File No: STD/1613

July 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

4-Pyrimidinecarboxylic acid, 3,4,5,6-tetrahydro-2-methyl-, (4S)- (INCI Name: Ectoin)

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

This Public Report is available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1613	Merck Pty Ltd	4-Pyrimidinecarboxylic acid, 3,4,5,6-tetrahydro-2-methyl-, (4S)- (INCI Name: Ectoin)	No	≤ 10 tonnes per annum	Component of cosmetics

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Human health risk assessment
Under the conditions of the occupational settings described, the notified 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

CONTROL MEASURES

Occupational Health and Safety

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

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

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

- If products and mixtures containing the notified 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.

Public Health

- As the notified chemical may potentially also be present in products meeting the definition of a therapeutic good, this report will be referred to the Therapeutic Goods Administration for their consideration.

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(1) of the Act; if
  - the concentration in cosmetic products is intended to exceed 3%;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from component of cosmetics, 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.

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

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

ASSESSMENT DETAILS

This notification has been conducted under the cooperative arrangement with the Australian Therapeutic Goods Administration (TGA). The health hazard assessment component of the TGA report was provided to NICNAS and, where appropriate, used in this assessment report. The other elements of the risk assessment and recommendations on safe use of the notified chemical were carried out by NICNAS and the Department of the Environment and Energy.

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Merck Pty Ltd (ABN: 80 001 239 818)
Ground Floor, Building 1
885 Mountain Highway
BAYSWATER VIC 3153

NOTIFICATION CATEGORY
Standard (Reduced fee notification): Chemical other than polymer (more than 1 tonne per year) – Assessed by comparable agency (TGA)

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for absorption/desorption, dissociation constant and flash point

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
TGA (2012)

NOTIFICATION IN OTHER COUNTRIES
Germany (1999 – 2002)

2. IDENTITY OF CHEMICAL

MARKETING NAME
RonaCare® Ectoin

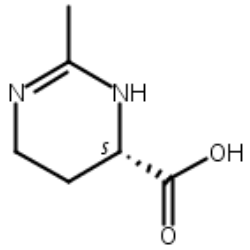
CAS NUMBER
96702-03-3

CHEMICAL NAME
4-Pyrimidinecarboxylic acid, 3,4,5,6-tetrahydro-2-methyl-, (4S)-

OTHER NAME(S)
Ectoin (INCI Name)
Ectoine

MOLECULAR FORMULA
$C_6H_{10}N_2O_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
142.16 Da

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

3. COMPOSITION

DEGREE OF PURITY
94 - 96%

IMPURITIES (> 1% BY WEIGHT)

Chemical Name	4-Pyrimidinecarboxylic acid, 1,4,5,6-tetrahydro-5-hydroxy-2-methyl-, (4S,5S)-
CAS No.	165542-15-4 *Weight %* ≤ 5.0%
Hazardous properties	Not listed in Hazardous Chemical Information System (HCIS). According to the classification provided by companies to ECHA in CLP notifications this substance causes serious eye irritation, causes skin irritation and may cause respiratory irritation.

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white powder

Property	Value	Data Source/Justification
Melting Point	Decomposes without melting at ≥ 270 °C	Measured
Boiling Point	Decomposes without boiling at ≥ 270 °C	Measured
Density	1,568 kg/m^3 at 20 °C	Measured
Vapour Pressure	3.29×10^{-8} – 8.24×10^{-8} kPa at 25 °C	Calculated using Modified Watson Correlation
Water Solubility	390 g/L at room temperature	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year at 25°C (at pH 4 ,7, 9)	Measured
Partition Coefficient (n-octanol/water)	log Pow ≥ -3	Calculated
Adsorption/Desorption	Not determined	Expected to be mobile in soils based on its high water solubility
Dissociation Constant	Not determined	Expected to be ionised under environmental conditions (pH 4-9)
Particle Size	MMD* = 103.5 µm Inhalable fraction (< 100 µm): 48% Respirable fraction (< 10 µm): 6%	Measured
Flash Point	Not determined	Decomposition observed at ≥ 270 °C
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400 °C	Measured

Explosive Properties	Not explosive	Measured
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidative properties

* MMD = Mass Median Diameter

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia in the neat form or as a cosmetic premix at ≤ 60% concentration.

