Existing Chemical
Secondary Notification
Assessment Report
STD/735S

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Australian Government
Department of Health
National Industrial Chemicals
Notification and Assessment Scheme

D-glucitol, 1-deoxy-1-(methylamino)-, N-C10-16 acyl derivatives

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

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Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This scheme was established by the Industrial Chemicals (Notification and Assessment) Act 1989 (the Act) to aid in the protection of the Australian people and the environment by assessing the risks of industrial chemicals, providing information and making recommendations to promote their safe use. NICNAS assessments are carried out by staff employed by the Australian Government Department of Health in conjunction with the Australian Government Department of the Environment and Energy.

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

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Director NICNAS

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Background

D-glucitol, 1-deoxy-1-(methylamino)-, N-C10-16 acyl derivatives, Chemical Abstracts Service (CAS) Number 173145-38-5, was notified by Procter and Gamble Australia Pty Ltd (Procter and Gamble) in 1999.

NICNAS assessed the notified chemical as NA/735, 'C12-14 linear glucose amide' under a standard notification category and it is now listed on the Australian Inventory of Chemical Substances (AICS).

Original assessment of the notified chemical was for use in a finished domestic dishwashing detergent, without reformulation.

In 2015, Clariant Australia Pty Ltd (the applicant), advised NICNAS of its intention to import the notified chemical for use in personal care products and household cleaning products, following reformulation in Australia. The new introducer's proposed introduction volumes significantly exceeded those previously assessed.

Secondary notification was required in accordance with section 65 of the Act because the risk management measures previously recommended by NICNAS in the original report may no longer manage the risks arising from the new exposure scenarios.

This secondary notification assessment focuses on the new data provided and reviews the potential risks to human health and the environment from exposure to the notified chemical under the new circumstances.

Exempt information (Section 66 of the Act)

No application for exempt information was made.

Importation/manufacturing volume and uses

The notified chemical is a UVCB substance as defined in section 6 of the Act. It was assessed for introduction to Australia as a non-ionic surfactant (at a concentration of 1.43%) in a finished domestic dishwashing liquid with no reformulation occurring in Australia. In the new chemical assessment, the import volume was notified as 27 tonnes per annum. Today, Procter and Gamble no longer imports the original products and has no plan to reintroduce the notified chemical in the near future.

The applicant has submitted the following information for the secondary notification assessment on C12-14 linear glucose amide:

- it will be imported into Australia at significantly increased volumes, up to 162 tonnes per annum
- it will be imported at higher concentrations than initially assessed (up to 55%)
- the notified chemical will be reformulated for proposed different end use as a surfactant in:
    - rinse-off cosmetic products at ≤ 7% concentration
    - household cleaning products at ≤ 12% concentration.

Human health effects

The applicant submitted a toxicological study for reproductive toxicity for the secondary notification assessment. The study found the notified chemical is not toxic to fertility or development but has the potential to cause adverse effects in parental animals upon prolonged exposure (reduction in body weight gains and in feed consumption in females).

These results confirm the findings of a developmental toxicity study submitted for the new chemical assessment: the notified chemical did not cause developmental toxicity at a dose where maternal toxicity was observed.

Based on data submitted at the time of the new chemical assessment, toxicokinetic studies showed a major component (C12 glucose amide) of the notified chemical and a C18 analogue are both readily absorbed from the digestive tract and distributed widely throughout tissues, but absorption of the major component through the skin is minimal.

The notified chemical has low acute oral and dermal toxicity in animals. Although no acute inhalation toxicity data were available, an analogue (D-glucitol, 1-deoxy-1-(methylamino)-, N-C8-10 acyl derivs., CAS No. 1591782-62-5), notified as a new chemical in 2016, has been found to be harmful if inhaled. Therefore, there is the potential for the notified chemical to cause toxic effects through inhalation.

The notified chemical is a slight skin irritant in rabbits and a severe and persistent irritant to rabbit eyes. It is not a skin sensitiser in guinea pigs.

In a subchronic oral study in rats, the notified chemical has the potential to cause clinical changes and effects on body weight gain on prolonged exposure consistent with the findings of the reproductive toxicity study.

The notified chemical was not genotoxic in a number of in vitro and in vivo studies, although positive results were observed in an in vitro study of chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of metabolic activation at a concentration where significant toxic effects occurred.

Occupational exposure and health risks

The notified chemical was originally introduced in dishwashing liquid at a concentration of 1.43% in bottles with no reformulation or repackaging occurring in Australia. Workers employed in the transport of the bottles (shipped in cartons within containers) and retail workers involved in handling the dishwashing liquid at sales outlets were not expected to be exposed to the chemical except in case of an accidental spill.

New operational procedures for formulating and handling the C12-14 linear glucose amide were notified for this secondary notification assessment, and thus the occupational health risks needed to be reassessed. Based on the studies submitted at the time of the new chemical assessment, the notified chemical is a slight skin irritant, and is severely irritating to eyes. Imported C12-14 linear glucose amide (at concentrations of 20% to 55%), will be blended locally into reformulated products (containing $\leq$ 12% notified chemical) for distribution to sales outlets.

Occupational exposure during transport and warehousing is limited to accidental release. There is potential for dermal, ocular and inhalation exposure to the notified chemical ($\leq$ 55% concentration) during reformulation and associated activities at the blending sites. Exposure to the notified chemical is expected to be minimised with automated processes and the use of personal protective equipment (PPE); therefore, the risk to the health of workers from the handling of the notified chemical is not considered to be unreasonable.

There is also potential for exposure to the notified chemical in end-use products (≤ 12% concentration) in professions where services involve the application of cosmetic products to clients or in the cleaning industry. At the time of the original assessment, the end-use product (1.43% concentration) was not in occupational use. The main route of exposure is expected to be dermal, while ocular exposure is also possible.

Professional end-users may use some PPE to minimise repeated exposure and, therefore, in combination with good hygiene practices, the 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 (see Public exposure and health risks below).

The notified chemical has slight skin irritant and severe eye irritant potential. However, the hazards associated with eye and skin irritation are likely to be offset by the low proposed use concentration (≤ 12%) and use pattern of the notified chemical. Consequently, the potential risk from the use of the notified chemical is considered to be low.

Public exposure and health risks

For this secondary assessment, the chemical was notified for use in consumer applications in rinse-off cosmetic and household cleaning products, at up to 12% concentration.

For these uses, the principal route of exposure will be dermal, while accidental ocular exposure is also possible. The notified chemical has slight skin irritant and severe eye irritant potential. The main risk of irritation is expected from use of cosmetic products containing the notified chemical. However, given the low proposed use concentration in cosmetics (maximally 7%) and use in rinse-off cosmetics only, significant irritation is not expected. The eye irritation risk associated with use of the notified chemical in consumer products may be further minimised by the inclusion of directions for use, to warn against eye contact.

The public may also experience repeated exposure to the notified chemical at up to 12% concentration through use of a range of rinse-off cosmetics and household cleaning products. Consumer health risks estimated for systemic toxicity associated with repeated use of these products were not considered to be unreasonable.

Environmental effects

No new ecotoxicity studies on aquatic species, namely fish, daphnia and algal toxicity studies, were provided for the secondary notification assessment. Therefore, the ecotoxicity results in the new chemical assessment are reproduced in this secondary notification assessment. These results indicate the notified chemical is acutely toxic to aquatic life but is not expected to have lasting toxic effects.

Previously unseen studies for inhibition in sewage bacteria were provided for the secondary notification. The results suggest the notified chemical may be slightly inhibitory to certain species of sewage bacteria. These microbial data are not typically used to predict the environmental effects of chemicals and, therefore, do not affect the environmental effects conclusions made for the notified chemical in this secondary notification, consistent with the conclusion in the new chemical assessment (NICNAS, 2000).

The notified chemical is considered to be acutely toxic to fish, aquatic invertebrates and algae based on the ecotoxicity data provided in the original new chemical notification (NICNAS, 2000). Therefore, the notified chemical is formally classified as 'Acute Category 2: Toxic to aquatic life' under the *Globally Harmonized System of Classification and Labelling of*

Chemicals (GHS) (United Nations, 2009). The provided ecotoxicity data in the original new chemical notification indicate that the notified chemical is not chronically harmful to aquatic life and, therefore, it is not formally classified for its long term hazards under the GHS (United Nations, 2009).

The notified chemical is not expected to be bioaccumulative based on the measured log P_{OW} = 2.3. Additionally, biodegradation studies submitted for the new chemical notification indicated that the notified chemical and close analogues are biodegradable. Two additional biodegradability studies submitted for the secondary notification indicated the notified chemical is ready biodegradable. The notified chemical is not considered to be stable enough for bioaccumulation.

