File No: STD/1660

January 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

1-Octanamine, *N,N'*-(1, 10-decanediyldi-1(4*H*)-pyridinyl-4-ylidene)bis-, hydrochloride (1:2) (INCI Name: Octenidine HCl)

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/1660	Schulke Australia Pty Ltd	1-Octanamine, *N,N'*-(1, 10-decanediyldi-1(4*H*)-pyridinyl-4-ylidene)bis-, hydrochloride (1:2) (INCI Name: Octenidine HCl)	Yes	< 0.4 tonne per annum	Component of cosmetic products

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute toxicity, oral (Category 4)	H302 - Harmful if swallowed
Skin corrosion/irritation (Category 2)	H315 – Causes skin irritation

The environmental hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute Category 1	H400 –Very Toxic to aquatic life

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, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Acute toxicity, oral (Category 4): H302 - Harmful if swallowed
  - Skin corrosion/irritation (Category 2): H315 – Causes skin irritation

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

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:
  - Enclosed, automated processes, where possible
  - Local exhaust ventilation and/or appropriate extraction systems where possible

- 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 processes:
  - Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:
  - 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 SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

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

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

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 exceeds or is intended to exceed 0.3% in cosmetic products;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component in cosmetic products, 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

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Schulke Australia Pty Ltd (ABN: 49 605 683 172)
Suite 3, level 2
2 – 4 Lyonpark Road
MACQUARIE PARK NSW 2113

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: other names, analytical data, degree of purity, residual impurities, additives/adjuvants, import volume, and identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are not varied.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2011)
European Union (2018)

2. IDENTITY OF CHEMICAL

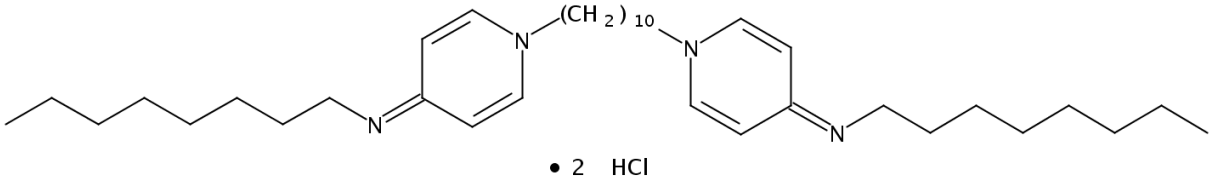
CHEMICAL NAME
1-Octanamine, *N,N'*-(1, 10-decanediyldi-1(4*H*)-pyridinyl-4-ylidene)bis-, hydrochloride (1:2)

MARKETING NAME(S)
Octenidine HCl (INCI name)

CAS NUMBER
70775-75-6

MOLECULAR FORMULA
$C_{36}H_{62}N_4.2ClH$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
623 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 98%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: yellowish crystalline powder

Property	Value	Data Source/Justification
Melting Point	218.8 – 219.3 °C	Measured
Boiling Point	Decomposes at > 221 °C at 101.3 kPa	Measured
Density	1,046 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.7198 × 10^{-14} kPa at 20 °C	Calculated with EpiSuite
Water Solubility	14.2 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Negligible	Measured
Partition Coefficient (n-octanol/water)	log Pow = 1.5 at 20 °C	Measured, but is likely to have surfactant properties based on the structure
Adsorption/Desorption	K_{oc} = 2.3 × 10^5 - 1.5 × 10^6 at 20 °C	Measured
Dissociation Constant	No pKa determinable between pH 0.8 and 13.7	Measured
Particle Size	Not determined	Particle size expected to be > 2,000 µm
Flash Point	Not determined	Expected to be high as the notified chemical is a salt in solid form that does not melt below 221 °C
Flammability	Not determined	Expected to be high as the notified chemical is a salt in solid form that does not melt below 221 °C
Autoignition Temperature	Not determined	Expected to be high as the notified chemical is a salt in solid form that does not melt below 221 °C.
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	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 expected to be stable under normal conditions of use.

Physical hazard classification
Based on the submitted limited physico-chemical data depicted in the above table, the notified chemical cannot be classified 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 at ≤ 20% concentration as a component of a powder preparation for use in cosmetic products.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Schulke Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in a powder form as a component of a preparation at ≤ 20% concentration in fibre boxes with an inner polyethylene pouch. Finished consumer products containing the notified chemical at ≤ 0.3% concentration will be packaged in containers suitable for retail sale.

USE
The notified chemical acts as an antimicrobial and/or emulsifying agent in cosmetic products. The final consumer products will contain the notified chemical at ≤ 0.3% concentration.

OPERATION DESCRIPTION

Reformulation
At the customer facilities, the notified chemical at ≤ 20% concentration will be formulated into end-use products. The reformulation procedure will likely vary depending on the nature of the formulated products, and may involve both automated and manual transfer steps. However, in general, it is expected that the reformulation processes will involve blending operations that will be highly automated and use closed systems with adequate ventilation, followed by automated filling of the reformulated products into containers of various sizes.

End-use
The finished cosmetic products containing the notified chemical at ≤ 0.3% concentration will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application of products could be by hand, spray 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)*
Blending Operators	< 0.25	260
Filling Operators	< 0.01	260

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the notified chemical as a component of powder preparations (at ≤ 20% concentration) or as a component of end-use products (at ≤ 0.3% concentration) only in the event of accidental rupture of containers. The notifier states that such exposures will be minimised through the use of personal protective equipment (PPE) including protective coveralls, chemical resistant gloves, safety glasses and respiratory protection where appropriate.

Formulation of end products
During reformulation, dermal and ocular exposure of workers to the notified chemical (at ≤ 20% concentration) may occur during weighing and transfer stages, blending, quality control analysis, packaging of materials and cleaning and maintenance of equipment. Given the calculated low vapour pressure (2.7198×10^{-14} kPa at 20 °C) and particle size (> 2,000 μm) of the notified chemical, inhalation exposure is not expected unless dusts, mists or aerosols are formed. The notifier states that exposure is expected to be minimised through the use of PPE such as coveralls, goggles, impervious gloves and face masks which contain a particle filter.

Beauty salons
Exposure to the notified chemical in end-use products (at ≤ 0.3% concentration) may occur in professions where the services provided involve the application of cosmetic products to clients. The principal route of exposure will be dermal, while ocular and inhalation exposure are also possible. Such professionals may use 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 finished products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of a wide range of cosmetic products containing the notified chemical at ≤ 0.3% concentration. The principal route of exposure will be dermal, while ocular and inhalation exposure are also possible, particularly if the products are applied by spray.

Data on typical use patterns of cosmetic products in which the notified chemical is proposed to be used are shown in the following table (SCCS, 2015). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. A dermal absorption value of 0.135% was used for the notified chemical for calculation purposes (see Section 6.2 for further information). A lifetime average female body weight (BW) of 64 kg (enHealth, 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	0.3	1	0.000495
Face cream	1540	0.3	1	0.000097
Hand cream	2160	0.3	1	0.000137
Deodorant (non-spray)	1500	0.3	1	0.000090
Deodorant (aerosol spray)	1430	0.3	1	0.000095
Shampoo	10460	0.3	0.01	0.000007
Conditioner	3290	0.3	0.01	0.000002
Shower gel	18670	0.3	0.01	0.000012
Hand wash soap	20000	0.3	0.01	0.000013
Hair styling products	4000	0.3	0.1	0.000025
Total				**0.000973**

C = concentration; RF = retention factor.
Daily systemic exposure = Amount × C (%) × RF × dermal absorption (%) / body weight (64 kg)

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above table that contain the notified chemical. This would result in a combined internal dose of 0.000973 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 = 800 mg/kg bw; harmful
Rat, acute inhalation toxicity	LC50 > 4.1 mg/L/4 hour; low toxicity at 0.1% concentration
Rabbit, skin irritation	non-irritating at 0.1% concentration
Rabbit, skin irritation	irritating
Human, tolerability test	no evidence of irritation at 0.3% concentration
Rabbit, eye irritation	non-irritating at 0.1% concentration
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days.	NOEL = 32 mg/kg bw/day NOAEL = 128 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian cell gene mutation test (mouse lymphocytes)	non genotoxic
Genotoxicity – *in vivo* mammalian erythrocyte micronucleus test (mouse)	non genotoxic

Toxicokinetics, metabolism and distribution

An *in vivo* absorption, distribution and excretion study (in accordance with OECD TG 417) in rats dosed with 10 mg/kg bw or 100 mg/kg bw ^{14}C-radiolabelled notified chemical was performed. The notified chemical was

poorly absorbed from the gastrointestinal (GI) tract into the systemic circulation, with 95% and 88% (low- and high-dose group, respectively) excreted unabsorbed within the faeces over the study period. The total systemic absorption of the test substance (adding the amount in the organs, the urine and the cage wash) was 1.56% for the low (10 mg/kg bw) dose group and 1.51% for the high (100 mg/kg bw) dose group.