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

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

PORT OF ENTRY
Melbourne

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia via air and/or sea in its neat form packaged in 1 kg polyethylene bottles or in 5 kg cardboard boxes with polyethylene inner liners. The notified chemical and products containing the notified chemical will then be distributed in Australia via road transport.

USE
The notified chemical will be used as a component of leave-on cosmetic skin care products at ≤ 3% concentration.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia in its neat form or as a cosmetic premix at ≤ 60% concentration for reformulation into leave-on cosmetic skin care products at ≤ 3% concentration. Cosmetic premixes may also be formulated within Australia from the neat notified chemical for further reformulation into cosmetic products.

Reformulation
The procedures for incorporating the notified chemical into cosmetic premixes and finished cosmetic products will vary depending on the nature of the products being formulated. Both manual and automated steps will likely be involved. In general, it is expected that for the reformulation process the notified chemical will be weighed and transferred to a mixing vessel where it will be blended to form cosmetic premixes or finished cosmetic products. This will be followed by automated filling of the reformulated products into containers of various sizes. For cosmetic premixes containing the notified chemical, further reformulation will be required to produce finished cosmetic products. The blending operations are expected to be highly automated and use closed systems and/or adequate ventilation. During the formulation process, samples of the notified chemical, the premixes or finished cosmetic products will be taken for quality control testing.

End Use
The finished cosmetic products containing the notified chemical at ≤ 3% concentration will be used by consumers and professionals such as beauticians. Depending on the nature of the products, application of the finished leave-on skin care products is expected to be by hand or through the use of an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse	0.5	100 - 500
Quality control	0.5	100 - 150
Formulators	6	100 - 500
Packaging	6	100 - 150
Retailers and beauty professionals	1	330

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical at up to ≥ 94% concentration only in the unlikely event of an accidental spill or rupture of packaging.

Reformulation
Exposure to the notified chemical at up to ≥ 94% concentration may occur during weighing and transfer stages, quality control analysis and cleaning and maintenance of equipment. The primary route of exposure is expected to be dermal whilst ocular exposure is also possible. Inhalation of the notified chemical is not expected due to its very low vapour pressure, unless dusts, mists or aerosols are formed during the process. Exposure is expected to be minimised through the use of exhaust ventilation and/or automated/enclosed systems as well as through the use of personal protective equipment (PPE) such as coveralls, eye protection, impervious gloves and respiratory protection (as proposed by the notifier).

End-use
Exposure to the notified chemical at ≤ 3% concentration in end-use products may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. workers in beauty salons). The principal route of exposure will be dermal, while ocular exposure is also possible. Such professionals may use some PPE 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 the same products containing the notified chemical.

6.1.2. Public Exposure
Public exposure to the notified chemical is expected to be widespread and frequent through daily use of cosmetic products containing the notified chemical at ≤ 3% concentration. The principal route of exposure will be dermal, while ocular exposure is also possible.

Data on typical use patterns of product categories in which the notified chemical may be used are shown in the following table (SCCS, 2012; ACI, 2010). For the purposes of the exposure assessment via the dermal route, Australian use patterns for the various product categories were assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed for the notified chemical (ECHA, 2014). A lifetime average female body weight (BW) of 64 kg (eńHealth, 2012) was used for calculation purposes.

Cosmetic products (dermal exposure)

Product type	**Amount** (mg/day)	**C** (%)	**RF** (unitless)	**Daily systemic exposure** (mg/kg bw/day)
Body lotion	7820	3.000	1.000	3.6656
Face cream	1540	3.000	1.000	0.7219
Hand cream	2160	3.000	1.000	1.0125
Total				**5.4000**

C = concentration (%); RF = retention factor.
Daily systemic exposure = (Amount × C × RF × dermal absorption)/body weight

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 5.4 mg/kg bw/day.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test.	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOEL = 300 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	non genotoxic

Toxicokinetics

The notified chemical is a pyrimidine derivative. Analogues of pyrimidine such as fluoroacil, floxuridine and cytarabine have antimetabolite activity. The pyrimidine analogues are reported to inhibit the biosynthesis of pyrimidine nucleotides or to mimic these natural metabolites to such an extent that they interfere with vital cellular functions, such as the synthesis or function of nucleic acids (Goodman et al., 1996). It is not clear however, whether the notified chemical itself has any antimetabolite activity or is dermally absorbed.