Environmental exposure and risks

The notified chemical will be used in personal care products and household cleaning products. The majority of the notified chemical is expected to be released to sewers from these uses. At sewage treatment plants, the notified chemical is expected to be partially removed from the water column by adsorption to sediment or sludge due to its surface activity. Based on the maximum import volume and assessed use pattern, the release of the notified chemical to surface waters is not expected to reach ecotoxicologically significant quantities in the aquatic environment.

On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

Recommendations

This section provides the recommendations arising from the secondary notification assessment of the notified chemical, and incorporates the applicable recommendations from the new chemicals assessment report (NICNAS, 2000). No recommendations to minimise occupational exposure to the notified chemical during reformulation or to minimise public exposure during cosmetic use were made at that time as such uses were not notified.

The hazard classifications presented below are according to the GHS (United Nations, 2009), whereas the classification presented in the new chemical assessment report (NICNAS, 2000) was according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999). No environmental classification was made at that time.

New recommendations are directed principally at:

- regulatory bodies
- importers
- reformulators of the notified chemical.

Implicit in these recommendations is the implementation of best practice to minimise occupational exposure.

Recommendations to national bodies

Based on the assessment findings, the notified chemical is recommended to Safe Work Australia for classification and labelling according to the GHS (United Nations, 2009) as below:

- Serious eye damage/eye irritation (Category 1): H318 - Causes serious eye damage
- Acute Aquatic Toxicity (Category 2): H401 – Toxic to aquatic life.

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

- Concentration ≥ 3%: Causes serious eye damage
- 1% ≤ Concentration < 3%: Causes serious eye irritation

Public health recommendations

The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemical for listing on the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP).

Recommendations to importers and state and territory governments

Hazard communication

Labels

Importers of the notified chemical should update their labels to reflect the new hazard identified by this assessment. In addition, importers should review their labels for compliance

with the *Labelling of workplace hazardous chemicals - Code of practice* (Safe Work Australia, 2011).

Safety Data Sheets (SDS)

Under the *Model Work Health and Safety Regulations* (Safe Work Australia, 2016a) and the Commonwealth, state and territory regulations introduced in accordance with these model regulations, employees must have easy access to the Safety Data Sheet (SDS) for hazardous substances at their workplace. The SDS, previously called Material Safety Data Sheet (MSDS), provides information to those who use the hazardous substance.

Importers of the notified chemical should:

- update their SDS to reflect the new hazard identified by this assessment
- review their SDS for compliance with the *Preparation of Safety Data Sheets for hazardous chemicals - Code of practice* (Safe Work Australia, 2016b)
- ensure that employees exposed to the chemical have easy access to a copy of the SDS.

Control measures

Occupational controls

A person conducting a business or undertaking (PCBU) 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
- adequate general ventilation and local exhaust ventilation.

A PCBU at a workplace should implement the following safe work practices to minimise occupational exposure to the notified chemical during reformulation processes:

- avoid contact with eyes
- avoid formation of mists/aerosols.

A PCBU 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:

- eye protection
- respiratory protection if mist/aerosol formation is expected.

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

If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the GHS (United Nations, 2009) 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

- product formulators should exercise due care when using the notified chemical in cosmetic products given its potential ability to enhance the dermal penetration of other chemicals in the formulation

- consumer products containing the notified chemical should be labelled with a warning against eye contact, and directions on first aid measures if the product contacts the eyes (e.g. avoid contact with eyes; in case of contact with eyes, rinse immediately with plenty of water and seek medical advice).

Environment

The assessment conclusion made in this secondary notification is based on the release of the notified chemical through sewers. Any direct release of the notified chemical to surface waters should be avoided.

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 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 Act) an introducer (importer/manufacturer) of the notified chemical/polymer, has post-assessment regulatory obligations to notify NICNAS when any of these circumstances change.

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

(1) Under Section 64(1) of the Act; if

- the concentration of the chemical is intended to exceed 7% in rinse-off cosmetic products or 12% in household cleaning products
- the chemical is intended to be used in products involving spray applications.

or

(2) Under Section 64(2) of the Act; if

- the function or use of the chemical has changed from a component of rinse-off cosmetic products, household cleaning products , or is likely to change significantly
- the amount of chemical being introduced has increased, or is likely to increase, significantly
- if 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.

Abbreviations and acronyms

Act, the	Commonwealth *Industrial Chemicals (Notification and Assessment) Act 1989*
AICS	Australian Inventory of Chemical Substances
bw	body weight
calc.	calculated
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary cells
cmc	critical micelle concentration
conc	concentration
DA	dermal absorption rate
DL	day of lactation
DOC	dissolved organic carbon
EbC50	EC50 (see below) in terms of reduction of biomass
EC50	median effective concentration or half maximal effective concentration
ErC50	EC50 (see above) in terms of reduction of growth rate
F1	first filial/offspring generation
g	gram
GD	gestational day
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
hazard	inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent; intrinsic property of a substance to cause harm
HPLC	high-performance liquid chromatography
HCIS	Hazardous Chemical Information System
IC50	half maximal inhibitory concentration
IR	infrared
ISO	International Organization for Standardization
K_{OC}	organic carbon normalised adsorption coefficient
K_{OW} (or P_{OW})	octanol-water partition coefficient (also see P_{OW})
kg	kilogram
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
m^2	square metre

m^3	cubic metre
µg	microgram
µm	micrometre
M	molar (mol/L)
mg	milligram
mg/cm³	milligrams per cubic centimetre
mg/kg bw/d	milligram per kilogram bodyweight per day
min	minute
mL	millilitre
mN/m	millinewton per metre
mM	millimolar
MOE	margin of exposure
mol	mole
MSDS	(Material) Safety Data Sheet, also see SDS
ND	new data/recommendation/information
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NOHSC	National Occupational Health and Safety Commission
OECD	Organisation for Economic Co-operation and Development
P	Parental
Pa	pascal
PCBU	a 'person conducting a business or undertaking' (PCBU) in WHS Act
PEC	predicted environmental concentration
P_{OW} (or K_{OW})	octanol-water partition coefficient (also see K_{OW})
PNEC	predicted no effect concentration
PR	retained product rate
PT	percentage transfer
ppm	parts per million
risk	probability or likelihood of harm and the likely extent of the harm; the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent
RF	retention factor
RQ	risk quotient
SD	Sprague Dawley (rats)
SDS	Safety Data Sheet (also see MSDS)

SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
SWA	Safe Work Australia
TG	test guideline
TOC	total organic carbon
US EPA	United States Environmental Protection Agency
UV	ultraviolet
UVCB	(chemical of) Unknown or Variable Composition, complex reaction products or Biological material
VIS	visible
WHS	Workplace Health and Safety (www.safeworkaustralia.gov.au)
w/v	weight to volume

1. Introduction

1.1 Background

Data submitted for the original assessment on use, exposure and toxicity are summarised in this report in the relevant sections. Details of the studies provided for assessment as a new chemical are reproduced in the Appendix. New data submitted for this assessment are discussed in detail and identified by the abbreviation **ND**.

1.2 Declaration

A notice was published in the Chemical Gazette of 2 August 2016, requiring a secondary notification of D-glucitol, 1-deoxy-1-(methylamino)-, N-C10-16 acyl derivatives, in accordance with Section 65(2) of the Act. The declaration required the provision of any information relevant to assessment of the notified chemical not covered in the new chemical assessment, and included the following:

- trade name(s) under which the chemical is marketed by the introducer
- annual import volumes of the chemical
- the concentration of the chemical in imported and end-use products
- proposed end-uses of products containing the chemical
- composition data on the different alkyl chain lengths that comprise this UVCB chemical
- any additional physicochemical data that are available for the chemical
- description of the reformulation/repacking process and disposal of wastes resulting from the process
- description of end-uses, and disposal of any wastes, for uses not covered in the original assessment, or if procedures have changed since the original assessment.
- The percentage of the total imported volume of the chemical that is expected to be released as:
    - residues in empty containers (both from import and in end-use)
    - accidental spills and leaks
    - washings from equipment used to reformulate the product(s).
- any additional toxicology data that are available for the chemical, or a suitable analogue
- full ecotoxicological and environmental fate studies on the chemical, or a suitable analogue
- any additional environmental studies that are available for the chemical, particularly any field monitoring studies related to sewage treatment.

1.3 Objectives

The objectives of this assessment are to review the new data made available since the publication of the new chemical assessment report and, where appropriate, to revise the

original assessment to:

- re-assess the human health hazards associated with the notified chemical
- re-assess the environmental hazards associated with the notified chemical
- re-assess the risks of adverse effects resulting from exposure to workers and the general public from the use of the notified chemical
- based on the above, make appropriate recommendations to control exposures and/or reduce potential health risks for workers and the general public, as required.