The repeat dose toxicity study on the notified chemical mentions a radiolabelled study (not sighted) where the gastrointestinal absorption was < 1% of the administered dose (Sterling-Winthrop, 1985).

In an *in vivo* dermal absorption study (in accordance with OECD TG 427) in rats with the notified chemical (at 0.1% concentration in a product formulation), the majority of the applied dose (65 - 81%) of the radioactively labelled notified chemical was rinsed off the application site at the end of the 6 hour exposure period, with 11.4% of the notified chemical remaining in/on the treated skin area (immediately after exposure). The notified chemical was predominantly found in viable skin (7.6%). After the 6 hour exposure period, < 0.05% of the dose was systemically absorbed (penetration rate < 0.015 µg/cm^2/h). During the seven days after exposure, the amount of notified chemical available for absorption through the skin decreased (8.64 ± 4.38%) and a very low increase in systemic absorption was observed. The systemic absorption after 168 hours was 0.135%, although this value does not include radioactive material that may have been absorbed and was lost as carbon dioxide via respiration, contained in organs or in cage materials.

Acute toxicity
In acute toxicity studies in rats, the notified chemical was found to be harmful via the oral route (at a concentration of 94.1%) and of low toxicity via inhalation (at a concentration of 0.1%).

Irritation
In a skin irritation study in rabbits with the notified chemical (at 94.1% concentration), slight to well-defined erythema was observed in all animals developing immediately after exposure or during the 4 hours following exposure. After 24 hours, all animals exhibited well-defined erythema and slight to moderate oedema, with no recovery indicated up to 72 hours after exposure with erythema increasing from moderate to severe in two animals. Scab formation was observed in all animals at the day 7 observation and persisted in 1/3 animals to at least 7 weeks after exposure (observation period terminated). All animals recovered from erythema and oedema within the 28 day observation period. Based on the persistence of scab formation in one animal, the study authors concluded that the notified chemical (at 94.1% concentration) may have a potential corrosive effect on the skin.

A study in human test subjects found the notified chemical to be non-irritating at 0.3% concentration. In a study conducted in rabbits, the notified chemical was non-irritating when tested at a concentration of 0.1%.

A study in rabbits found the notified chemical to be non-irritating to the eyes at 0.1% concentration.

Sensitisation
Due to the irritating, and potentially corrosive effects of the notified chemical on the skin, three preliminary studies were performed to determine the use concentration for the guinea pig maximization test. During the induction phase, animals were exposed to the notified chemical at concentrations 0.0125% (intradermal exposure) and 1% (topical exposure). Animals were then challenged with the notified chemical at 0.5% concentration. A sensitisation response was observed in 15% of the animals exposed to the test substance. However, this is insufficient for classification of the notified chemical as a sensitiser under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*.

Repeated dose toxicity
In a 90 day repeated dose oral toxicity study in mice exposed to the notified chemical at doses of 32, 64, 128 and 256 mg/kg/day, the No Observed Adverse Effect Level (NOAEL) was established as 128 mg/kg bw/day based on a decrease in liver weights and serum urea levels in male animals in the high dose group.

Mutagenicity/Genotoxicity
The notified chemical was found to be non-mutagenic in a bacterial reverse mutation assay and non-clastogenic in an *in vivo* mouse lymphocyte mutation test and an *in vivo* mouse micronucleus test.

Health hazard classification

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

Hazard classification	*Hazard statement*
Acute toxicity, oral (Category 4)	H302 - Harmful if swallowed
Causes skin irritation (Category 2)	H315 – Causes skin irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical is harmful if swallowed and a skin irritant. The potential for the notified chemical to cause corrosive effects cannot be ruled out.

Reformulation

Exposure of workers to the notified chemical at ≤ 20% concentration may occur during blending operations. The main route of exposure is expected to be dermal. While the notified chemical is considered to be harmful via the oral route, ingestion is unlikely under the occupational settings described. However skin and eye irritation effects cannot be ruled out. Therefore, caution should be exercised when handling the notified chemical during reformulation processes.

Provided that the stated control measures are in place to minimise worker exposure, including the use of automated processes and PPE (impervious gloves, goggles, and coveralls), the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use

Beauty care professionals will handle the notified chemical at ≤ 0.3% concentration, similar to public use. Therefore the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

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

6.3.2. Public Health

Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic products containing the notified chemical at ≤ 0.3% concentration. The main route of exposure is expected to be dermal, while ocular and inhalation exposure are also expected where products are applied by spray.

Irritation

The notified chemical is a skin irritant with a potential for corrosive effects. The notified chemical was non-irritating at 0.3% concentration in a human study and at 0.1% concentration in a study conducted in rabbits. Irritant effects are therefore not expected at the proposed use concentration of ≤ 0.3%.

Repeated dose toxicity

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 of 0.000973 mg/kg bw/day (see Section 6.1.2). A NOAEL of 128 mg/kg bw/day (based on administered dose) was derived from a 90-day repeated dose oral toxicity study. Adjusting for bioavailability (oral absorption of 1.56%) gives a NOAEL based on systemic absorption of 2 mg/kg bw/day. Using the systemic NOAEL of 2 mg/kg bw/day, the MoE was estimated to be 2,055. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical is to be imported into Australia as part of a chemical mixture at ≤ 20% concentration that will then be formulated into various cosmetic products. Except in the case of accidental spills and leaks, there is unlikely to be significant release of the notified chemical to the environment from either transport to or within Australia, or storage within factory facilities. The reformulation process is expected to occur within a fully enclosed environment. Any waste containing the notified chemical generated during reformulation (such as wash waters, residues in empty import containers and spilt materials) will be collected into an on-site industrial waste tank. Material in this tank will be removed by an approved waste collection company for appropriate disposal.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used in cosmetic products that are applied to the skin. After application, release of the chemical to the environment will occur from washing of skin into wastewater, and hence into sewage. Release into surface water is expected to be minimal.

RELEASE OF CHEMICAL FROM DISPOSAL

It is likely that only a small amount (< 5%) of any cosmetic mixture will remain in a product container after final use. 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

The submitted biodegradability study indicates that the notified chemical is degradable in domestic sewage (100% biodegradation in five days). For details of the study, please see Appendix C. Further, a log Pow value of 1.5 suggests that bioaccumulation is unlikely.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. It is assumed that 100% of the total import volume of the notified chemical is released to the sewer nationwide over 365 days per year, and there is removal of the notified chemical from degradation, adsorption and volatilisation, modelled using SimpleTreat 3.0 (Struijs 1996) during sewage treatment processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	400	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	400	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	1.10	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	24.4	million
Removal within STP	87%	
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.029	µg/L
PEC - Ocean:	0.0029	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.029 µg/L may potentially result in a soil concentration of approximately 0.19 µg/kg (0.029 × 1000/150). Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years is expected to be approximately 0.97 µg/kg and 1.9 µg/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 (acute)	EC50 (96 h) = 0.08 mg/L	Very toxic to fish
Daphnia toxicity (acute)	EC50 (48 h) = 0.0052 mg/L	Very toxic to invertebrates
Daphnia reproduction (chronic)	EC50 (21 days) > 5.57 µg/L	Unclassified
Algal toxicity	EC50 (72 h) = 0.0151 mg/L	Very toxic to algae or other aquatic plants

The results from ecotoxicological investigations on the notified chemical indicate that it is very acutely toxic to aquatic life. Therefore, the notified chemical is formally classified as "Acute category 2. "Very toxic to aquatic life" under the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive acute endpoint for aquatic invertebrates and an assessment factor of 50 as data is available for acute toxicity for three trophic levels and chronic toxicity for a single trophic level.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Daphnia	0.0052	mg/L
Assessment Factor	50	
Mitigation Factor	1	
PNEC:	0.104	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.029	0.104	0.28
Q - Ocean:	0.0029	0.104	0.028

The Risk Quotients (Q = PEC/PNEC) for discharge of treated effluents containing the notified chemical have been calculated to be < 1 for both river and ocean compartments. This indicates that, based on the amount of the notified chemical to be imported each year and its use pattern, it is unlikely to reach ecotoxicologically significant concentrations in surface waters. The notified chemical is not expected to bioaccumulate. Hence, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 218.8 – 219.3 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Capillary method.
Test Facility	GAB (2003a)