Given the low molecular weight of the notified chemical (142.16 Da), there is potential for absorption across biological membranes; however, dermal absorption may be limited by the hydrophilic nature of the notified chemical as demonstrated by its high water solubility (390 g/L) and partition coefficient (log Pow $\geq$ -3).

Acute toxicity

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

Irritation and sensitisation

Based on studies conducted in rabbits, the notified chemical is slightly irritating to eyes but non-irritating to skin. In the eye irritation study all animals displayed diffuse erythema and conjunctival discharge in the time period up to 1 hour after application. After 24 hours, only one animal showed a slight erythema (score 1). All signs of irritation were resolved at the 48 hour observation period.

In the guinea pig maximisation test, the notified chemical did not show evidence of skin sensitisation when tested up to 25% concentration.

Repeated dose toxicity

In a 28-day repeated dose oral (gavage) toxicity study, rats were treated with the notified chemical at 0, 100, 300 or 1000 mg/kg bw/day. Test substance-related adverse effects observed included lower mean body weights and increased total bilirubin in males and increased bile acid levels in both sexes at 1000 mg/kg bw/day. These effects persisted throughout the 14-day recovery period. No treatment related effects were noted at the lower doses. A No Observed Effect Level (NOEL) was established as 300 mg/kg bw/day for the notified chemical.

Mutagenicity/Genotoxicity

The notified chemical tested negative in two bacterial reverse mutation assays and negative in an *in vitro* mammalian cell gene mutation test.

Health hazard classification

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical is expected to be of low hazard presenting only as a slight eye irritant.

Reformulation

During reformulation, workers may be at risk of slight eye irritation effects when handling the notified chemical at up to ≥ 94% concentration. The notifier anticipates that worker exposure will be limited through the use of engineering controls such as enclosed systems, automated processes and local exhaust ventilation. The use of appropriate PPE (coveralls, imperious gloves and eye protection) will also be used to limit worker exposure.

End-Use

Workers involved in professions where the services involve application of cosmetic products containing the notified chemical to clients (*e.g.* beauty salon workers) may be exposed to the notified chemical at ≤ 3% concentration. Dermal, and to a lesser extent, ocular exposure may occur. PPE may be employed by workers to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the risk to such workers is expected to be of a similar or lesser extent than that for consumers using the various products containing the notified chemical.

Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health

Members of the public will experience widespread and frequent exposure to the notified chemical at ≤ 3% concentration through daily use of cosmetic products. The main route of exposure is expected to be dermal with some potential for accidental ocular exposure.

Local Effects

The notified chemical is slightly irritating to eyes. Given the low proposed use concentration (≤ 3%) irritation effects are not expected.

Systemic effects

The repeat 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 5.4 mg/kg bw/day (see Section 6.1.2). Using a NOEL of 300 mg/kg bw/day derived from a 28 day repeated dose oral toxicity study, the margin of exposure was estimated to be 56. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences. Based on the exposure assumptions in this assessment (refer Section 6.1), the MoE of 100 corresponds to a NOAEL of 540 mg/kg bw/day. Considering that 300 mg/kg bw/day corresponds to a NOEL rather than a No Observed Adverse Effect Level (NOAEL), and the conservative exposure assumptions used in estimating the MoE, the MoE of 54 is considered to be acceptable.

Overall, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 3% concentration in cosmetic 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

The notified chemical will be imported neat or as a component of cosmetic premixes for reformulation into cosmetic products. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and leaks. In the event of spills, the product containing the notified chemical is expected to be collected with adsorbents, and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve blending operations that will be highly automated, and is expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. Wastes containing the notified chemical generated during

reformulation include equipment wash water, residues in empty import containers and spilt materials. It is estimated by the notifier that up to 0.5% of the import volume of the notified chemical (or up to 50 kg) may be released from reformulation processes. This will be collected and released to sewers in a worst case scenario, or disposed of to landfill in accordance with local government regulations. Empty import containers are expected to be recycled or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE
The notified chemical is expected to be released to the aquatic compartment through sewers during its use in cosmetic products.