1.4 Peer review

During all stages of preparation, this report has been subject to internal peer review by NICNAS.

1.5 Applicant

Following the secondary notification declaration of D-glucitol, 1-deoxy-1-(methylamino)-, N-C10-16 acyl derivatives, one company applied for assessment of this chemical.

In accordance with the Act, NICNAS provided the applicant with a draft copy of the report for comment during the corrections/variations phase of the assessment. The applicant details are as follows:

Clariant Australia Pty Ltd

296-324 Ferntree Gully Road

Level 3, 3 Acacia Place

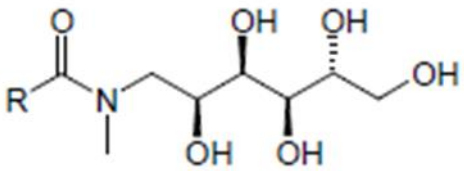
Notting Hill VICTORIA 3168

1.6 Exempt information

No application for exempt information was made.

2. Chemical identity, physical and chemical properties

2.1 Chemical identity

Chemical name:	D-glucitol, 1-deoxy-1-(methylamino)-, N-C10-16 acyl derivatives
CAS number:	173145-38-5
Marketing names:	Dawn dishwashing liquid (1.43% notified chemical) GAS-2EM Surfactant (51% notified chemical) GlucoTain LiquiFlex (20-55% notified chemical) (**ND**) GlucoTain Plus (20-55% notified chemical) (**ND**) GlucoTain Clean (20-55% notified chemical) (**ND**)
Other names:	C12-14 linear glucose amide GS-base E-4194.01 C12/14 GS-base (**ND**) Lauroyl/Myristoyl Methyl Glucamide (**ND**) Lauryl Methyl Glucamide (**ND**) Glucamide 24 (**ND**) Chemical in Glucamide 1218 (**ND**)
Molecular formula:	$C_{19}H_{39}O_6N$ (based on C12 acyl group) (**ND**)
Structural formula:	The notified chemical is a UVCB substance. Following is a generalised structure with the components: R = C_9H_{19} (0.7%), $C_{11}H_{23}$ (71.6%), $C_{13}H_{27}$ (23.7%), $C_{15}H_{31}$ (4.0%) (**ND**).  Where major components R = C11-, C13-alkyl group
Molecular weight:	377.53 g/mol (based on C12 acyl group) (**ND**)
Method of detection and determination:	UV/Visible spectroscopy Infrared spectroscopy ^{1}H NMR spectroscopy ^{13}C NMR spectroscopy
Spectral data:	

UV/Vis	A single absorption peak was observed in methanol solutions at 207 nm in neutral to acidic conditions or at 213 nm in basic conditions (**ND**)
IR	3350, 2916, 2849, 1619, 1077, 1056, 1024, 658 cm^{-1} (**ND**)
^{1}H NMR	0.9 (triplets), 1.3-1.6 (multiplets), 2.3 – 2.5 (multiplets), 2.9/3.1 (singlets), 3.4 – 4.1 (multiplets), 3.3 (solvent), 4.8 (solvent) ppm (**ND**)
^{13}C NMR	13, 22-33, 37, 51-52, 63, 69-73, 175, 47 (solvent) ppm (**ND**)

The spectra are consistent with the structure and purity of the notified chemical (Clariant, 2013a; **ND**).

2.2 Composition

The notified chemical is a mixture of linear glucose amides (N-Alkanoyl N-methylglucamine) with the general structural formula depicted above. At the time of the new chemical assessment, incorrect structural formula, molecular formula and molecular weight were submitted by the notifier and subsequently erroneously published in the new chemical assessment report, whereby the substance was depicted without the N-methyl group (i.e. NH was depicted instead). New data submitted for the secondary notification have been included in the Table above to correct these errors (Clariant, 2013a; **ND**).

At the time of the new chemical assessment, the notified chemical (E-4194.01) comprised the dodecoyl (C12) derivative (around 74% by weight) as the major component with a significant presence of the tetradecoyl chain (C14, 25%) and smaller amounts of the decoyl (C10) and hexadecoyl (C16) components (each present at around 0.5%).

The applicant reported that the notified chemical (Glucamide 24) to be introduced into Australia will have the composition range and typical batch (SN 134/13) composition as shown in the Table below (Clariant 2013b; **ND**). Although the purity and alkyl chain distribution of Glucamide 24 and E-4194.01 are very similar, a comparison of the impurity profile of the notified chemical showed some minor differences.

Constituents	Glucamide 24 (**ND**)		E-4194.01
	Range	SN 134/13	
N-Alkanoyl N-methylglucamine (%)	85-98	94.0	93
Alkyl chain distribution (calc. as %)			
C8	< 1	< 0.1	-
C10	< 2	0.7	0.4
C12	65-75	71.6	73.6
C14	21-30	23.7	24.9

C16	0-8	4.0	0.5

Hazardous impurities:

Chemical name : Methanol (**ND**)
CAS number 67-56-1 *Weight %* < 0.1

Hazardous properties	Based on the HCIS this chemical has the following classification:	
	Acute toxicity, oral (Category 3)	H301 - Toxic if swallowed
	Acute toxicity, dermal (Category 3)	H311 - Toxic in contact with skin
	Acute toxicity, inhalation (Category 3)	H331 - Toxic if inhaled
	Specific target organ toxicity (single exposure) (Category 1)	H370 - Causes damage to organs

Other impurities (≥ 1% by weight)

Chemical name	*Weight %*		
	Glucamide 24 (**ND**)		E-4194.01
	Range	SN 134/13	
N-methylglucamine (CAS number: 6284-40-8)	< 4	1.4	2
Fatty acid methyl ester (CAS number unknown)	< 1	< 0.1	1
Fatty acid (CAS number unknown)	< 8	1.5	-
Ester amides (CAS number unknown)	-	-	1
Propylene glycol (CAS number unknown)	< 2	0.4	-
Water (CAS number: 7732-18-5)	< 3	0.8	-
Sodium soap (CAS number unknown)	-	-	3
Unknown constituents (CAS number unknown)	< 2	1.9	-

2.3 Physical and chemical properties

The physical and chemical data for the notified chemical (E-4194.01) assessed by NICNAS

in the new chemical assessment report (NICNAS, 2000) are shown in the table below with full details of the tests submitted presented in Appendix A. The new data submitted for SN 134/13 are indicated as **ND**.

Summary of the notified chemical's physical and chemical properties

Property	Value	Data Source/justification
Appearance at 20°C and 101.3 kPa	Colourless, coarse, powder (**ND**)	
Melting point	76.5 – 113.3°C	Measured
Boiling point	Could not be determined (decomposition)	Measured.
Density	1140 kg/m^3 at 20°C	Measured
Vapour pressure	1.6 × 10^{-6} Pa at 25°C (**ND**)	Measured
Water solubility	0.032 ± 0.004 g/L at pH 5.7 at 20°C (**ND**) 0.030 ± 0.004 g/L at pH 5.7 at 20°C (active content) (**ND**)	Measured
n-Octanol solubility	28 g/L at 20°C (**ND**)	Measured
Hydrolysis as a function of pH	< 10 % hydrolysis after 5 days at 50°C at pH 4, 7 and 9	Measured
Partition coefficient (n-octanol/water)	log P_{OW} = 2.97 at pH 5.7 at 20°C (**ND**)	Calculated from measured solubilities in n-octanol and water; expected to partition to phase boundaries based on surfactant properties
Surface tension	31.7 mN/m at 20°C	Measured
Adsorption/ desorption	Log K_{OC} = 1.96	Estimated
Dissociation constant	Not applicable	The notified chemical contains no acidic or basic functional groups
Particle size	Not determined	Not relevant as notified chemical is only imported in solution

Flash point	Not applicable	Notified chemical not volatile
Flammability	Not highly flammable	Measured
Autoignition temperature	Not auto flammable	Measured
Explosive properties	Not explosive	Measured
Oxidising properties	Not oxidising	Measured

Comments on physical and chemical properties

The notified chemical used for physicochemical testing in the new studies (SN 134/13) was described as a colourless powder (Clariant, 2013a,b; 2014a,b,c; **ND**) as opposed to a white solid used in studies submitted for the new chemical assessment.