Boiling Point Decomposes at > 221.0 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Siwoloboff method. Boiling point was not determined. Test substance decomposed at 221.0 °C.
Test Facility	GAB (2003b)

Density 1,046 kg/m^3 at 20 °C

Method	EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Pycnometer.
Test Facility	GAB (2003c)

Water Solubility 14.2 g/L at 20 °C (pH 7)

Method	OECD TG 105 Water Solubility (Flask method) EC Council Regulation A.6 (92/69)
Remarks	Water solubility was determined at three different temperatures (10 °C, 20 °C, 30 °C) and four different pH values (3.84, 5.65, 7.02, 9.02 - mean measured values over 72 h for the three temperatures). At each pH value, solubility increased with increasing temperature, with maximum solubility found with the pH values of 5.65 and 7.02 (near neutral pH).
Test Facility	GAB (2003d)

Hydrolysis as a function of-pH Negligible

Method	OECD TG 111 Hydrolysis as a function of pH EC Method C.7
Remarks	A preliminary test was carried out at the pH values of 4.0, 7.0 and 9.0 at 50 °C. Less than 10% hydrolysis occurred after 120 hours. The results indicate that hydrolysis of octenidine dihydrochloride is negligible in the pH range 4-9, and the half-life in this pH range expected to be greater than one year.
Test Facility	GAB (2003e)

Partition Coefficient (n-octanol/water) log Pow = 1.5 at 20 °C

Method	OECD TG 123 Partition Coefficient (slow stirring) EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Partition of octenidine dihydrochloride between water and n-octanol after 1, 3, 7 and 14 days of stirring was determined by HPLC of the water phase and the oxidation of nitrogen in the n-octanol phase. Log Pow values were calculated after 14 days.
Test Facility	Schulke and Mayr GmbH (2010)

Adsorption/Desorption $K_{oc} = 2.3 \times 10^5 – 1.5 \times 10^6$ at 20 °C

Method	OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method
Remarks	The adsorption/desorption behaviour of octenidine dihydrochloride was evaluated in five top soils. The test item was stable under the test conditions. For both the adsorption and desorption tests, no correlation between the organic content of a soil and the calculated coefficient value could be observed, whereas these tests did demonstrate correlations with

	cation exchange capacity. The results indicate that for the test item ionic linkage to a soil matrix may be a more important sorption mechanism than binding to soil organic matter.
Test Facility	Fraunhofer (2007a)

Dissociation constant No pKa determinable between 0.8 and 13.7

Method	OECD TG 112 Dissociation constants in water
Remarks	Preliminary tests evaluating octenidine dihydrochloride found that the titration method was appropriate. Five determinative tests were then performed. No pKa determinable between 0.8 and 13.7.
Test Facility	Siemens (2007)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical at 94.1% concentration

METHOD: Groups of 10 young adult male, Sprague-Dawley albino rats, ranging in weight from 100 to 130 grams (average of 116 grams), were used in these studies. They were medicated once orally by stomach tube or injected intravenously via the tail vein at a rate of 1 mL/minute. In the oral study the rats were fasted approximately 4 hours before medication and 10 controls were medicated orally with 1% aqueous gum tragacanth. In the intravenous study, the rats were not fasted, and 10 unmedicated rats were used as controls. The two studies were done on different days and all rats were housed in double cages, 10 per cage, for observation. Survivors were observed for 7 days after medication, and then autopsied. Autopsies were also done on those that died. In this report, the day of medication was considered Day 0, the next day (24 hours later) was day 1, 48 hours was Day 2 etc.

Species/Strain	Rat/Sprague-Dawley
Vehicle	Intravenous exposure: Distilled water Oral exposure: 1% aqueous gum tragacanth
Remarks - Method	Results for individual rats were not included.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg)*	*Mortality*
Intravenous Exposure:			
1 (vehicle control)	10 M	0	0/10
2	10 M	5.00	0/10
3	10 M	7.94	2/10
4	10 M	12.60	8/10
Oral Exposure:			
5 (vehicle control)	10 M	0	0/10
6	10 M	500	1/10
7	10 M	794	4/10
8	10 M	1,260	10/10
9	10 M	2,000	10/10
10	10 M	3,160	10/10

7 Day LD50	*Intravenous exposure*: 10.0 (8.2 – 12.5) mg/kg, terms of salt 9.4 (7.7 – 11.8) mg/kg, terms of base *Oral exposure*: 800 (650 – 970) mg/kg, terms of salt 753 (612 – 913) mg/kg, terms of base
Signs of Toxicity	*Intravenous exposure:* There were 10 deaths, 9 found the morning after medication and 1 on day 2. *Oral exposure*: 35 deaths – 8 on the morning after exposure, 14 on day 2, 4 on day 3, 3 on day 4, 4 on day 5, 2 on day 6
Effects in Organs	*Intravenous exposure*: No gross tissue changes, attributable to exposure to the test substance were observed in those that died. Animals that survived to 7 days – in 3/8 survivors at 7.94 mg/kg and in both of the survivors at 12.6 mg/kg, the tails was necrotic (black and/or tip

sloughing). No other gross tissue changes were observed.

Oral exposure:
Of the 35 animals that died, the lungs of 8 animals were congested throughout. Pitted areas and apparent thickening of the glandular portion of the stomach and adhesions of the stomach to the liver were observed in 12 animals (2 at 794 mg/kg, 6 at 1,260 mg/kg and 4 at 2,000 mg/kg). In 2 of these animals, the stomach contained black material, possibly clotted blood. In another rat that died, a large area of congestion was found in the glandular part of the stomach.

Animals that survived to 7 days (9/10 in group 6 and 6/10 in group 7) no gross tissue changes attributable to exposure to the test substance were observed except in 2 rats at 794 mg/kg (group 7) in which adhesions of stomach to the liver and spleen, and thickening, perforations, and pitted areas in the glandular portion of the stomach were found.

Remarks - Results

Intravenous exposure:
No symptoms were observed at 5 mg/kg or in the control group. Ataxia and partial decrease in motor activity were observed 5 to 10 minutes after injection in all rats at the high dose level and in one rat at the middle dose level. Shortly thereafter, the middle-dose rat and one high-dose rat developed jerky leg movements of short duration and dyspnea, followed, in the latter rat, by a complete absence of motor activity and loss of righting reflex. These two rats and 6 other at high dose level were found dead the next morning. Also, at that time the tail was purple-black in 2 rats at the middle dose level and 1 at the high dose level; this persisted until the end of the study. All other symptoms were gone on day 2.

At 24 hours, a mean body weight gain of 8% had occurred in the controls while there was no change at 5 mg/kg and losses of 6% had occurred at both 7.94 and 12.6 mg/kg. At 7 days, total weight gains of 43%, 35%, 21% and 17% were seen for the same group.

Oral exposure:
Symptoms included wet matted fur, dyspnea, ataxia, partial to complete absence of motor activity and brown exudate around the eyes and nares. Except for the wet matted hair, which was seen only on the day of medication, symptoms were not observed until the morning after exposure. Loose stools were also seen on Day 1 in the cages of the rats given 794 or 1,260 mg/kg, 1 rat appeared emaciated on day 3, and 4 at 1,260 kg/mg and 1 at 794 mg/kg appeared emaciated and/or smaller than normal on day 2. No adverse effects observed in the control group.

At 24 hours, mean body weight gains of 12% and 7% had occurred in the control and 500 mg/kg groups; there was no change at 794 mg/kg, and there were losses of 5%, 8% and 9% respectively, at 1,260, 2,000 and 3,160 mg/kg. At 7 days, total weight gains of 41%, 36% and 27% respectively were noted for the control, 500 and 794 mg/kg groups.

CONCLUSION The notified chemical is toxic via the oral route.

TEST FACILITY Sterling-Winthrop (1976)

B.2. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical at 0.1% concentration.

