.2. Bioaccumulation

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 305 Bioconcentration: Flow-through Fish Test.

Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	Exposure: 55 days Depuration: 49 days
Auxiliary Solvent	None
Concentration Range	Nominal: 2 and 20 µg/L
Analytical Monitoring	Thin Layer chromatography, Scintillation counters
Remarks - Method	Conducted in accordance with the above guidelines. Two dose levels were prepared from the stock solution of ^{14}C-labelled notified chemical by dilution in dimethylformamide (DMF) to a daily amount of 5 mg/25 mL (high dose of 20 µg/L) and 0.5 mg/25 mL (low dose of 2 µg/L). The fish were continuously exposed to the ^{14}C-labelled notified chemical at an average low dose concentration of 1.60 µg/L and an average high dose concentration of 13.7 µg/L for 55 days at 14 -17 °C, pH 6.9 - 8.0 and an

	oxygen concentration of 4.2 – 9.4 mg/L. During 49 days depuration (56 – 104 days) the respective values were 14 – 18 ºC, 7.1 – 7.8 and 6.6 – 8.7 mg/L. The fish lipid residue was determined after drying at 105 ºC and subsequent cooling in an exicator to constant weight.
Bioconcentration Factor	874 ± 340
Remarks - Results	Plateau levels were reached after 25 days (low dose) and 39 days (high dose), depuration occurred with a half-life of about 9 – 13 days. Based on the actual concentration of the parent item in exposure water and the plateau levels in fish, the parent value amounted to 589 – 600 for low dose and to 1023 – 1284 for the high dose. On average the BCF value for the notified chemical amounted to 874 ± 340. The uptake of the notified chemical in fish was partially reversible. The fat content in the fish at day 11 was 27.1 mg/g fish and 29.0 mg/g for low and high dose, respectively. At day 55, the lipid content was higher for both dose level and amounted to 41.7 mg/g and 48.3 mg/g for low and high dose, respectively. BCF values in fat were similar for both days. Validity criteria for the test were satisfied with no significant deviation from the guidelines.
CONCLUSION	Under Australian PBT criteria, the notified chemical has low potential for bioaccumulation with BCF of <2000.
TEST FACILITY	RCC (2003)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – semi-static Commission Directive 92/69/EEC, C.1 Acute Toxicity for Fish – semi static Test
Species	Rainbow trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC/MS
Remarks – Method	According to the above guidelines, the test was conducted at nominal concentrations of 0, 4.6, 10, 22, 46, 100 mg/L under semi-static conditions for a period of 96 h. Note that the dispersion of the notified chemical in the test water remains not stable over 48 hours, therefore a semi-static test was chosen with test medium renewal every day to keep the dispersion of notified chemical as stable as possible during 96 h. The controls were kept in dilution water. Seven fish per test solution were observed for symptoms of intoxication and mortality after 3, 24, 43, 72, and 96 hours. Test conditions were: water temperature of 14 - 15°C, pH 7.7 - 8.1, 8.8 - 9.7 mg O_2/L, 8 hours dark and 16 hours light period.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*

Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0		7	0	0	0	0	0
4.6	n.a	7	0	0	0	0	0
10	n.a	7	0	0	0	0	0
22	14	7	0	0	0	0	0
46	34	7	0	0	0	0	0
100	75	7	0	0	1	3	3

n.a = not applicable

LC50 = 75 mg/L at 96 hours

NOEC = 34 mg/L at 96 hours

Remarks – Results The LC50 value is not applicable as this value is above the solubility. The LC50 was calculated by linear extrapolation between the log-concentration and the percent mortality. Note that the notified chemical form aggregates or droplets at high concentration and results are therefore considered appropriate only up to its solubility. Validity criteria for the test were satisfied with no significant deviation from the guidelines. However, the study results are reliable with restrictions and accurately measured concentration up to the limit of water solubility is considered appropriate for toxicity. The use of solubility limit for toxicity will restrict the possibility of physical effects on fish due to the excess undissolved notified chemical at higher concentrations.

CONCLUSION The notified chemical is not expected to be harmful to fish up to its limit of solubility in water

TEST FACILITY RCC (1999n)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test (48-hour static test)
Commission Directive 92/69/EEC, Part C.2 Acute Toxicity for Daphnia - Immobilisation Test (48 hour static test)

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring HPLC and UV/Vis detection
Remarks - Method Test was conducted in accordance with above guidelines. Twenty daphnids in two groups (10 each) were tested in control and six test concentrations in a 100 mL glass beaker. The control group was kept in dilution water. The test was performed in a temperature-controlled room with a 16 h light to 8 h dark photoperiod. Oxygen concentrations in the test media and the control were at least 8.6 mg/L, the pH values ranged from pH 7.9 to 8.1. The 24-hour and 48-hour EC50 and the 95% confidence limits were calculated by moving Average Interpolation. The NOEC was determined directly from the raw data.