The vapour pressure of the notified chemical was re-evaluated using an effusion method incorporating isothermal thermogravimetric analysis (Siemens, 2015; **ND**). Evaporation rates were measured at several temperatures and linear regression analysis used to determine the vapour pressure. The vapour pressure was determined to be much lower (1.6×10^{-6} Pa) than previously reported (1.4 Pa) at 25°C, but confirms the notified chemical still possesses low volatility. At the time of the new chemical assessment, the notifier had reported the value of 1.4 Pa as high due to the presence of volatile impurities in the test sample.

The water solubility of the notified chemical was re-evaluated using the determination of critical micelle concentration (cmc) via surface tension by the plate method (Clariant, 2014b; **ND**). The cmc was determined as the break point in a plot of surface tension vs. concentration measured and, for ecotoxicological purposes, taken to be the water solubility. The water solubility was determined to be lower (0.030 g/L, based on active content, at pH 5.7) than previously reported (0.140 g/L) at 20 °C; however, the latter value was determined using the shake flask method (OECD TG 105) and not measured as the cmc. The use of the cmc as the water solubility limit has been recommended in guidance on regulatory compliant K_{OW} determination for surfactants (ECHA, 2015) in order to avoid unrealistically low K_{OW} values. Based on the new data, the notified chemical is slightly water soluble.

New data are available for the n-octanol solubility of the notified chemical; however, the value is not significantly different (28 g/L; Clariant, 2014a; **ND**) from that submitted at the time of the new chemical assessment (24.9 g/L; NICNAS, 2000) and is therefore not discussed further.

As the notified chemical is surface-active, OECD TG 107 (shake flask method) and OECD TG 117 (HPLC method) are not applicable and thus the partition coefficient was calculated as the quotient of the n-octanol and water solubility. Based on the new data provided, the partition coefficient of the notified chemical was slightly lower (log P_{OW} = 2.97; Clariant, 2014c; **ND**) than that calculated at the time of the new chemical assessment (log P_{OW} = 2.3; NICNAS, 2000). However, this is largely due to the difference in measured water solubility as discussed above.

3. Importation and use

3.1 Importation

The notified chemical was originally assessed for introduction to Australia as a component of a finished domestic dishwashing liquid. The annual introduction volume of the notified chemical in these products was 27 tonnes and no reformulation occurred in Australia. However, these products have not been imported in the previous two years and the original notifier has no plan to reintroduce the notified chemical in the near future.

The notified chemical will now be imported by sea as a component of the products, GlucoTain LiquiFlex, GlucoTain Plus and GlucoTain Clean, at 20-55% concentration as a liquid. It will not be manufactured within Australia. The products containing the notified chemical at 20-55% concentration will be imported in 1 tonne IBCs and will be transported from the wharf to warehouses for storage and distribution. The imported products containing the notified chemical will be distributed to formulators for reformulation of rinse-off cosmetic and household cleaning products (**ND**).

The maximum introduction volume of the notified chemical over the next five years will be up to 162 tonnes per annum (**ND**) as compared to a maximum annual introduction of 27 tonnes per annum as originally assessed.

3.2 Existing use

The notified chemical is a non-ionic surfactant component of a domestic dishwashing liquid at a concentration of 1.43 %. This product has not been imported recently and the original notifier has no plans to import it in the near future. The original notifier is not an applicant to this secondary notification.

3.2 New uses (ND)

Proposed new uses for the notified chemical include its use as a co-surfactant in shampoo, shower and bath gel, hand soaps (liquid and bar soaps), and skin cleansers at concentrations ranging from 0.5 to 7%.

The notified chemical will also be used in household cleaning products (such as dishwashing and laundry liquids and hard surface cleaners) at ≤ 12% concentration. The finished products containing the notified chemical are not intended for use in aerosol forming applications.

The finished rinse-off cosmetic and household cleaning products containing the notified chemical at ≤ 7% and ≤12% concentration, respectively, will be distributed nationwide for retail and consumer use.

4. Exposure

New information on the use of C12-14 linear glucose amide provided for the secondary notification assessment has significantly altered public and occupational exposure. Therefore, the public and occupational exposure sections have been updated from the new chemical assessment report.

Two previously unseen environmental fate studies, based on the test substance and an analogue, were submitted to NICNAS for the secondary notification assessment, and the new data confirm that the notified chemical is ready biodegradable.

4.1 Occupational exposure

4.1.1 Operational description

The imported products containing the notified chemical, GlucoTain LiquiFlex, GlucoTain Plus and GlucoTain Clean (containing 20-55 % notified chemical), will be distributed to formulators for reformulation of rinse-off cosmetic and household cleaning products.

At the reformulation sites, metering pumps will be used to transfer either GlucoTain LiquiFlex, GlucoTain Plus or GlucoTain Clean from the original containers into vats where they will be blended with other raw materials. Blending will be carried out in enclosed and automated systems. Once blending is complete, quality assurance (QA) workers will take aliquots of samples for laboratory analysis. An automated and metered process will be applied to dispense the finished products into individual consumer size packaging.

The finished rinse-off cosmetic and household cleaning products containing the notified chemical at ≤ 12% concentration will be distributed nationwide for retail and consumer use.

The table below summarises the number and category of workers.

Category of worker	Exposure duration (hours/day)	Exposure frequency (days/year)
Transport and storage:		
Stevedores	2-3	10-15
Transport workers	6	260
Warehousing workers	6	260
At reformulation site:		
Reformulation process workers	4	260
Quality assurance workers	4	260
Maintenance workers and cleaners	1	260

End-users:

Retail workers	1	260
Professional users (e.g. beauticians)	1	260

4.1.2 Estimates of occupational exposure

Transportation and storage

Stevedores, transport and warehouse workers may come into contact with the notified chemical at up to 55% concentration, only in the event of an unlikely accidental rupture of containers.

Reformulation

During reformulation into cosmetic and household cleaning products, dermal, ocular and inhalation exposure of workers to the notified chemical at ≤ 55% concentration may occur. 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 appropriate).

End-use

Exposure to the notified chemical in end-use products at ≤ 12% concentration may occur in professions where the services provided involve the applications of cosmetic products to clients (e.g. hair dressers and workers in beauty salons) or in the cleaning industry. Spray (i.e. aerosol-forming) applications involving the use of the notified chemical are not expected. The main route of exposure is, therefore, expected to 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.

4.2 Public exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of rinse-off cosmetics or household cleaning products at concentrations of ≤ 7% and ≤ 12 % respectively. The principal route of exposure will be dermal, while ocular exposure is also possible.

For the purposes of the exposure assessment via the dermal route, data on typical use patterns of product categories in which the notified chemical may be used are shown in the following tables (ACI, 2010; Cadby et al., 2002; SCCS, 2012). Australian use patterns for the various product categories are assumed to be similar to those in Europe. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for the calculations. Based on the poor dermal absorption of the C12 glucose amide through rat skin (P&G, 1991b), a dermal absorption (DA) of 10% for the notified chemical in humans was assumed.

Direct dermal exposure

Cosmetic products

Product type	Use Amount (mg/day)	C (%)	RF	DA (%)	Daily Systemic Exposure (mg/kg bw/day)
Facial cleanser	800	7	0.01	10	0.0009
Shampoo	10,460	7	0.01	10	0.0114
Conditioner	3,920	7	0.01	10	0.0043
Shower gel	18,670	7	0.01	10	0.0204
Hand wash soap	20,000	7	0.01	10	0.0219
Total					**0.0589**

Daily systemic exposure = (Use amount × C × RF × DA)/BW, where C = use concentration, RF = retention factor, DA = dermal absorption rate, BW = average bodyweight

Household cleaning products

Product type	*Frequency (use/day)*	*C (%)*	*Contact Area (cm^2)*	*Product Use C (g/cm^3)*	*Film Thickness (cm)*	*Time Scale Factor*	*DA (%)*	*Daily systemic exposure (mg/kg bw/day)*
Laundry liquid	1.43	12	1980	0.01	0.01	0.007	10	0.0004
Dishwashing liquid	3	12	1980	0.009	0.01	0.03	10	0.0030
All-purpose cleaner	1	12	1980	1	0.01	0.007	10	0.0260
Total								**0.0294**

Daily systemic exposure = (frequency × C × contact area × product use C × film thickness × time scale factor × DA)/BW, where C = concentration, DA = dermal absorption rate, BW = average bodyweight

Indirect dermal exposure (from wearing clothes)

Household cleaning products

Product type	*Amount (g/use)*	*C (%)*	*PR (%)*	*PT (%)*	*DA (%)*	*Daily systemic exposure (mg/kg bw/day)*
Laundry liquid	230	12	0.95	10	10	0.0410

Product type	*Amount (g/use)*	*C (%)*	*PR (%)*	*PT (%)*	*DA (%)*	*Daily systemic exposure (mg/kg bw/day)*
Fabric softener	90	12	0.95	10	10	0.0160
Total						**0.0570**

Daily systemic exposure = (amount × C × PR × PT × DA)/BW, where C = use concentration, PR = retained product rate, PT = percentage transfer, DA = dermal absorption rate

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 0.145 mg/kg bw/day.