METHOD OECD TG 403 Acute Inhalation Toxicity
EC Council Regulation No 92/69/EEC B.2 Acute Toxicity (Inhalation)

Species/Strain	Rat/Sprague-Dawley Crl:CD (SD) IGS BR
Vehicle	None.
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol.
Particle Size	Mean mass median aerodynamic diameter - 2.01 μm. Inhalable fraction (< 4 μm) – 85.8% Geometric standard deviation – 1.91.
Remarks - Method	GLP compliant. No significant deviations from the protocol. Test atmosphere concentrations decreased during the exposure period. Following characterisation of the test atmosphere in the absence of animals, the study authors determined that the decrease in test concentration from 5 mg/L to 4.08 mg/L during the exposure period was due to the presence of the animals in the exposure chamber. The study authors attributed this effect to the low percentage of active ingredient in the test material. It was considered that it would only take a small uptake of the active ingredient by each animal to significantly affect the results of exposure. Chemical analysis used the active ingredient contained in each sample removed from the exposure chamber (during the exposure period) to determine the actual concentration of test material in the exposure chamber. The lack of observations seen in animals throughout the study period and the fact that the animals appeared normal from day 1 post-exposure emphasises the fact that conducting a further exposure in an attempt to achieve a concentration of 5 mg/L would not alter the observations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 M, 5 F	33.2	4.08 ± 0.78	0/10

LC50	> 4.08 mg/L/4 hours
Signs of Toxicity	Increased respiratory rate, hunched posture, pilo-erection and wet fur were noted following exposure. Recovery from effects from Day 1 post-exposure.
Effects in Organs	No macroscopic abnormalities detected.
Remarks - Results	Bodyweight development was as expected.

CONCLUSION The notified chemical (at a concentration of 0.1%) is of low toxicity via inhalation.

TEST FACILITY SafePharm (2008)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical at 0.1% w/w concentration

METHOD Similar to OECD TG 404 Acute Dermal Irritation/Corrosion

Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Vehicle	None.
Observation Period	3 days
Type of Dressing	Occlusive
Remarks - Method	Only reactions that differ from the untreated control contact areas (shaved and abraded) were recorded.

RESULTS

Remarks - Results	No signs of erythema or oedema were recorded in any of test or control

animals.

CONCLUSION The notified chemical is non-irritating to the skin at 0.1% w/w concentration.

TEST FACILITY IBR (1983a)

B.4. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion
EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation)

Species/Strain	Rabbit/SPF albino Chbb:HM
Number of Animals	3 F
Vehicle	Moistened with water
Observation Period	49 days
Type of Dressing	Semi-occlusive
Remarks - Method	GLP compliant. No deviations from the protocol.

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	2	2.7	2.7	2	< 14 days	0
Oedema	2.3	2.7	2.7	3	< 14 days	0

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

Remarks - Results

Immediately after exposure, one animal showed very slight erythema with recovery at the 1 hour observation, with whitish spots (potentially from residues of the test substance) visible at the 1 and 4 hour observations. Slight to well-defined erythema was observed in the remaining two animals.

One animal showed well-defined erythema and moderate oedema at the 24, 48 and 72 observations. The remaining two animals showed well-defined erythema and slight oedema at the 24 hour observation, increasing to moderate to severe erythema and moderate oedema at the 48 and 72 hour observations.

Seven days after exposure, moderate to severe erythema and moderate oedema and scab formation was observed for all animals. After 14 days, the scabs on all animals began to partially fall off. At 21 days after exposure, the scab on animal one was still coming off and granulation tissue was visible underneath. The scabs on the remaining two animals had dropped off and the granulation of tissue underneath was visible. At the 28 day observation the scab on one animal was still dropping off. However, no skin reactions were observed in the remaining two animals.

The scab on one animal remained for 7 weeks following exposure to the test substance, and as reversibility of effects was not foreseeable the study was terminated.

Recovery from erythema and oedema was observed in all animals. However, the study authors considered that the persistence of scab formation in one animal may be indicative of tissue damage and the test substance may have a potential corrosive effect on the skin.

CONCLUSION	The notified chemical is irritating to the skin with potential corrosive effects.
TEST FACILITY	Frey-Tox (2007a)

B.5. Skin irritation – human volunteers – tolerability test

TEST SUBSTANCE	Notified chemical at 0.3% concentration
METHOD	Tolerability test
Study Design	The test substance containing the notified chemical at 0.3% concentration was tested undiluted and at a 1:1 dilution for any skin irritant effect on 40 volunteers in the 24 hour patch test.
Study Group	34 F, 6 M; average age 47.8 years
Vehicle	Unknown
Remarks - Method	No evidence of skin irritation in the volunteers. Occluded. The size of the patch chamber was not provided. No details on the volume of test substance applied. Results recorded at 24, 48, and 72 hours
RESULTS	
Remarks - Results	Twenty-four hours after exposure, 13 subjects showed a faint erythema indicating slight irritation due to the plasters used to adhere the patch to the skin. At 48 and 72 hours after exposure – none of the test subjects showed any changes in the test area; no allergic reactions were observed following exposure.
CONCLUSION	The test substance was non-irritating under the conditions of the test.
TEST FACILITY	IVDK (2014)

B.6. Irritation – eye

TEST SUBSTANCE	Notified chemical at 0.1% w/w concentration
METHOD	Similar to OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	6
Observation Period	7 days
Remarks - Method	None.
RESULTS	
Remarks - Results	No signs of irritation were recorded at the 24, 48 or 72 hour observations. Slight conjunctival redness was observed in all animals at one, two and eight hours following exposure to the test substance. A slight increase in conjunctival secretion was observed in three animals at the one and two hour observations, with the effect persisting in two of these animals at the 8 hour observation. All animals showed recovery from conjunctival effects at the 24 hour observation. No irritant effects were observed in the cornea or iris.
CONCLUSION	The notified chemical is non-irritating to the eye at 0.1% w/w concentration.
TEST FACILITY	IBR (1983b)

B.7. Skin sensitisation – Guinea Pig Maximization Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation - GPMT EC Directive 92/69 part 6B Skin Sensitisation - GPMT
Species/Strain	Guinea pig/SPF Albino/Crl:HA
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: < 0.00625% topical: 0.25%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
Vehicle	Water
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-Hexylcinnamaldehyde..
INDUCTION PHASE	Induction Concentration: intradermal: 0.0125% topical: 1%
Signs of Irritation	Skin irritation and necrotic reactions
CHALLENGE PHASE	
1st challenge	Topical: 0.5%
Remarks - Method	GLP compliant. No deviations from the protocol. Freund's complete adjuvant used with intradermal injections Test substance was supplied in a powdered form.

Three preliminary studies were performed to determine the use concentration for the main study. Animals in the first study exhibited strong irritant effects (light greenish discolouration and necrosis following intradermal exposure, and well-defined, moderate to severe and necrosis with dark green to black discolouration observed in a dose-dependent manner following topical exposure) at an intradermal concentration range of 0.625% – 5.0% or a topical concentration range of 25% - 100% preventing selection of appropriate test concentrations.

The test concentration range was decreased (intradermal: 0.1% to 0.5%; topical: 2% - 20%) for the second study. However, partially necrotic skin (necrotic reactions were higher in animals exposed to the test substance at ≥ 0.25% concentration following intradermal exposure and well-defined erythema (with papule formation), moderate to severe erythema, including the presence of brownish necrosis with some weeping observed in a dose-dependent manner following topical exposure) was observed on the animals following exposure.

Following a further reduction in test concentration (intradermal: 0.00625% – 0.05%; topical: 0.125% - 1%), animals exposed to the test substance at 0.00625%, 0.0125%, and 0.025% concentrations exhibited very slight erythema at 24 and 48 hour observations, while animals exposed to 0.05% concentration of the test substance exhibited well-defined erythema at the 24 and 48 hour observations following intradermal exposure. Animals exposed to the test substance topically exhibited no irritation at 0.125% and 0.25%, but very slight erythema at 0.5% and 1% (including development of papules).

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after 1st Challenge*	
		24 h	*48 h*
Test Group	0.5%	0/20	3/20
Control Group	0.5%	0/10	0/10

Remarks - Results

Following exposure to the test substance in the challenge phase, skin irritation was not observed in animals in the test group at the 24 hour observation. However, three (3/20) animals exhibited slight or discrete erythema and papules at the 48 hour observation. Animals in the negative control group did not exhibit signs of irritation.

Negative control areas on animals in the control and test groups did not show signs of irritation.

All animals made the expected gains in body weight over the study period.

CONCLUSION

There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY

Frey-Tox (2007b)

B.8. Repeat dose toxicity

TEST SUBSTANCE

Notified chemical (at 94.1% concentration)

METHOD

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

Species/Strain: Mouse/Charles river CD1

Route of Administration: Oral –diet

Exposure Information: Total exposure days: 90 days
Dose regimen: 7 days per week
Post-exposure observation period:

Vehicle: Rodent maintenance diet

Remarks - Method: Quality assurance statement provided.