RESULTS

Concentration mg/L	*Number of D. magna*	*Number Immobilised*

Nominal	*Actual*		*24 h [acute] 14 d [chronic]*	*48 h [acute] 21 d [chronic]*
Control	n.a	20	0	0
0.10	n.a	20	0	0
0.32	0.18	20	0	0
1.0	0.40	20	2	4
3.2	2.4	20	5	13
10	6.6	20	6	13
32	17	20	17	20

n.a = not applicable

EC50 = 1.8 mg/L at 48 hours

NOEC = 0.18 mg/L at 48 hours

Remarks - Results

Validity criteria for the test were satisfied with no significant deviation from the guidelines. No significant immobility or mortality of the test organisms and other sign of intoxication were observed up to and including the concentration of 0.18 mg/L. At the concentration of 0.40 mg/L, 4 daphnids were immobile showing 20% immobility. The immobility rate increased from 65% at the test concentration of 2.4 and 6.6 mg/L to 100% at the test concentration of 17 mg/L. Note that the dispersion of the notified chemical proved to be not stable during the test period of 48 hours and the notified chemical was found to form droplets on the surface of the test medium adhering to the test vessels. This results in the decrease in nominal concentrations of the notified chemical in the test medium as confirmed by analytically determined concentrations at the start and end of incubation period. Therefore, the reported biological results are related to the total mean measured bioavailable notified chemical concentrations (calculated as the average over all measurements per test concentration). The results are considered appropriate as the mean measured concentrations for toxicity is below the solubility limit (CMC = 8.5 mg/L). Therefore, the 48 h EC50 of 1.8 mg/L within the range of solubility limit is considered appropriate for reliability of toxicity test.

CONCLUSION

The notified chemical is toxic to aquatic invertebrates up to its solubility limit.

TEST FACILITY

RCC (1999o)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 201 Alga, Growth Inhibition Test.
Commission Directive:92/69/EEC, C.3 Algal Inhibition Test.

Species *Scenedesmus subspicatus*
Exposure Period 72 hours
Concentration Range Nominal: 0.32 to 100 mg/L
Actual: 0.19 to 78 mg/L
Auxiliary Solvent None
Water Hardness 24 mg $CaCO_3$/L
Analytical Monitoring HPLC and UV/Vis detection, Coulter Counter
Remarks - Method Conducted in accordance with the above guideline at six nominal concentrations of 0.32, 1.0, 3.2, 10, 32, and 100 mg/L, and a control. The test design included three replicates per test concentration and six replicates in the control. The test medium of the highest test concentration of nominal 100 mg/L was prepared by dispersing 70 mg

notified chemical in 700 mL test water by ultrasonic treatment for 30 minutes and intense stirring for 30 minutes each at room temperature. Test media were prepared just before inoculation of the algae. The algal cell densities in the sample were determined twice by counting with an electronic particle counter (Coulter Counter). Test conditions were: 22°C, continuously illuminated at mean light intensity of about 8020 Lux, pH 7.9 – 8.7. The calculated mean biomass and the mean growth rate at the test concentrations were tested on significant differences to the control values by a Dunnet-test.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
2.6	0.19	126	0.19

Remarks - Results

The results are related to the total mean measured concentrations. These were 0.19 mg/L (0.32 mg/L nominal), 0.68 mg/L (1.0 mg/L nominal), 2.6 mg/L (3.2 mg/L nominal), 7.5 mg.L (10 mg/L nominal), 23 mg/L (32 mg/L nominal), and 78 mg/L (100 mg/L nominal).
The test media were visually clear at nominal concentration of 0.32 to 3.2 mg/L. At nominal concentration of 10 mg/L, a slight turbidity and at the nominal concentration of 32 and 100 mg/L a distinct turbidity was observed. The ErC50 value should be taken with caution, since the inhibition of growth rate up to the highest test concentration was lower than 50%. Note that the notified chemical form aggregates or droplets at high concentration and results are therefore considered appropriate only up to its solubility. However, results are not applicable as EbC50 value for biomass is not generally considered for toxicity classification whereas ErC50 value for growth rate is much greater than the solubility limit. Validity criteria were satisfied with no significant deviation from the test guidelines.

CONCLUSION

Considering the effect on growth rate, the notified chemical is not harmful to algae up to its solubility limit.

TEST FACILITY

RCC (1999p)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Commission Directive 87/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum: Activated sludge from domestics wastewater treatment plant
Exposure Period: 3 hours
Concentration Range: Nominal: 10 - 1000 mg/L

Remarks – Method

The activated sewage sludge was exposed to the notified chemical at different nominal concentrations (10, 32, 100, 320 and 1000 mg/L) for 3 hrs. Two controls (deionized water, wastewater and inoculum, without notified chemical) and the reference item 3,5-dichlorophenol (positive control) were also tested in parallel. Test conditions were: 20 ± 1°C, pH 7.1 - 7.9 and permanent aeration.

RESULTS

EC50	>1000 mg/L at 3 h
Remarks – Results	All validity criteria for the guideline were satisfied and no significant deviations from the guidelines were reported. Up to and including the concentration of nominal 1000 mg/L the notified chemical had no inhibitory effect on the respiration rate of activated sludge after the incubation period of three hours. Concentrations in excess of 1000 mg/L were not tested. The 3 hour EC50 of the reference item was calculated to be 7.0 mg/L. Results shows no bacterial toxicity of the notified chemical under test conditions.
CONCLUSION	The notified chemical is not expected to inhibit respiration of waste water microorganisms.
TEST FACILITY	RCC (1999q)

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