4.3 Environmental exposure

4.3.1 Releases

Release of chemical at site

The notified chemical will be imported as a component of raw material for reformulation into finished cosmetic and personal care products and household cleaning 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 inert material, and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve transfer of the raw material containing the notified chemical into blending vessels using metering pumps, followed by blending operations that will be highly automated and expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. The reformulation process will be followed by automated filling of the formulated products into end-use containers of various sizes. Wastes containing the notified chemical generated during reformulation include equipment wash water, spilt materials, and empty import containers. Wastes are expected to be collected and disposed of to landfill in accordance with local government regulations.

Release of chemical from use

The majority of the notified chemical is expected to be released to sewer across Australia as a result of its use in various cosmetic formulations and personal care products, which will be washed off the hair and skin of consumers or disposed of following cleaning activities. A small proportion of the notified chemical is expected to be disposed of to landfill as residue in empty end-use containers.

Release of chemical from disposal

A small proportion of the notified chemical may remain in end-use containers once the consumer products are used up. Wastes and residues of the notified chemical in empty containers are likely either to share the fate of the container and be disposed of to landfill, or be released to sewer when containers are rinsed before recycling through an approved waste management facility.

4.3.2 Environmental fate

Biodegradation studies submitted for the new chemical notification (NICNAS, 2000) indicated that the notified chemical is biodegradable. Two additional biodegradability studies, one for the notified chemical and one for the analogue (N-[1-14C] Oleoyl-N-methylglucamine), were submitted for this secondary notification. The studies are summarised in this section as new data (ND).

The notified chemical is considered to be ready biodegradable based on the ready biodegradation study, conducted on the notified chemical in accordance to OECD test guideline (TG) 301 B (LISEC, 1995). The analogue biodegradation test was based on the elimination of the 14C labelled test substance by analysis of 14C-activity in the effluent (Dr U Noack-Laboratorien, 2015). The test was conducted according to OECD TG 303 A, which is not a standard ready biodegradation test. Therefore, the results are used as weight of evidence to confirm that the notified chemical is ready biodegradable.

Following its use in cosmetics, personal care products and household cleaning products, the majority of the notified chemical is expected to enter the sewer system, before potential release to surface waters nationwide. Based on its surface activity, the notified chemical is expected to adsorb to suspended matter or sludge at wastewater treatment plants when it enters sewer systems. Notified chemical that remains in treated waste water and then enters receiving waters is expected to rapidly biodegrade. Sludge containing the notified chemical may be applied to agricultural soils or be disposed of to landfill as waste. In soil or land, the notified chemical is not expected to be mobile based on its low water solubility and surface activity.

The notified chemical is not expected to have high potential to bioaccumulate based on the log P_{OW} and surface activity. In the aquatic and soil compartments, the notified chemical is expected to degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

4.3.2.1 Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved/Total Organic Carbon (DOC/TOC)
Remarks - Method	The organic carbon content was not measured at the start of the test due to analyser malfunction, and the starting measurement was taken on the second day. The starting organic carbon content should be 10 mg Carbon/L but was measured to be 8.7 mg Carbon/L on the second day. This deviation was not deemed to have had a significant impact on the validity or integrity of the study.

RESULTS

Test substance		*Aniline (reference substance)*	
Day	% Degradation	Day	% Degradation
6	21.4-21.9	6	24.5
14	64.4-64.6	14	77.1
20	77.7-78.2	20	87.3
28	83.7-84.4	28	91.6
29	84.5-85.2	29	92.3

Remarks - Results: All validity criteria for the test were satisfied. The percentage degradation of the reference compound surpassed the threshold level of 60% by 14 days (77.1%). Therefore, the tests indicate the suitability of the inoculum. The degree of degradation of the test substance after 28 days was 84.9%. As the test substance is surface active, the 10-day window is not applicable. Therefore, the test substance is considered to be ready biodegradable according to the OECD (TG 301 B) guideline.

CONCLUSION: The notified chemical is ready biodegradable.

TEST FACILITY: LISEC (1995)

4.3.2.2 Ready biodegradability

TEST SUBSTANCE: Analogue (N-[1-14C] Oleoyl-N-methylglucamine)

METHOD: OECD TG 303 A: Simulation Test - Aerobic Sewage Treatment (Activated Sludge Units)

Inoculum: Activated sludge

Exposure Period: 28 days

Auxiliary Solvent: None

Analytical Monitoring: Liquid scintillation counting (LSC) & flow scintillation analysis (FSA)

Remarks - Method: The test was conducted in accordance with the test guidelines

	above, and in compliance with GLP standards and principles.
RESULTS	The ultimate degradation was in the range 97.2 – 99.6% with a mean value of 98.5%.
Remarks - Results	As the use of ^{14}C-labelled substances is not described in the OECD TG 303 A, the following validity criteria of OECD TG 314 was adapted for the present test design: Total recovery of radioactivity should normally range from 75 % to 115 % in each individual sample, and average total mass balance should normally range from 85 % to 110 %. If mass balances are significantly below this range, this may be due to the inability to efficiently trap $^{14}CO_2$ from a continuous flow-through system, inability to recover degradation products or the loss of degradation products to glassware or volatilisation. The biodegradation was evaluated based on the composition of the ^{14}C-activity in the effluent. Based on the analysis of the test substance in the effluent the mean elimination rate of the total influent concentration was calculated to be 99.0% in a continuously operating activated sludge unit. Biodegradation of >80% was already achieved after two days. It is concluded that the degradation of the test substance is almost ultimate.
CONCLUSION	The analogue chemical and, by inference, the notified chemical are ready biodegradable.
TEST FACILITY	Dr U Noack-Laboratorien (2015)

4.3.3 Predicted environmental concentration (PEC) (ND)

The predicted environmental concentration (PEC) has been calculated to assume a worst case scenario, with 100% release of the notified chemical into sewer systems and that none of the notified chemical will be removed by sewage treatment processes. The release is assumed to be nationwide over 365 days per year.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	162,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	162,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	443.84	kg/day
Water use	200.0	L/person/day

Population of Australia (Millions)	22.613	million
Removal within STP	0%	Mitigation
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	98.14	µg/L
PEC - Ocean:	9.81	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 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 98.14 µg/L may potentially result in a soil concentration of approximately 0.65 mg/kg in one year. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 3.27 mg/kg and 6.54 mg/kg, respectively.

5. Hazard assessment

5.1 Physicochemical and human health hazard assessment

This section contains a summary of all the data relevant to the physicochemical and human health hazard assessment of the notified chemical, with a focus on new data. The robust summaries of the toxicological data available for the assessment of the notified chemical as a new chemical are presented in Appendix B of this report.

No new data relevant to the physicochemical hazards of the notified chemical were submitted for the secondary notification assessment. Therefore, the physicochemical hazard assessment section has been reproduced from the new chemical assessment report without significant modification.

The robust summary of a newly submitted human health study on the notified chemical is presented in this section and designated as **ND**.

5.1.1 Physicochemical effects assessment

Based on the submitted physicochemical data, the notified chemical is not recommended for hazard classification according to the GHS (United Nations, 2009), as adopted for industrial chemicals in Australia.

5.1.2 Human health effects assessment

The results from toxicological investigations conducted on the notified chemical at the time of the new chemical assessment (NICNAS, 2000) are summarised in the following Table and text. The robust study summaries of these data are provided in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	severely irritating
Guinea pig, skin sensitisation – Buehler test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 100 mg/kg bw/day
Rat, repeat dose oral toxicity – 13 weeks	NOAEL = 200 mg/kg bw/day
Mutagenicity – bacterial reverse mutation (2 studies)	non mutagenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	non mutagenic
Genotoxicity – *in vitro* chromosomal aberration test	genotoxic*
Genotoxicity – *in vivo* mammalian bone marrow chromosome aberration test in the rat	non genotoxic
Developmental toxicity – prenatal study in the rat	NOEL ≥ 363 mg/kg bw/day for developmental toxicity; NOAEL = 150 mg/kg bw/day for maternal toxicity

LD50 = median lethal dose; NOAEL = no observed adverse effect level; NOEL = no observed effect level.
* not biologically significant due to the lack of reproducibility and the aberrations were within the historical control range.

A newly submitted study on reproductive toxicity (Argus, 2000) provided for the secondary notification assessment is briefly summarised in the following table and the robust study summary provided below.