Study was designed to establish a maximum dietary dose of the notified chemical for use in a carcinogenic study. Two related preliminary studies were referenced in submitted report's conclusion: Preliminary Assessment of the Toxicity of [notified chemical] following Daily Oral Administration to Mice over 13 weeks; and Absorption of ^{14}C-Octenidine from either feed or from aqueous solution after oral administration to mice.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	20 M, 20 F	0	0/20 M, 0/20 F
low dose	20 M, 20 F	32	0/20 M, 0/20 F
mid dose -1	20 M, 20 F	64	0/20 M, 0/20 F
mid dose - 2	20 M, 20 F	128	1/20 M, 1/20 F
high dose	20 M, 20 F	256	0/20 M, 0/20 F

Mortality and Time to Death

Two mice in the 128 mg/kg bw/day group were found dead (1 female in week 1 and 1 male in week 6). No macroscopic abnormalities were recorded in the male while the female exhibited fluid filled trachea and congestion of the lungs. Both animals exhibited minimal congestion of the liver and the female also exhibited an inflammatory cell infiltration. The effects observed were not considered related to the animals exposure to the test substance by the study authors.

Clinical Observations

Three males in the 256 mg/kg bw/day group and three males in the 128 mg/kg bw/day dose group showed signs of slight distension of the abdomen during the latter half of the study. However, gaseous distension or other reason for this effect was not confirmed following microscopic examination. No other clinical observations were recorded.

Females in the 32 mg/kg bw/day dose group (↓ 14.8%), males in the 64 mg/kg bw/day dose group (↓ 17.9%) and males in the 256 mg/kg bw/day dose group (↓ 17.1%) showed a slight, but statistically significant, lack of bodyweight gain. All other animals exhibited body gains similar to those observed in the control group.

Animals exposed to the test substance showed similar levels of food consumptions as animals in the control group with no statistically significant differences.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

A treatment related decrease in serum globulin (% shown in brackets) with a corresponding effect on serum levels of total protein was observed in male animals in the 64 mg/kg bw/day (↓ 9.1%), 128 mg/kg bw/day (↓ 18.2%) and 256 mg/kg bw/day (↓ 18.2%) dose groups. Female animals in the 32 mg/kg bw/day and 64 mg/kg bw/day dose groups showed a statistically significant increases in serum globulin of 20% and 26.7% respectively. A significant decrease in serum urea (↓ 22.1%) was also observed in high-dose males and the study authors considered that this may have been related to the decrease in serum globulin.

Differences in the percentage of neutrophils, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total bilirubin, levels of creatinine, globulin and urea, haemoglobin, platelet count and red blood cell count, between controls and exposed animals which were statistically significant did not show a dose-response relationship, were small differences only, or were exhibited in one dose group and one sex only. The study authors considered these differences to have arisen by chance.

Effects in Organs

No significant differences in the macroscopic appearance of organs between animals exposed to the test substance and animals in the control group were observed.

Liver weights of males in the 256 mg/kg bw/day dose group were significantly lower (↓ 12.7%) than those of controls. No significant differences at the microscopic level were observed.

Differences in the absolute and relative heart, kidney, testes and ovary weights which were statistically significant did not show a dose-response relationship, were small differences only, or were exhibited in one dose group and one sex only. The study authors considered these differences to have arisen by chance.

Remarks – Results

A change in endogenous gut flora leading to marked gaseous distension and decreased metabolic efficiency and a decrease in the normal rate of bodyweight gain was linked to exposure to the test substance in a preliminary study (Preliminary Assessment of the Toxicity of [notified chemical] following Daily Oral Administration to Mice over 13 weeks). A second preliminary study (Absorption of ^{14}C-Octenidine from either feed or from aqueous solution after oral administration to mice) which compared absorption of the notified chemical from the diet and from aqueous solution showed that absorption of the notified chemical was similar in either case, but were both less than 1% of the total administered. However, while this study showed a decreased incidence of gaseous distension of the gastrointestinal tract, reduced bodyweight gain, reduced efficiency of food utilisation and low serum globulin in males in the 64 and 128 mg/kg bw/day dose groups and 256 mg/kg bw/day dose group were seen as a feasible indication of poor nutrient absorption from the gastro-intestinal tract by the study authors and was considered to be treatment related. The lack of bodyweight gain in females in the 32 mg/kg bw/day dose group was considered to be by chance as the effect was not observed in other treated females or males in the 32 mg/kg bw/day dose group.

The study authors established a No Observed Effect Level of 32 mg/kg bw/day. The slight reduction in bodyweight gain while considered to be treatment related by the study authors showed no real dose response relationship. The slight reduction in bodyweight gain is predominantly due to the anti-microbial effects of the notified chemical on the gut flora, and hence is not a systemic effect, and is also not relevant to exposure pathways other than oral. Additionally although there were statistically significant decreases in the serum globulin levels in male animals there were larger increases in the female animals that were not dose dependent.

No information on the historical control levels was provided but considering the large increases seen in the female animals the toxicological relevance of the decreases in the male animals is likely to be low. The decrease in liver weights and serum urea levels in male animals in the 256 mg/kg bw/day dose group was considered to be potentially adverse.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 128 mg/kg bw/day in this study, based on adverse effects in males at the higher dose.

TEST FACILITY Sterling-Winthrop (1985)

B.9. Oral absorption

TEST SUBSTANCE ^{14}C-labelled [pyridine-2,6-^{14}C] octenidine dihydrochloride and non-labelled octenidine dihydrochloride

METHOD
- Species/Strain: Rat/Wistar [Crl:WI(WU)]; male
- Vehicle: Water
- Route of Administration: Oral - gavage
- Study Duration: Groups 1 and 3 – 7 days; Groups 2 and 4 – 24 hours
- Remarks - Method: GLP compliant. No signification deviations from the study protocol.

^{14}C-measurements were taken from the following tissues and organs:
Groups 1 and 3 – urine, faeces, lungs, liver, kidneys, stomach, small intestine, large intestine.
Groups 2 and 4 – blood

RESULTS

Group	*Number and Sex of Animals*	*Dose* *test substance (mg/kg bw)*	*radioactivity (MBq/kg BW)*	*Mortality*
1	4 M	10	5	0/4
2	4 M	10	5	0/4
3	4 M	100	5	1/4
4	4 M	100	5	0/4

Time (hours)	*Mean percentage (%) of radioactive dose recovered in* *Faeces*	*Urine*	*Cage Wash*	*Mean percentage (%) of total radioactive dose recovered*
Low Dose (Group 1)				
24	83.86 ± 17.96	0.9217 ± 0.3721	0.2413 ± 0.3677	85.03 ± 18.66
48	10.55 ± 7.338	0.0848 ± 0.0956	0.0261 ± 0.0466	10.66 ± 7.284
72	0.4109 ± 0.4591	0.0413 ± 0.0494	0.0326 ± 0.0596	0.4848 ± 0.4634
96	0.1913 ± 0.1534	0.0283 ± 0.0336	0.0152 ± 0.0304	0.2348 ± 0.1975
120	0.0565 ± 0.0557	0.0239 ± 0.0313	0	0.0804 ± 0.0814
144	0.0565 ± 0.0687	0.0174 ± 0.0293	0.0087 ± 0.0174	0.0826 ± 0.1110
168	0.0304 ± 0.0379	0.0087 ± 0.0123	0.0065 ± 0.0130	0.0457 ± 0.0616
Total of the means	95.15 ± 14.48	1.117 ± 0.5589	0.3184 ± 0.510	96.62 ± 17.00
*High Dose (Group 2)**				
24	53.84 ± 25.44	0.8553 ± 0.0646	0.0979 ± 0.0396	66.58 ± 11.66
48	16.72 ± 12.79	0.1064 ± 0.1003	0.0340 ± 0.0399	20.50 ± 12.78
72	1.091 ± 1.040	0.0319 ± 0.0175	0.0575 ± 0.0986	0.6156 ± 0.2408
96	0.5021 ± 0.7576	0.0213 ± 0.0110	0.0021 ± 0.0043	0.1504 ± 0.1575
120	0.0362 ± 0.0401	0.0149 ± 0.0081	0.0085 ± 0.0120	0.0624 ± 0.0714
144	0.0447 ± 0.0512	0.0085 ± 0.0070	0.0087 ± 0.0170	0.0596 ± 0.0889
168	0.0319 ± 0.0473	0.0085 ± 0.0120	0.0043 ± 0.0085	0.0511 ± 0.0812

Time (hours)	*Mean percentage (%) of radioactive dose recovered in*			*Mean percentage (%) of total radioactive dose recovered*
	Faeces	*Urine*	*Cage Wash*	
Total of the means	88.01 ± 16.56	1.089 ± 0.1408	0.1490 ± 0.1381	88.01 ± 11.86