RELEASE OF CHEMICAL FROM DISPOSAL
It is estimated by the notifier that a maximum of 1% of the import volume of the notified chemical (or up to 100 kg), may remain in containers once the consumer products are used up. Wastes and residues of the notified chemical in empty containers are likely to either share the fate of the container and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Following its use in cosmetic products in Australia, the majority of the notified chemical is expected to enter the sewer system, before potential release to surface waters nationwide. Based on the result of the biodegradability study, the notified chemical is considered readily biodegradable (93% in 28 days). For details of the environmental fate studies, please refer to Appendix C. Based on its high water solubility, release to surface waters may occur as limited partitioning to sludge and sediment is expected under environmental pH. The notified chemical is not expected to bioaccumulate due to its low calculated partition coefficient (log $K_{OW} \geq -3$) and ready biodegradability.

The majority of the notified chemical will be released to sewer after use. A small proportion of the notified chemical may be applied to land when effluent is used for irrigation, or when sewage sludge is used for soil remediation. The notified chemical may also be applied to land when disposed of to landfill as collected spills and empty container residue. The notified chemical in surface waters, landfill, soil and sludge are expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assuming a worst case scenario, with 100% release of the notified chemical into sewer systems nationwide and no removal within sewage treatment plants (STPs).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	10,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	27.40	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	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:	6.06	µg/L
PEC - Ocean:	0.61	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m2/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 1,500 kg/m3). Using these assumptions, irrigation with a concentration of 6.06 µg/L may potentially result in a soil concentration of approximately 40.39 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 0.2 mg/kg and 0.4 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	96 h E_rC50 > 100 mg/L	Not harmful to algae
Algal Toxicity	96 h E_rC50 > 100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	3 h IC50 > 1000 mg/L	Not inhibitory to microbial respiration

Based on the above ecotoxicological endpoints, the notified chemical is not expected to be harmful to aquatic life. 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 using the acute endpoint from fish. A safety factor of 100 was used given acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish).	100.00	mg/L
Assessment Factor	100.00	
Mitigation Factor	1.00	
PNEC:	1,000.00	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	6.06	1,000	0.006
Q - Ocean	0.61	1,000	0.001

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters, based on its maximum annual importation quantity. The notified chemical is considered readily biodegradable, and is expected to have a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in cosmetic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposes without melting at ≥ 270 °C

Method OECD TG 102 Melting Point/Melting Range, July 27 1995.
Remarks Capillary method was used in this test.

At about 270 °C, the colour of the test item changed from white to beige. At about 300 °C the colour turned to light brown and at 325 °C the sample became brown. At about 335 °C, the test item began foaming and at 345 °C the residue test item was liquid. At this temperature the test was stopped because melting of the test item was not detectable. The authors of this study concluded that the notified chemical does not melt under the conditions of the test as the test item decomposed before melting.
Test Facility RCC (1999a)

Boiling Point Decomposes without boiling at ≥ 270 °C

Method OECD TG 103 Boiling Point, July 27 1995.
Remarks Capillary method was used in this test.

At about 270 °C, the colour of the test item changed from white to beige. At about 300 °C the colour turned to light brown and at 325 °C the sample became brown. At about 335 °C, the test item began foaming and at 345 °C the residue test item was liquid. At this temperature the test was stopped because boiling of the test item was not detectable. The authors of this study concluded that the notified chemical does not boil under the conditions of the test as the test item decomposed before boiling.
Test Facility RCC (1999b)

Density 1,568 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids, July 27 1995.
Remarks Pycnometer method
Test Facility RCC (2001a)

Vapour Pressure $3.29 \times 10^{-8} – 8.24 \times 10^{-8}$ kPa at 25 °C

Method OECD TG 104 Vapour Pressure, July 27 1995.
Remarks The study authors considered the vapour pressure of the notified chemical to be below the measurement range for experimental determination. As such, the vapour pressure of the notified chemical was calculated by the study authors using the Modified Watson Correlation (Lyman et al., 1990), taking the decomposition range of 335 to 345 °C as the lower limit for a boiling point.
Test Facility RCC (1999c)

Water Solubility 390 g/L at room temperature

Method OECD TG 105 Water Solubility.
Remarks Flask Method. The water solubility was not corrected for the purity of the test item.
Test Facility RCC (1999d)

Hydrolysis as a Function of pH $t_{1/2}$ > 1 year at 25°C (pH=4 ,7, 9)

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*t½ (years)*
4	25	> 1
7	25	> 1
9	25	> 1

Remarks HPLC method. Less than 10% hydrolysis was observed after 5 days at 50 °C at pH 4, 7 and

9 and therefore the estimated half-life at 25°C is > 1 year.