Endpoint	*Result and Assessment Conclusion*
Toxicity to Reproduction – One generation study in the rat (**ND**)	NOAEL ≥ 350 mg/kg bw/day for reproductive and developmental toxicity; NOAEL = 150 mg/kg bw/day for repeat dose toxicity

Toxicokinetics

Absorption, distribution and elimination tests showed that the C12 component and the C18 glucose amide analogue (D-Glucitol, 1-deoxy-1-[methyl(1-oxoisooctadecyl)amino]-, CAS number 139385-52-7) to the notified chemical are readily absorbed from the digestive tract and distributed widely throughout tissues. In contrast, absorption of the C12 component through the skin is minimal (0.5% of the applied dose absorbed over 72 hours) with the principal route of elimination via the urine.

Acute toxicity

The acute oral toxicity of the notified chemical in rats is low (LD50 > 2000 mg/kg bw) and the acute dermal toxicity of the notified chemical in rabbits is low (LD50 > 2000 mg/kg bw). No test report for acute inhalation toxicity was provided. However, an analogue (D-glucitol, 1-deoxy-1-(methylamino)-, N-C8-10 acyl derivs., CAS No. 1591782-62-5) has been found to be harmful if inhaled (NICNAS, 2016; **ND**). Therefore, the potential of the notified chemical to cause toxicity effects through inhalation cannot be ruled out.

Skin irritation

The notified chemical is slightly irritating to rabbit skin, with Grade 1 erythema and oedema persisting for up to 7 days.

Eye irritation

The notified chemical was a severe irritant to rabbit eyes in pure form, with vascularisation of the cornea observed in one out of the three animals tested. The irritation scores provided from this test could not be directly assessed against the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) as the quantity instilled in the eyes was less than 10 mg, rather than the 100 mg normally used in the OECD test. The irritation scores were below the level leading to classification as an eye irritant, but instillation of larger quantities may have resulted in higher scores. The irritation was found to be persistent, with effects seen in all animals at 4 days, and persisting up to 35 days in one animal.

Sensitisation

The notified chemical was not a skin sensitiser in a non-adjuvant type test in guinea pigs.

Repeated dose toxicity

In a 28-day oral study in rats, a NOAEL of 100 mg/kg bw/day was established. At doses of 500 and 1000 mg/kg bw/day, a number of histological changes to the stomach lining were observed. Reduced food consumption and decreased bodyweight were also noted, along with

a number of clinical chemistry changes that the study authors attributed to the poor nutritional status of these animals. Twelve of the twenty rats receiving 1000 mg/kg bw/day died or were sacrificed in extremis during the study. At the higher doses, breathing difficulties were also observed.

In a 13-week oral study in rats, breathing problems were observed in animals treated with 50 mg/kg bw/day and above. Clinical chemistry and haematology parameters were changed in animals treated with 200 and 500 mg/kg bw/day. These changes were considered to be due to the poor general condition of the animals. Six out of twenty animals treated with 500 mg/kg bw/day died of treatment-related causes during the study. No macroscopic or microscopic changes could be found during necropsy to explain the reasons for the deaths. On the basis of increased mortality and morbidity at 500 mg/kg bw/day, the study authors concluded that the NOAEL was 200 mg/kg bw/day in this study, as all findings at this dose were slight and there were no changes in blood or urinalysis parameters indicative of toxicity. However, based on clinical signs and the slight, transient effects on body weight gain at 200 mg/kg bw/day, a NOEL of 50 mg/kg bw/day was established.

Genotoxicity

The notified chemical gave negative results in two in vitro mutagenicity tests (*Salmonella typhimurium* reverse mutation assay, and mouse lymphoma forward mutation assay) in the presence and absence of S9 metabolic activation. Positive results were observed in an in vitro study of chromosomal aberrations in CHO cells in the absence of metabolic activation, although the study authors concluded that the results were not biologically significant because of lack of reproducibility in repeat tests, and because the values were within historical control ranges. This conclusion is supported by the absence of genotoxicity in an in vivo study of cytogenicity in rat bone marrow cells was negative.

Reproductive toxicity

A reproductive toxicity study (Argus, 2000; **ND**) was submitted for the secondary notification assessment, and the robust study summary follows:

5.1.2.1 Toxicity to reproduction – One generation study

TEST SUBSTANCE	Notified chemical (96.2% in purity)
METHOD	OECD TG 416 Two-Generation Reproduction Toxicity Study; EC Directive 92/69/EEC B.34 One-Generation Reproduction Toxicity Test
Species/Strain	Rat/Crl:CD (SD)/GS BR VAF/Plus
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 70 days prior to mating, through cohabitation (maximum of 21 days) and until the day before sacrifice (maximum 122 days for males and 128 days for females) Dose regimen: Once daily 7 days per week
Vehicle	Deionized water

Remarks - Method The design protocol was based on a two-generation study (OECD TG 416) however study progression determined that data from a one-generation study only were needed.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	50 (25 M/25 F)	0	0/50
Low dose	50 (25 M/25 F)	15	0/50
Mid dose	50 (25 M/25 F)	150	6/50
High dose	50 (25 M/25 F)	350	0/50

Mortality and Time to Death

No parental animals died because of treatment related to the test substance. In the mid-dose group, two males (days 9 and 27) and four females (day 15) died because of intubation accidents.

Effects on Parental (P) animals:

Clinical observations

At doses of 150 and 350 mg/kg bw/day, excessive salivation, breathing rales and a red perioral substance (males only) were observed. Although the study authors considered these clinical signs test substance-related, NICNAS does not regard these findings as indicative of systemically mediated toxicity but more likely consistent with local effects caused by gavage-related reflux (and accidental aspiration) of irritating material. Such reflux-induced effects and their differentiation from direct test substance-related toxicity have been more recently reviewed (Damsch et al., 2011). Non-dose-dependent incidences of chromodacryorrhoea and chromorhinorrhoea were also noted.

Body Weight and Food Consumption

In males, decreases in body weight gains (11%) and terminal body weights (7.7%) in the 350 mg/kg bw/day group were considered to be related to the test substance. Absolute and relative feed consumption values were unaffected by dose.

For females, average body weights, body weight gains and absolute and relative feed consumption values during the pre-cohabitation period were comparable among the four dosage groups. A significant decrease in maternal body weight gains (15.3%) and absolute feed consumption (9.3%) was observed in the 350 mg/kg bw/day group for the entire gestation period. Although absolute body weights were slightly decreased (≤ 6.7%) in the 350 mg/kg bw/day group on each day of the lactation period, maternal body weight gains were increased (23%, not statistically significant) over the controls for the entire lactation period. Absolute and relative feed consumption values during the lactation period were comparable among the four dosage groups.

Effects in Organs

In males, no biologically important differences occurred among the four dosage groups for the weights of the left and right epididymides, left cauda epididymis, left and right testes, seminal vesicles (with and without fluid), pituitary and brain or the ratios of these organ weights to the terminal body weights or brain weights.

In females, the weights of the pituitary, brain, left and right ovary and uterus and the ratios

of these organ weights to the terminal body weight or absolute brain weight did not differ significantly among the four dosage groups.

Reproductive/Developmental Effects

In males, no statistically significant or biologically important differences occurred in the number or the percentage of motile sperm, the number of non-motile sperm, and the sum of the motile and non-motile sperm. Cauda epididymal sperm counts were 86%, 90% and 80% and density values were 93%, 88% and 85% of the control group values in the low, mid and high dose groups respectively. These non-statistically significant reductions were not considered to be biologically important because mating and fertility parameters were comparable among the groups. There were no test substance-related microscopic changes observed in the reproductive tissues of male rats at the top dose or in the testes of rats that failed to reproduce.

In females, the test substance did not affect oestrous cycling. The precoital index, the fertility and mating indices and the number of rats with confirmed mating dates during the first and second week of cohabitation were comparable in the four groups. All females were mated.

There were 23 (92.0%), 23 (92.0%), 21 (100%) and 22 (88.0%) pregnant dams in the 0 (Vehicle), 15, 150 and 350 mg/kg bw/day groups, respectively. One rat in the top dose group did not deliver a litter by gestation day (GD) 25, and had only one implantation site in utero, but this event was considered unrelated to the test substance. One or more live-born pups were delivered by every other pregnant dam (there were 21 to 23 litters delivered in each group). Natural delivery observations were unaffected by the test substance. The gestation index, the number of dams delivering litters, the duration of gestation, averages for implantations and dams with stillborn pups were comparable among the four groups and did not significantly differ.

There were no test substance-related microscopic changes observed in the brain, pituitary or reproductive tissues of female rats in the 350 mg/kg bw/day group. Microscopic examination of the ovaries of rats that failed to reproduce revealed no findings that could be correlated with this infertility.