** Note: data from animal 3104 and 4104 was excluded from the mean*

Time (hours)	*Mean percentage (%) of radioactive dose recovered in blood*	
	Low dose (Group 2)	*High dose (Group 4)*
2	0.0708 ± 0.0555	0.0183 ± 0.0217
4	0.0103 ± 0.0133	0.0121 ± 0.0154
8	0.0040 ± 0.0081	0.0197 ± 0.0104
24	0.0110 ± 0.0149	0.0012 ± 0.0023
Mean over time	0.0961 ± 0.0565	0.0513 ± 0.0265

Organ	*Mean percentage (%) of radioactive dose recovered in organs*	
	Low dose (Group 1)	*High dose (Group 3)*
Lung	0.0007 ± 0.0007	0.0003 ± 0.0001
Kidneys	0.0114 ± 0.0151	0.078 ± 0.0025
Liver	0.0992 ± 0.0445	0.2142 ± 0.0593
Stomach	0.0023 ± 0.0010	0.0201 ± 0.0068
Small Intestine	0.0052 ± 0.0026	0.0183 ± 0.0019
Large Intestine	0.0005 ± 0.0007	0.0013 ± 0.0012
All organs	0.12 ± 0.06	0.27 ± 0.07

Note: data from animal 3104 was excluded from the mean

Remarks – Results

One animal in Group 3 (high-dose) exhibited poor health including reduced food and water consumption following exposure to the test substance. This animal was sacrificed on Day 3 of the study period and replaced with an animal from Group 4 (high-dose). Results from these animals were not included in the mean calculations. There were no other unscheduled deaths. No other animals exhibited similar health effects following exposure. No treatment related effects were noted during necropsy of animals in the low- and high-dose groups (group 1 and 3). All surviving animals made the expected body weight gains.

Limit of quantification (LOQ) for organ, blood and faeces samples was assumed to 94 dpm (background mean calculated as 40.82 ± 17.73 dpm), and 50 dpm for urine and cage wash samples (background mean calculated as 32.96 ± 5.55 dpm).

The test substance was predominantly excreted via the faeces in the low- and high-dose groups (95.15 ± 14.49% and 88.01 ± 16.56% respectively) within the first 24 (83.87 ± 17.97% and 53.84 ± 25.44%, low- and high-dose groups respectively) and 48 hours (10.55 ± 7.338% and 16.71 ± 12.79% low- and high-dose groups respectively). Excretion of the test substance via urine was much less (1.117 ± 0.5589% and 1.009 ± 0.1408%, low- and high-dose groups respectively), with the majority again excreted within the first 24 hours. Recovery of the tests substance recovered from cage wash samples showed a similar pattern to urine (0.3184 ± 0.5110% and 0.1490 ± 0.1381% recovered from low- and high-dose groups respectively, with the majority recovered after 24 hours).

Less than 0.1% of the test substance was recovered from blood over the 24 hour study period (Groups 2 and 4). Recovery was highest after 2 hours in the low-dose group (0.0708 ± 0.0555%) and 8 hours in the high-dose group (0.0197 ± 0.0104%).

The liver of animals in the high-dose group (Group 3) exhibited the highest recovery of test substance (0.2142 ± 0.0593%). Mean recovery of the test substance across all organs was 0.12 ± 0.06% for animals in the low-dose group and 0.27 ± 0.07% for animals in the high-dose group.

Recovery rates (of radioactive material) of the dose applied were 96.46 ± 15.04% and 89.33 ± 16.62% (low- and high-dose groups respectively). The recovery rate in the high-dose group is below that recommended under the test guideline (< 90%). The study authors considered that this low rate may be due to analytical loss. In addition, the study authors noted that the animal in the high-dose group that died prematurely exhibited reduced food and water consumption leading to a corresponding reduction in faecal excretion during the first 48 hours of the study period (and before the animal was sacrificed). Based on the observations in other animals, the lack of faecal matter from this animal was expected to reduce the level of radioactive material recovered.

The total systemic absorption of the test substance (adding the amount in the organs, the urine and the cage wash) was 1.56% for the low (10 mg/kg bw) dose group and 1.51% for the high (100 mg/kg bw) dose group.

CONCLUSION The notified chemical is not expected to show significant systemic bioavailability following oral exposure under the conditions of the test.

TEST FACILITY Fraunhofer (2010)

B.10. Dermal Absorption

TEST SUBSTANCE ^{14}C-labelled [pyridine-2,6-^{14}C] octenidine dihydrochloride (0.1 wt%)

METHOD
- Species/Strain: Rat/Wistar (Crl:WU); male
- Vehicle: Formulation ingredients of octenidine product (99.9%) without octenidine dihydrochloride
- Exposure Period: 6 hours
- Observation Period: 0, 18, 42 and 162 hours post exposure
- Type of Dressing: Non-occlusive
- Remarks - Method: GLP compliant. No signification deviations from the study protocol.

The application area (~ 9.6 cm^2) was defined using an O-ring affixed to the clipped skin using cyanoacrylate glue. An amount of 10.40 µL/cm^2 (86.4 µg of test substance) was applied to the skin within the O-ring. At the end of the exposure period, the treated skin was washed with an aqueous solution of soap and a swab. Swabs containing the soap solution were retained. A fresh dressing was applied for animals within the 24, 48 and 168 hour observation groups.

No analysis of cage wash performed.

RESULTS

Group	*Number and Sex of Animals*	*Observation period post exposure (hours)*
Control	2 M	0
1	4 M	0
2	4 M	18
3	4 M	42
4	4 M	162

Group	*Mean percentage of radioactive dose recovered in*				
	Swabs	*Gauze covers*	*O-rings*	*Spacers*	*Adhesive plaster*
1	65.2 ± 13.7	1.7 ± 2.0	1.37 ± 1.35	0.21 ± 0.39	-
2	81.2 ± 7.5	4.9 ± 3.0	0.55 ± 0.69	0.11 ± 0.12	1.08 ± 1.30
3	71.3 ± 4.2	4.8 ± 4.2	0.41 ± 0.34	0.03 ± 0.02	0.87 ± 0.40

Group	Mean percentage of radioactive dose recovered in				
	Swabs	*Gauze covers*	*O-rings*	*Spacers*	*Adhesive plaster*
4	70.7 ± 10.7	9.2 ± 10.7	0.44 ± 0.15	0.08 ± 0.05	1.71 ± 0.77
Mean percentage of unabsorbed dose:					
	72.1 ± 10.5	5.2 ± 6.0	0.69 ± 0.81	0.11 ± 0.2	1.2 ± 0.9
Mean percentage (%) of administered dose that was unabsorbed:				78.93 ± 9.78	

Group	Mean percentage (%) of radioactive dose recovered in		Percentage (%) of administered dose that was absorbable
	Stratum corneum	*Viable skin*	
1	3.86 ± 3.79	7.54 ± 5.36	11.41 ± 6.71
2	1.06 ± 0.87	4.24 ± 0.45	5.29 ± 0.91
3	1.18 ± 0.56	5.72 ± 1.58	6.89 ± 1.45
4	0.29 ± 0.08	8.34 ± 4.44	8.64 ± 4.38
Total	1.60 ± 2.24	6.46 ± 3.60	8.06 ± 4.35

Hours after exposure	Mean radioactive dose (Bq) recovered in	
	Faeces	*Urine*
6	2.75 ± 9.25	7.71 ± 5.85
24	3.82 ± 13.05	43.16 ± 26.38
48	0.04 ± 0.13	12.28 ± 6.27
72	49.00 ± 33.16	0.26 ± 0.39
96	24.92 ± 19.21	11.29 ± 5.82
120	16.57 ± 15.46	13.85 ± 6.65
144	43.68 ± 49.92	8.73 ± 3.23
168	23.07 ± 46.11	49.14 ± 9.98
Total mean excreted dose (Bq)	44.95 ± 98.70	66.56 ± 65.36
Mean percentage (%) of administered dose	0.017 ± 0.038	0.026 ± 0.025

Hours after exposure	*Mean radioactive dose (Bq) recovered from blood*	*Mean percentage (%) of total dose*
3.5	51.9 ± 67.4	$0.02 \pm 7.7 \times 10^{-6}$
4	10.7 ± 15.6	$0.004 \pm 1.6 \times 10^{-6}$
6	0	0
12	32.7 ± 65.4	$0.013 \pm 4.8 \times 10^{-6}$
24	4.4 ± 8.9	$0.002 \pm 6.6 \times 10^{-7}$
48	97.1 ± 46.6	$0.037 \pm 1.4 \times 10^{-5}$
168	16.2 ± 32.5	$0.006 \pm 2.4 \times 10^{-6}$
Mean percentage (%) of administered dose:	0.012 ± 0.019	

Remarks - Results

Mean recovery of the test substance was between 79% and 97%. The majority of the applied dose (72.1 ± 10.5%) was rinsed off the skin at the end of the 6 hour exposure period. The mean amount of test substance present in the stratum corneum and viable skin (dermis) after 6 hours of exposure was 3.86 ± 3.79% and 7.54 ± 5.36%, respectively. The percentage of test substance in the stratum corneum decreased over time (1.06 ± 0.87%, 1.18 ± 0.56% and 0.29 ± 0.08% at 18, 42 and 162 hours (respectively) after washing).