Test Facility RCC (2001b)

Partition Coefficient (n-octanol/water) log Pow ≥ -3

Method EEC Directive 92/69, Part A, Methods for the determination of physico-chemical properties

Remarks In the preliminary test, a very good solubility in water and a poor solubility in n-octanol was found indicating a partition coefficient below -2. Thus, a main test according to OECD guidelines 107/117 could not be applied. Therefore, the partition coefficient of the notified chemical was estimated using the solubility data in n-octanol as obtained in the preliminary test according to EEC Directive 92/69.

Test Facility RCC (1999e)

Particle Size

MMD* = 103.5 µm
- inhalable fraction (< 100 µm) = 48%
- respirable fraction (< 10 µm) = 6%

Method EC, Directorate General XII-JRC "Particle Size Distribution, Fibre Length and Diameter Distribution" ECB/TM, February 1996.

Mean Range (µm)	*Mass (%)*
< 6.91	5
< 22.12	10
< 103.50	50
< 187.78	90

* Mass median diameter

Remarks Laser scattering/diffraction method. The test substance was dispersed in maize oil. The particle size distribution of the test substance was found in this study to range from approx. 0.5 µm to 300 µm.

Test Facility RCC (2001c)

Flammability Not highly flammable.

Method EEC Directive 92/69, A.10 Flammability (Solids), December 1992.

Remarks The study authors noted that the test substance pile could be ignited with a flame during the preliminary test. However, the test item could not sustain a burning reaction. After approximately 30 seconds, the superficial and local flame extinguished. No smoke or soot could be observed. No main test was performed.

Test Facility RCC (1999f)

Autoignition Temperature > 400 °C

Method EEC Directive 92/69, A.16 Relative Self-Ignition Temperature for Solids, December 1992.

Remarks The study authors reported that the notified chemical showed no relevant exothermic reaction and is therefore not auto-flammable according to the criteria of the test guideline.

Test Facility RCC (2001d)

Explosive Properties Not explosive

Method UN Recommendations on the Transport of Dangerous Goods (Manual of Tests and Criteria, Annex 6, Orange Book, 3rd Edition, 1999).

Remarks The explosive properties of the notified chemical were estimated by appraising its molecular structure, estimating its oxygen balance and performing calorimetric tests (using differential scanning calorimetry).

Test Facility RCC (2001e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity, February 24 1987. EC Directive 84/449/EEC B.1 Acute Oral Toxicity, April 25 1984.
Species/Strain	Wistar Rat/Hoe:WISKf(SPF71)
Vehicle	Deionised water
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	Squatting posture, drawn-in flanks, stilted gait, bristling fur and irregular respiration lasting up to 48 hours in both groups.
Effects in Organs	No abnormalities detected at post-mortem.
Remarks - Results	No mortality occurred. All animals made expected body weight gains during the study.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Hoechst (1990a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity, February 24 1987 EC Directive 84/449/EEC B.3 Acute Dermal Toxicity, April 25 1984.
Species/Strain	Rat/Wistar
Vehicle	0.9% sodium chloride solution
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Many animals from both groups displayed dry and rough skin from day 3 to day 8 following 24 hour exposure to the test substance. In some cases, this was accompanied by fine to coarse scaling, erythema and scab formation.
Signs of Toxicity - Systemic	None observed during the study.
Effects in Organs	No abnormalities detected at post-mortem.
Remarks - Results	No impairments in body weight development were observed during the study.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.