Effects on 1st Filial Generation (F1)

All litter parameters were unaffected by the test substance. These included the number of pups found dead or presumed cannibalized from day of lactation (DL) 1 to 21, pup body weights, surviving pups, percent male pups and live litter sizes at weighing. The number of pups found dead or presumed cannibalized was significantly increased in the 350 mg/kg bw/day dosage group on DL 1. This increase was not considered test substance-related because the viability and lactation indices were comparable among the groups.

All clinical and necropsy observations in the offspring were considered unrelated to the test substance.

Necropsy of pups that were found dead revealed no milk in stomach in 3, 2, 1 and 1 pups from the four respective dosage groups. All pups appeared normal at necropsy on DLs 4 and 21.

Remarks – Results

Clinical signs observed at 150 and 350 mg/kg bw/day (salivation, rales and a red perioral

substance) were judged by NICNAS to be local effects associated with oral gavage administration. Systemic toxicity in the 350 mg/kg bw/day groups was reflected in attenuation in body weight gains and transient but slight reduction in feed consumption (females). No adverse effects on fertility or development occurred at 350 mg/kg bw/day, the highest dose tested.

CONCLUSION

Although the study authors established the NOAEL as 15 mg/kg bw/day based on adverse clinical effects seen at a dose of 150 mg/kg bw/day in this study, NICNAS regards the NOAEL for systemic toxicity as 150 mg/kg bw/day based on effects on body weight gains at a dose of 350 mg/kg bw/day. The NOAEL for reproductive and developmental toxicity is greater than 350 mg/kg bw/day.

TEST FACILITY Argus (2000)

Developmental toxicity

In a developmental toxicity study, the notified chemical did not cause developmental toxicity at a dose where maternal toxicity was observed. The NOEL for developmental toxicity was determined to be 363 mg/kg bw/day (the highest dose tested) while the NOAEL for maternal toxicity was found to be 150 mg/kg bw/day based on decreased bodyweight gain at the higher dose.

Effects observed in humans

A patch test in human volunteers showed that the notified chemical was a mild skin irritant. Several skin irritation and sensitisation studies in humans have been performed using the C12 glucose amide component and formulations containing this component. In general, the formulation studies were provided by the notifier in table form and the full details were not available. Consistent with the results of the animal studies for the notified chemical, the C12 component was found to be a slight skin irritant under the conditions of the tests but was not a skin sensitiser.

5.1.3 Hazard classification

The new chemical assessment report (NICNAS, 2000) concluded that the notified chemical had the potential to cause serious damage to eyes and recommended a hazard classification of R41 according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999). According to the GHS (United Nations, 2009), as adopted for industrial chemicals in Australia, the notified chemical should be classified as presented in the following table.

Hazard classification	Hazard statement
Serious eye damage/eye irritation (Category 1)	H318 - Causes serious eye damage

5.2 Environmental hazard assessment

This section contains a summary of all the data relevant to the environmental hazard assessment of the notified chemical, with a focus on new data. The robust summaries of the data available for the assessment of the notified chemical as a new chemical (NICNAS, 2000) are reproduced in the Appendix of this report.

5.2.1 Environmental effects assessment

Summary of ecotoxicity data for the notified chemical or analogue

Endpoint	*Result (nominal)**	*Assessment Conclusion*
Acute toxicity to fish – Zebra fish (notified chemical)	LC50 (96 h) = 7.4 mg/L NOEC (96 h) = 5.6 mg/L	Acutely toxic to fish
Acute toxicity to fish – Fathead minnow (C14 component)	LC50 (96 h) = 2.9 mg/L NOEC (96 h) = 1.2 mg/L	Acutely toxic to fish
Chronic toxicity to fish– Fathead minnow (notified chemical)	LOEC (35 day) = 10 mg/L NOEC (35 day) = 5 mg/L	Not chronically harmful to fish
Acute toxicity – Daphnia (notified chemical)	EC50 (48 h) = 18 mg/L NOEC (48 h) = 10 mg/L	Acutely harmful to *Daphnia*
Acute toxicity – Daphnia (C14 component)	EC50 (48 h) = 5 mg/L (95 % confidence 3.3-9.2 mg/L) NOEC (48 h) = 3.3 mg/L	Acutely toxic to *Daphnia*
Chronic toxicity – Daphnia (notified chemical)	EC50 (21 day) = 6.8 mg/L LOEC (21 day) = 10 mg/L NOEC (21 day) = 5 mg/L	Not chronically harmful to *Daphnia*
Algal toxicity growth (notified chemical)	EbC50 (72 h) = 14 mg/L ErC50 (72 h) = 30 mg/L NOEC (72 h) = 5.6 mg/L	Acutely harmful to algae
Algal toxicity growth (C14 component)	EbC50 (72 h) = 3.9 mg/L NOEC (72 h) = 2.9 mg/L	Acutely toxic to algae
Inhibition of bacterial respiration (notified chemical)	NOEC ≈ 10 mg	Some inhibition of respiration
Acute toxicity - Earthworm (notified chemical)	LC50 (14 day) > 1000 mg/kg (dry soil) NOEC (14 day) = 1000 mg/kg (dry soil)	Not toxic to earthworms
Growth test on plants (notified chemical)	NOEC (17 day) = 320 mg/L	Not toxic to plants

* Results listed are nominal test concentrations, but in several of the tests the solution concentrations were also measured, and where appropriate, the results in terms of the measured concentration are presented in Appendix C.

LC50 = median lethal concentration; NOEC = no observed effect concentration; LOEC = lowest observed effect concentration; EC50 = median effective concentration; EbC50 = EC50 in terms of reduction of biomass.

New studies (**ND**) on microbial species were provided for the secondary notification assessment and are briefly summarized below followed by robust summaries of the studies.

Endpoint	*Result (nominal)*	*Assessment Conclusion*
Inhibition of bacterial respiration (notified chemical)	EC50 (3 h) = > 71 mg/L NOEC (4 h) = 8.9 mg/L	Some inhibition of bacterial respiration
Inhibition of bacterial nitrification (notified chemical)	EC50 (4 h) = > 142 mg/L NOEC (4 h) = > 142 mg/L	Not inhibitory to bacterial respiration
Acute toxicity – bacterial growth (notified chemical)	EC50 (17 h) = > 140 mg/L	Not inhibitory to bacterial respiration

The result of 3 h EC50 ≥ 71 mg/L is considered to be less reliable, as discussed in the following study summary. Based on the results 4 h EC50 ≥ 142 mg/L and 17 h EC50 ≥ 140 mg/L, the notified chemical is not inhibitory to bacterial respiration.

5.2.1.2 Inhibition of microbial activity (ND)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 1.1-140 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. The respiration rate was determined by measurement of Biochemical Oxygen Demand during the test after 3 hours of exposure.
RESULTS	
IC50	> 71 mg/
NOEC	8.9 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The results of this study may not be reliable as a maximum inhibition of 30% was found at a test substance concentration of 35 mg/L and the inhibition was less at the higher concentrations. Furthermore, it was reported that no inhibition was found at the highest concentration of 140 mg/L which may be due to a less than complete dissolution of the test substance. The 3 h EC50 was determined to be > 71 mg/L based on nominal concentrations.

CONCLUSION	Some inhibition of respiration.
TEST FACILITY	TNO (1997a)

5.2.1.3 Inhibition of microbial activity (ND)

TEST SUBSTANCE	Notified chemical
METHOD	ISO 9509 'Water quality-Method for assessing the inhibition of nitrification of activated sludge microorganisms by chemicals and waste waters'.
Inoculum	Activated sludge
Exposure Period	4 hours
Concentration Range	Nominal: 1.1-142 mg/L Actual: Not determined
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Allylthiourea (ATU) was used as the reference control. 100 mL sample of each mixture was taken and filtered through a GF/C glass fibre filter after 4 hours of incubation. Samples were then analysed on the same day for nitrogen contents.
RESULTS	
IC50	> 142 mg/L
NOEC	142 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The EC50 with respect to bacterial growth after 4 hours was > 142 mg/L. No inhibiting effects were observed at the highest concentration tested.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	TNO (1997b)

5.2.1.4 Inhibition of microbial activity (ND)

TEST SUBSTANCE	Notified chemical
METHOD	ISO 10712 'Water quality- Pseudomonas putida growth inhibition test'.

Inoculum	Bacterium (*Pseudomonas putida*)
Exposure Period	17 hours
Concentration Range	Nominal: Actual: Not determined
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The incubation temperature in the final test and reference test was between 23.4 and 25.0°C. The deviation was not deemed to have had a significant impact on the validity or integrity of the study. 3,5-dichlorophenol was used as the reference control. The growth of the bacterium was determined after 17 hours by measuring the increase in light absorbance of the culture at 605 nm.