The percentage of test substance in viable skin showed a decrease 18 hours after washing (4.24 ± 0.45%). However, this value increased over the remainder of the observation period (5.72 ± 1.58% and 8.34 ± 4.44% 42 hours and 162 hours (respectively) after washing).

The trend observed in viable skin was reflected in the overall percentage of administered dose that was absorbable (11.41 ± 6.71%, 5.29 ± 0.91%, 6.89 ± 1.45% and 8.64 ± 4.38% at the observations made 0, 18, 42 and 162 hours (respectively) after washing) indicating that dermal absorption of the test substance from the stratum corneum increases over time, although not significantly. Overall the mean percentage of absorbed dose was 8.06 ± 4.35%.

Of the test substance that was absorbed, < 1% was recovered from the faeces, urine, and blood (0.017%, 0.027% and 0.012% respectively). The amount of absorbed ^{14}C-labelled [pyridine-2,6-^{14}C] octenidine dihydrochloride increased as the exposure time increased and was 0.0082%, 0.013%, 0.061% and 0.135% for groups 1, 2, 3 and 4, respectively. Therefore, the systemic absorption after 168 hours was 0.135%, although this value does not include radioactive material that may have been absorbed and was lost as carbon dioxide via respiration, contained in organs or in cage materials.

Recovery rates (of radioactive material) of the dose applied were below that recommended under the test guideline. The study authors considered that the low rates (< 90%) observed in some animals may be due to analytical loss (limit of quantification was recorded as 66 dpm). No cage wash was performed and this may account for some of the loss in recovery of radioactivity.

One animal removed the gauze covering the exposure site prior to washing (before the end of the exposure period) and the study authors considered that this contributed to the low overall rate of recovery observed in this animal. This animal was not included in the calculations looking at total recovery across all animals.

All animals made the expected body weight gains. As no gross tissue changes, attributable to exposure to the test substance were observed during necropsy, and no significant differences in organ weights were observed, recovery of the tests substance from organs was not performed.

CONCLUSION

The systemic absorption (cumulative amounts in the blood, urine and faeces) of ^{14}C-labelled [pyridine-2,6-^{14}C] octenidine dihydrochloride after 168 hours was 0.135%.

TEST FACILITY

Fraunhofer (2008)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE

Notified chemical (at 94.1% concentration)

METHOD

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

Species/Strain	*Salmonella typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
Metabolic Activation System	S9 fraction from Aroclor induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0.03, 0.1, 0.3, 1, 3 µg/plate b) Without metabolic activation: 0.03, 0.1, 0.3, 1, 3 µg/plate
Vehicle	Dimethyl sulfoxide/Ethanol
Remarks - Method	GLP compliant Preliminary assays established the dose range chosen in the two experiments. In the first assay (dose concentrations: 100, 333, 1,000, 3,333, and 10,000 µg/plate), complete inhibition was observed at all doses on both tester strains (TA100 and TA1538). In the second assay (dose concentrations: 1, 3, 10, 33, and 100 µg/plate), complete inhibition of the tester strains (TA100 and TA1538) was observed at the two highest doses with extensive and moderate inhibition observed at 10 µg/plate and 1 µg/plate respectively. The study was run twice as the negative control in the first test was outside the acceptance criteria (one standard deviation from the mean).

Positive controls: without metabolic activation – Sodium azide (TA1535, TA100), 9-aminoacridine (TA1537), 2-Nitrofluorene (TA1538, TA98); with metabolic activation – 2-anthramine.

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	> 3	> 3	negative
Present				
Test 1	≥ 10	> 3	> 3	negative

Remarks - Results

No significant dose related increase in the number of revertants, in the presence or absence of metabolic activation was observed.

No information was provided regarding the effect of the test substance on the background bacterial lawn, in the absence or presence of metabolic activation.

Positive and negative controls performed as expected.

CONCLUSION

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

TEST FACILITY

Pharmakon (1982)

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

TEST SUBSTANCE

Notified chemical (at 94.1% concentration)

METHOD

Similar to OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test

Species/Strain: Mouse (CD-1)

Cell Type/Cell Line: Lymphocytes/ L5178Y TK +/-

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

Vehicle: Deionized water

Remarks - Method

5 - 2,500 µg/ml; test substance remained soluble at 39 µg/ml, with a milky suspension formed at ≥ 78 µg/ml; 24 h after exposure, few cells survived the 5 µg/ml treatment and no survivors at ≥ 10 µg/ml. Toxicity test was repeated with lower concentration range 0.039 – 20 µg/ml; reduction in cell count at 5 µg/ml and complete lethality at 10 µg/ml; mutation assay initiated with a concentration range of 0.156 – 20 µg/ml.

Positive controls: without metabolic activation – ethylmethane sulfonate; with metabolic activation – dimethylnitrosamine.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.313, 0.625, 1.250, 2.5, 3.75	4	20
Present			
Test 1	2.5, 3.75, 5, 7.5, 10	4	20

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in: Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 5	> 3.75	> 3.75	negative
Present				
Test 1	≥ 5	> 10	> 10	negative

Remarks - Results	Three trials of the study were performed. However, the first test was contaminated and the second test failed to meet acceptance criteria (cloning efficiency (53%) was too low). Results from the third trial met all acceptance criteria. In the absence of metabolic activation, a significant increase in mutant frequency was observed at the lowest dose tested (0.313 µg/ml). However, this result was not considered biologically relevant by the study authors as the low number of total mutant clones observed at this dose combined with a low relative cloning efficiency (66.3%) may have artificially increased the mutant frequency especially when compared to the mutant frequency of the next highest dose, 0.625 µg/ml (where a higher number of mutant clones (39) and a higher relative cloning efficiency (99.4%) were observed). The frequency of mutation observed at doses ≥ 0.625 µg/ml were similar to background levels. No dose-response relationship in mutation frequency was observed. In the presence of metabolic activation, no significant increase in mutation frequency was observed. The positive and negative controls performed as expected in the presence and absence of metabolic activation.
CONCLUSION	The notified chemical was not clastogenic to mouse lymphoma cells treated *in vitro* under the conditions of the test.
TEST FACILITY	LBI (1979)

B.13. Genotoxicity – *in vivo* Mammalian Erythrocyte Micronucleus Test

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 474 Mammalian Erythrocyte Micronucleus Test
Species/Strain	Mouse (CD-1)
Route of Administration	Oral – gavage
Vehicle	Gum tragacanth (0.25% w/v)
Remarks - Method	GLP compliant. Animals were exposed to the test substance once. Samples of bone marrow were taken at 24, 48 and 72 hours after exposure (5 M and 5 F per group). Two preliminary dose-finding studies were performed. In the first preliminary test, animals were exposed to a concentration range of 312.5, 625, 1,250, 2,500 and 5,000 mg/kg (2 M, 2 F per group). Forty percent of animals had died at the 23 hour observation (including one male and one female in the low dose group) with all animals dead at the 72 hour observation. In the second preliminary test, animals were exposed to a concentration range of 32, 64, 128 and 256 mg/kg (5 M, 5 F per group). One male and one female in the low dose group, and one female exposed to 64 mg/kg died 30 minutes following exposure. A second female exposed to 64 mg/kg was also found dead 3 hours after exposure, two females were found dead at the 47 hour observation (exposed to ≥ 128 mg/kg) and one female exposed to 128 mg/kg was found dead at the 50 hour observation. No other deaths were observed in animals exposed to the test substance. One animal in the control group was found dead 25.5 hours after the start of the study. However, no other adverse effects were observed in the control group.

Based on the preliminary tests, the study authors determined that a dose of 32 mg/kg was not expected to cause any deaths.

Group	*Number and Sex of Animals*	*Dose (mg/kg)*	*Sacrifice Time (hours)*
I (vehicle control)	15 M, 15 F	-	24, 48, 72
II (low dose)	15 M, 15 F	32	24, 48, 72
V (positive control, M)	15 M, 15 F	2	24, 48, 72

M=mitomycin C.