TEST FACILITY	Hoechst (1990b)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion, 12 May 1981. EC Directive 84/449/EEC B.4 Acute Skin Irritation Toxicity, April 25 1984.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	0.9% sodium chloride solution
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations
RESULTS	
Remarks - Results	No signs of irritation were observed in the entire duration of the study following 4 hour exposure to the test substance.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	Hoechst (1991a)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion, 24 February 1987. EC Directive 84/449/EEC B.5 Acute Eye Irritation Toxicity, April 25 1984.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	72 hours
Remarks - Method	No significant protocol deviations
RESULTS	
Remarks - Results	One hour after application of the test substance, all animals displayed clear, colourless conjunctival discharge and diffuse conjunctival redness. In addition, 2/3 animals displayed slight conjunctival swelling. At the 24 hour observation, only 1/3 animals displayed signs of irritation (slight conjunctival reddening). No signs of irritation were detected by the 48 hour observation point.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Hoechst (1991b)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 406 Skin Sensitisation – Maximisation Test, May 12 1981. EC Directive 84/449/EEC B.6 Acute Skin Sensitisation Toxicity, April 25 1984.
Species/Strain	Guinea pig/Pirbright White
PRELIMINARY STUDY	Maximum non-irritating: intradermal: 0.2% topical: 25% (highest concentration evaluated)
MAIN STUDY	

Number of Animals	Test Group: 10 Control Group: 5
Vehicle	White Vaseline, DAB.
Positive control	Not reported
INDUCTION PHASE	Induction Concentration - intradermal: 1% (in viscous paraffin DAB) 1% (in 50% Freund's adjuvant) - topical: 25% (in white Vaseline DAB)
Signs of Irritation	Intradermal injection of 1% concentration of test substance in paraffin produced slight reddening and swelling. Intradermal injection of Freund's adjuvant (with and without test substance) produced clear reddening and swelling. Topical application of the test substance did not produce signs of irritation at sites which received the test substance at 1% concentration in paraffin. Topical application of the test substance on animals injected with Freund's adjuvant (with and without test substance) produced reddened, swollen and hard application sites. Some of these sites also displayed necrosis formation.
CHALLENGE PHASE	
1st challenge	topical: 25% (in white Vaseline DAB)
Remarks - Method	No significant protocol deviations. Test and control animals were challenged with the test substance 2 weeks after dermal induction. An additional "parallel" group of 5 animals were used in this study. These animals were treated in the same way as controls during the induction phase, but were challenged with the test substance (25% concentration in white Vaseline DAB) 1 week after dermal induction.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*	
		24 h	*48 h*
Test Group	25%	0/10	0/10
Control Group	25%	0/5	0/5

Remarks - Results	No skin reactions were observed after challenge treatment with the test substance in any of the animals, including those in the "parallel' group.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Hoechst (1991c)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents, July 27 1995. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral), September 30 1996.
Species/Strain	Rat/Wistar (HanBrl, 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	Distilled water
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M/5F	0	0/10
low dose	5M/5F	100	0/10
mid dose	5M/5F	300	0/10
high dose	5M/5F	1000	0/10
control recovery	5M/5F	0	0/10
high dose recovery	5M/5F	1000	0/10

Mortality and Time to Death
All animals survived the scheduled treatment or recovery periods.

Clinical Observations
One male treated at 300 mg/kg/day displayed localised alopecia during week 1. Miosis was noted during week 2 in a single male treated with 1000 mg/kg/day. Grip strength and locomotor activity of treated animals were similar to corresponding control animals during a functional observational battery test performed at week 4 of treatment.

Food consumption of treated animals was comparable to control animals. However, the mean body weight of males treated with 1000 mg/kg/day was lower than corresponding controls throughout the treatment and recovery periods attaining statistical significance on days 8 and 22 of treatment. The authors of this study consider these differences to be attributable to the test substance.

Laboratory Findings – Clinical Biochemistry, Haematology, Urinalysis
Haematology
At 300 mg/kg/day, females displayed prolonged activated partial thromboplastin times, significantly increased mean cell haemoglobin concentration, and significantly decreased platelet count compared to controls during the treatment period.

At 100 mg/kg/day, females displayed prolonged activated partial thromboplastin times and significantly increased mean cell haemoglobin concentration compared to controls during the treatment period.

The authors of this study do not consider these findings to be test substance-related because females treated with 1000 mg/kg/day and all males did not display any of the above effects.

Clinical Biochemistry
At 1000 mg/kg/day, total bilirubin levels were significantly increased in males after the treatment and recovery periods. Bile acid levels were noticeably increased in males and significantly increased in females treated at 1000 mg/kg/day after the treatment period. After the recovery period, bile acid levels were significantly increased in males and noticeably increased in females. The authors of this study consider these findings to be related to the test item.

Urinalysis
Males treated with 1000 mg/kg/day displayed significantly increased specific gravity and osmolality and significantly decreased pH after the treatment and recovery periods. These males also displayed significantly decreased urine volume after the recovery period. The authors of this study do not consider these effects to be related to the test item because these differences (when compared to controls) are within the limits of historical control data.

Effects in Organs
The absolute or relative organ weights recorded after 4 weeks treatment or 2 weeks recovery were considered to be unaffected by the test substance.

Macroscopic organ alterations noted during post-mortem included incompletely or non-collapsed lungs, accentuated liver pattern, renal pelvis dilation, uterus horn dilation, discolouration and discoloured foci in several organs. The authors of this study considered these alterations to be within the range of historical data of rats of this strain and age.

Microscopic organ alterations noted during post-mortem were considered by the authors of this study to be within the range of historical data of rats of this strain and age. Further information on these specific alterations (including raw data) was not provided.

Remarks – Results
Test substance-related adverse effects observed included lower mean body weights and increased total bilirubin in males and increased bile acid levels in both sexes at 1000 mg/kg bw/day. All these effects persisted throughout the recovery period.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 300 mg/kg bw/day in this study, based on the presence of treatment-related effects at 1000 mg/kg/ bw/day.

TEST FACILITY RCC (2001f)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test (salmonella typhimurium), May 26th 1983.
OECD TG 472 Bacterial Reverse Mutation Test (Escherichia coli), May 26th 1983.
Plate incorporation procedure

Species/Strain *S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System S9 fraction from Aroclor 1254-induced rat liver

Concentration Range in Main Test
a) With metabolic activation: 4 - 5000 µg/plate
b) Without metabolic activation: 4 - 5000 µg/plate

Vehicle Double-distilled water

Remarks - Method No deviations from the study plan

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	*Cytotoxicity in Preliminary Test**	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 10,000	-	> 10,000	Negative
Test 2	-	> 5000	> 5000	Negative
Present				
Test 1	> 10,000	-	> 10000	Negative
Test 2	-	> 5000	> 5000	Negative

* The highest concentration in the preliminary test was 10,000 µg/plate

Remarks - Results No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, in the presence or absence of metabolic activation. Positive controls performed as expected, confirming the validity of the test system.

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

TEST FACILITY Hoechst (1992)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 471 Bacterial Reverse Mutation Test, 21 July, 1997. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102 *E. coli*: WP2uvrA pkM101
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 5 - 5000 µg/plate b) Without metabolic activation: 5 - 5000 µg/plate
Vehicle	Double-distilled water
Remarks - Method	No significant protocol deviations

RESULTS

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

Remarks - Results	No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with the test substance at any dose level, in the presence or absence of metabolic activation. Positive controls performed as expected, confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Merck (1999a)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 476 In Vitro Mammalian Cell Gene Mutation Tests.
Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma/L5178 $TK^{(+/-)}$
Metabolic Activation System	S9 fraction from Aroclor 1254-induced rat liver
Vehicle	Double-distilled water
Remarks - Method	No deviations from the study plan were noted. The mouse lymphoma cells were tested with the test substance for potential to induce mutations at the TK locus. Preliminary experiments were conducted to determine the dose range for the main study.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	158, 500, 1580, 5000	3 h	48 h	7 – 10 days
Test 2	158, 500, 1580, 5000	24 h	48 h	7 – 10 days
Present				
Test 1	158, 500, 1580, 5000	3 h	48 h	7 – 10 days
Test 2	158, 500, 1580, 5000	3 h	48 h	7 – 10 days

All cultures were selected for metaphase analysis

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

Remarks - Results

The test substance did not induce any statistically significant increases in the mutant frequency at any tested concentration in each exposure group, with and without metabolic activation.

The positive and vehicle controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to the TK locus in the L5178 $TK^{(+/-)}$ mouse lymphoma cells treated *in vitro* under the conditions of the test.