RESULTS

Doses Producing Toxicity	> 32 mg/kg
Genotoxic Effects	negative
Remarks - Results	Animals treated with the test substance did not show any significant increases in the number of micronucleated polychromatic or normochromatic erythrocytes over the study period. Positive and negative controls performed as expected. There was no evidence provided that the notified chemical reached the bone marrow of the treated animals. Additionally absorption of the notified chemical through the gastrointestinal tract is expected to be low based on radio labelling studies that suggest it is less than 1% of the administered dose (see B.8 repeat dose toxicity for further details on the absorption).

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* mammalian erythrocyte micronucleus test.

TEST FACILITY Sterling-Winthrop (1984)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Biodegradability

TEST SUBSTANCE	Octenidine dihydrochloride
METHOD	OECD TG 301 D closed bottle test.
Inoculum	Domestic sewage
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical oxygen demand (ThOD)
Remarks - Method	The study was conducted as part of a wider study on the biodegradability of microbiocidal agents. The difference between the oxygen reduction in a blank solution and one of the test item were ascertained analytically. The concentration for the test item was 0.1 mg/L, which was considered to be below the concentration to cause microbial inhibition. .Sodium benzoate was used as a reference.

RESULTS

Biodegradability (% of ThOD)	
Day 5	*Day 28*
100	100

Remarks - Results	There is insufficient detail to determine whether the reference material sufficiently degraded and if the O_2 depletion in the inoculum blank was below 1.5 mg O_2/L.
CONCLUSION	Even though it is not clear to whether all validity criteria were met, based on 100% biodegradation in 5 days, the notified chemical is considered to be readily biodegradable.
TEST FACILITY	Zollner et al (1995)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Octenidine dihydrochloride (98.9%)
METHOD	OECD TG 203 Fish, Acute Toxicity Test with no significant deviations. Static.
Species	Zebra fish (*Brachydanio rerio*)
Exposure Period	96 h
Auxiliary Solvent	None.
Water Hardness	2.09 mmol $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	None.

RESULTS

Concentration mg/L Nominal	*Number of Fish*	*Mortality 96 h*
0	7	0
0.1	7	0
0.18	7	0
0.32	7	5

Concentration mg/L Nominal	*Number of Fish*	*Mortality 96 h*
0.58	7	7
1.05	7	7

EC50 0.08 mg/L (Calculation method not specified).

Remarks – Results Based on nominal concentration values, the EC50 was calculated to be 0.18 – 0.32 mg/L. However approximately 53% of the nominal concentration depleted over the 96 h of the experiment, implying a factor of difference between the nominal and measured concentrations of about 2. Results were hence based upon adjustments of the nominal concentration to 47%. The oxygen concentration was greater than 60%.

CONCLUSION The notified chemical is very toxic to fish.

TEST FACILITY Hydrotox GmbH (2003a)

C.2.2. Acute immobilisation test

TEST SUBSTANCE Octenidine dihydrochloride (98.9%)

METHOD OECD TG 202 *Daphnia* sp. Acute Immobilisation Test.
Static.

Species *Daphnia magna*
Exposure Period 48 h
Auxiliary Solvent Dimethylformamide (DMF 0.1 mL/L)
Water Hardness 144 mg $CaCO_3$/L (moderately hard)
Analytical Monitoring LC/MS/MS
Remarks - Method Four replicates of daphnia were exposed to five nominal test concentrations of the test substance, together with a negative control. An additional two vessels for each of the 3.0, 5.9 and 11.5 µg/L concentrations were used for chemical analysis. For determination of the EC100, vessels with concentrations of 11.5, 16.1 and 22.5 µg/L were used.

RESULTS

Concentration µg/L	*Number of D. magna*	*Number Immobilised*	
		24 h [acute]	*48 h [acute]*
0	20	0	0
3.0	20	0	0
4.2	20	0	0
5.9	20	0	0
8.2	20	3	4
11.5	20	3	11

EC50 5.2 – 7.2 µg/L (Measured)
NOEC 3.7 µg/L (Measured)
Remarks – Results Oxygen saturation was maintained between 8.2 – 8.5 mg/L. Similarly temperature, and pH were all maintained in acceptable ranges. The lowest concentration producing 100% immobility was nominally 22.5 µg/L (measured concentration of 14.2 µg/L). The deviation of the measured concentrations from the nominal concentrations was 63%; this was factored into the calculation of the EC50 (5.2 – 7.2 µg/L) and the highest concentration causing no immobility (3.7 µg/L). All validity criteria were met.

CONCLUSION The notified chemical is very toxic to invertebrates.

TEST FACILITY Hydrotox GmbH (2003b)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Octenidine dihydrochloride (100%)

METHOD OECD TG 211 *Daphnia* sp. Reproduction Test – semi static
Species *Daphnia magna*
Exposure Period 21 days *[chronic study]*
Auxiliary Solvent Dimethylformamide (DMF 0.1 mL/L)
Water Hardness 144 mg $CaCO_3$/L (moderately hard)
Analytical Monitoring LC/MS
Remarks - Method Five nominal test concentrations of the test substance were used: 0.15, 0.41, 1.10, 2.96, 8.00 µg/L, together with a negative control of dilution water only. Ten replicates of one *Daphnia* each were used for each concentration. *Daphnia* were exposed to the substance and the test solutions being exchanged three times each week. At the start of the test and at each renewal, samples of the two highest concentrations (2.96, 8.00 µg/L) were taken from the test preparation before distribution to each replicate, and at representative renewals samples were also taken from the 'aged' highest concentration. These samples were measured for the concentration of the chemical. (Analytical requirements restricted quantification to the two highest concentrations for the fresh media and the highest for the aged medium).

RESULTS

Nominal concentration (µg/L)	*Parental survival*	*Growth (mean length on day 21)*	*Cumulative offspring per female (mean)*	*Intrinsic rate of increase*
0	100	0.45 ± 0.02	89.6 ± 9.6	0.329 ± 0.012
0.15	100	0.46 ± 0.03	91.7 ± 6.9	0.340 ± 0.013
0.41	100	0.45 ± 0.01	95.3 ± 17.3	0.328 ± 0.012
1.10	100	0.44 ± 0.03	82.8 ± 11.1	0.328 ± 0.014
2.96	100	0.46 ± 0.02	91.0 ± 10.7	0.315 ± 0.023
8.00 (5.57 geometric mean)	100	0.47 ± 0.02	80.3 ± 17.0	0.318 ± 0.025

EC50 > 5.57 µg/L (measured)
NOEC Not comprehensively determined.
Remarks - Results The mean measured test concentrations of the freshly prepared test solutions of the two highest concentrations were in the range 78 – 105 % of the nominal concentration. The concentration of the aged solution of the highest concentration decreased to 43 – 63 % of its nominal starting concentration (8.00 µg/L). The geometric mean of the highest concentration was calculated to be 5.57 µg/L. Temperature, oxygen saturation, and pH were all maintained in acceptable ranges. A NOEC was determined but insufficient detail was provided to ascertain the accuracy of the result. All validity criteria were met.

CONCLUSION The notified chemical is not toxic under the conditions of the test.

TEST FACILITY Fraunhofer (2007b)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE Octenidine dihydrochloride (98.9%)

METHOD OECD TG 201 Alga, Growth Inhibition Test
92/69/EWG,C.3
Species Green alga (*Scenedesmus subspicatus*)
Exposure Period 72 hours

Concentration Range	Nominal: 0.13 – 2.0 mg/L Actual: 0.057 – 0.22 mg/L
Auxiliary Solvent	N,N-dimethylformamide (DMF) 1 mL/L
Water Hardness	140-144 mg $CaCO_3$/L
Analytical Monitoring	LC/MS/MS
Remarks - Method	The nominal concentrations 20, 36, 65, 117 and 210 µg/L of the chemical were used. These concentrations were based on a preliminary range study.

RESULTS

Biomass		*Growth*	
EbC50 *µg/L at 72 h*	*NOEC* *µg/L*	*<ErC50>* *µg/L at 72 h*	*NOEC* *µg/L*
15.1 (Measured) (8.8 – 22.3)*	8.4	23.1 (Measured) (13.4 – 39.1)*	8.4

* 95% confidence limit

Remarks - Results	Temperature and pH were all maintained in acceptable ranges In vessels without alga, the measured concentration of the substance at 72 h was 41.9 % of the nominal concentration, while in those vessels with alga the measured concentration of the substance at 0 was 30 – 37 % of the nominal concentration, and at 72 h below the level of detection. In order to take into consideration depletion of the substance, calculations were conducted with the figure of 41.9 % of the nominal concentrations. Cell concentration in the controls increased by a factor of 27.5.
CONCLUSION	The notified chemical is toxic to alga.
TEST FACILITY	Hydrotox GmbH (2003c)

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