TEST FACILITY

Merck (2001a).

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	Official Journal of the European Community No. L 383 A, Part C dated 29 December 92, Method C.4-E: Closed Bottle Test.
Inoculum	Activated sewerage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Not reported
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Toxicity control*		*Aniline*	
Day	*Mean Degradation (%)*	*Day*	*Mean Degradation (%)*	*Day*	*Mean Degradation (%)*
7	51	7	66	7	71
14	65	14	70	14	87
21	91	21	98	21	97
28	93	28	100	28	100

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound (aniline) surpassed the threshold level of 60% at 14 days (87%), and attained 100% degradation at 28 days. Therefore, the test indicates the suitability of the inoculums. The percentage degradation of the toxicity control surpassed the threshold level of 25% at 14 days (70%; 100% at 28 days), showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance at 28 days was 93%. Therefore, the test substance is considered to be readily biodegradable.
CONCLUSION	The notified chemical is considered to be readily biodegradable.
TEST FACILITY	Environmental (2000)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static.
Species	Brachydanio rerio (zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	10	0	0	0	0	0
100	103	10	0	0	0	0	0

LC50	> 100 mg/L at 96 hours.
NOEC	> 100 mg/L at 96 hours.
Remarks – Results	All validity criteria of the test guideline were satisfied. The analytically determined test material concentration after 96 hours was 103% of the nominal value. Thus, the nominal values correspond to the analytical values. The 96 h LC50 and NOEC for fish were determined to be >100 mg/L, based on nominal concentrations.

CONCLUSION The notified chemical is not harmful to fish.

TEST FACILITY Merck (2001b)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
0	0	20	0	0
100	89.7	20	0	0

EC50	> 100 mg/L at 48 hours
NOEC	> 100 mg/L at 48 hours
Remarks - Results	The validity criteria of the test guideline were not specified. All test media were clear and stayed unchanged throughout the study. The 48 h EC50 and NOEC for Daphnia were determined to be >100 mg/L, based on nominal concentrations.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates

TEST FACILITY Merck (1999b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test – Static

Species	Desmodesmus subspicatus (green alga)
Exposure Period	96 hours
Concentration Range	Nominal: 0 and 100 mg/L

	Actual: Not reported
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
mg/L at 96 h	*mg/L*	*mg/L at 96 h*	*mg/L*
>100	100	>100	100

Remarks - Results	All validity criteria of the test guideline were satisfied. The study was designed as a limit test in a closed static test system. Samples for analysis were taken directly after preparation and at the end of the exposure period. The analytically determined test material concentration after 72 hours was 106% of the nominal value. Thus, the nominal value corresponded to the analytical value. The 96 h EC50 and NOEC for algae were determined to be ≥100mg/L and 100 mg/L, based on nominal concentrations.

CONCLUSION The notified chemical is not harmful to algae.

TEST FACILITY Merck (2001c)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test – Static.

Species	*Desmodesmus subspicatus* (green alga)
Exposure Period	96 hours
Concentration Range	Nominal: 0 and 100 mg/L Actual: Not reported
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The nominal concentration of 100 mg/L was selected following the results from a previous study (Merck (2001b)).

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
mg/L at 96 h	*mg/L*	*mg/L at 96 h*	*mg/L*
>100	≥100	>100	≥100

Remarks - Results	All validity criteria of the test guideline were satisfied. The study was designed as a limit test in an open static test system. The analytically determined concentration of the freshly prepared test material was 105% of the nominal value. After 72 hours under experimental conditions the concentration was 107%. Thus, the nominal value corresponded to the analytical value. The 96 h EC50 and NOEC for algae were determined to be ≥100mg/L and 100 mg/L, based on nominal concentrations.

CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	Merck (2001d)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge.
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 320, 1000 mg/L Actual: Not determined
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. 3,5-Dichlorophenol was used as the reference control.
RESULTS	> 1000 mg/L at 3 h
IC50	100 mg/L at 3 h
NOEC	All validity criteria for the test were satisfied. The 3 h IC50 was determined to be > 1000 mg/L, based on nominal concentrations.
Remarks – Results	> 1000 mg/L at 3 hours
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	Institut (2001)

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