RESULTS

IC50	> 140 mg/L
NOEC	Not reported
Remarks – Results	All validity criteria for the test were satisfied. The EC10 and EC50 with respect to bacterial growth after 17 hours was > 140 mg/L. No inhibiting effects were observed at the highest concentration tested.
CONCLUSION	The notified chemical is not inhibitory to microbial activity
TEST FACILITY	TNO (1997c)

5.2.2 Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) has been calculated using the most sensitive chronic toxicity endpoint for fish (35 day NOEC = 5 mg/L). A safety factor of 10 was used given measured acute and chronic endpoints are available for aquatic life representing three trophic levels.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment

LC50 (Fish, 96 h)	5	mg/L
Assessment Factor	10	
PNEC	500	µg/L

5.2.3 Hazard classification

The environmental hazard classification according to the GHS (United Nations, 2009) is presented below. Under the GHS (United Nations, 2009) the notified chemical is considered to be toxic to fish, aquatic invertebrates and algae and is formally classified as 'Acute Category 2: Toxic to aquatic life'. Based on the chronic toxicity, ready biodegradability and low bioaccumulation potential of the notified polymer, it is not formally classified under the GHS for chronic toxicity. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Environmental hazard classification

Hazard classification	Hazard statement
Acute (Category 2)	H401 - Toxic to aquatic life

6. Risk characterisation

The public health, occupational health and environmental risk estimations have been updated to account for the new exposure scenarios resulting from the proposed changes to the use and introduction volume of the notified chemical.

6.1 Public health risk estimation

Cosmetic and household cleaning products containing the notified chemical at ≤ 12% concentration will be available to the public. The main route of exposure is expected to be dermal with some potential for accidental ocular or oral exposure.

Irritation

The notified chemical causes severe eye damage and irritation. The main risk of eye damage and irritation will be expected from the use of cosmetic products containing the notified chemical. Given the low proposed use concentration in cosmetics (i.e. ≤ 7%), use in rinse-off cosmetics only and likely dilution upon application, significant eye irritation effects are not expected. The eye irritation risk associated with use of the notified chemical in consumer products may be further minimised by the inclusion of appropriate labelling and directions for use to warn against eye contact.

Risk of repeated exposure

Members of the public may experience repeated exposure to the notified chemical up to 12% concentration through the use of a range of rinse-off cosmetic products and household cleaning products.

The new chemical assessment determined that, while the notified chemical was found to be of low acute toxicity via the oral and dermal routes, health effects after repeated dermal exposure could not be ruled out, particularly given the systemic toxicity observed following repeated oral exposure. Systemic toxicity upon repeated oral exposure was also observed in a reproductive toxicity study submitted for this assessment.

Estimation of the repeated dose toxicity potential of the notified chemical using the worst-case exposure scenario from the use of multiple products would result in a combined internal dose of 0.145 mg/kg bw/day (see Section 4.2). No dermal NOAEL was determined. An oral NOAEL of 150 mg/kg bw/day was established in the reproductive toxicity study based on a LOAEL of 350 mg/kg bw/day. Although a lower NOAEL of 100 mg/kg bw/day was established in a 28-day oral repeat dose toxicity study, this was based on a higher LOAEL of 500 mg/kg bw/day and was therefore not carried forward for quantitative risk estimation. Use of the NOAEL of 150 mg/kg bw/day resulted in a margin of exposure (MOE) of 1034. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences. Therefore, based on the available information and with appropriate labelling regarding risks associated with eye contact, the risk to the public from use of the notified chemical at ≤ 7% in rinse-off cosmetics and ≤ 12% in household cleaning products is not considered to be unreasonable.

6.2 Occupational health risk estimation

Based on the available information, the notified chemical causes serious eye damage and the potential for the chemical to cause acute inhalation toxicity cannot be ruled out.

Reformulation

Dermal, ocular and potentially inhalation exposure to the notified chemical at up to 55% concentration may occur during reformulation. The stated use by the notifier of PPE such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate) and engineering controls including automated/enclosed processes and local exhaust ventilation should minimise the risk for workers.

Provided that control measures stated by the notifier are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use

Cleaners and beauty care professionals may come into contact with products containing the notified chemical at ≤ 12% concentration. These products will also be available to the public. The risk to workers who regularly use these products is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical (for details of the public health risk assessment, see Section 6.1).

6.3 Environmental risk characterisation

The newly submitted ecotoxicity data for the notified chemical do not significantly affect the conclusions of the new chemical environmental effects assessment. However, as the PEC values have significantly increased compared to those derived for the new chemical assessment of the notified chemical, the related risk estimations have changed and are shown below. 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**
River:	98.14	500	**0.20**
Ocean:	9.81	500	**0.02**

The risk quotients for discharge of treated effluents containing the notified chemical to the aquatic environment indicate 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 ready biodegradable, and is expected to have a low potential for bioaccumulation. Based on the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

Appendix A: Physical and chemical properties

The physical and chemical properties data submitted for the new chemical assessment of the notified chemical are presented here.

Melting point	76.5 – 113.3°C
METHOD	OECD TG 102 Melting Point/Melting Range
Remarks	The melting point was determined using the capillary method. No significant protocol deviations.
TEST FACILITY	RCC Notox (1991a)
Boiling point	Could not be determined
METHOD	OECD TG 103 Boiling Point/Boiling Range
Remarks	The boiling point could not be determined using a melting point apparatus as the test substance decomposed on heating. The boiling point of the decomposition product was >250°C. No significant protocol deviations.
TEST FACILITY	RCC Notox (1991b)
Density	1140 kg/m^3 at 20°C
METHOD	OECD TG 109 Density of Liquids and Solids
Remarks	The relative density was determined using the pycnometer method No significant protocol deviations
TEST FACILITY	RCC Notox (1991c)
Vapour pressure	1.4 ± 0.1 Pa at 25°C
METHOD	OECD TG 104 Vapour Pressure Curve
Remarks	The vapour pressure was determined using the static manometric technique, and the vapour pressure data determined at 24.8, 31.1 and 37.7°C used to interpolate the vapour pressure at 25°C. No significant protocol deviations.
TEST FACILITY	RCC Notox (1991d)

Water solubility 140 ± 10 mg/L at 20.0 ± 0.5°C

METHOD OECD TG 105 Water Solubility

Remarks The water solubility was determined using the flask method, with analysis using High Performance Liquid Chromatography (HPLC). The pH of the solution was between 6.7 and 7.3.

No significant protocol deviations.

TEST FACILITY RCC Notox (1991e)

Hydrolysis as a function of pH < 10 % hydrolysis after 5 days at 50°C at pH 4, 7 and 9

METHOD EC Directive 84/449/EEC C.10 Abiotic Degradation: Hydrolysis as a Function of pH

Remarks HPLC was used for analysis of solution concentrations.

No significant protocol deviations.

TEST FACILITY RCC Notox (1991f)

Partition coefficient (n-octanol/ water) log P_{OW} = 2.3

METHOD OECD TG 117 Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method

Remarks As the test substance is a surface-active substance, the main study was not performed. The partition coefficient was determined as an estimation of the ratio of the solubility of the test substance in n-octanol (this study) to that in water (RCC Notox, 1991e).

TEST FACILITY RCC Notox (1991g)

Surface tension 31.7 mN/m at 20°C

METHOD OECD TG 115 Surface Tension of an Aqueous Solution

Remarks The surface tension was determined using the ring method and a 100 mg/L solution.

No significant protocol deviations.

TEST FACILITY RCC Notox (1991h)

Flammability Not highly flammable

METHOD EC Directive 84/449/EEC A.10 Flammability (solids)

Remarks The test material could not be ignited under the conditions of the test.

No significant protocol deviations.

TEST FACILITY RCC Notox (1991i)

Autoignition temperature Not auto flammable

METHOD EC Directive 84/449/EEC A.16 Auto-Flammability (solids-determination of relative self-ignition temperatures)

Remarks No autoignition observed below the melting point of the test substance

No significant protocol deviations.

TEST FACILITY RCC Notox (1991j)

Explosive properties Not explosive

METHOD EC Directive 84/449/EEC A.14 Explosive Properties

Remarks Not explosive by thermal stress, shock or friction.

No significant protocol deviations.

TEST FACILITY RCC Notox (1991k)

Oxidising properties Not oxidising

METHOD EC Directive 84/449/EEC A.17 Oxidizing Properties

Remarks Oxidising properties were determined by comparing the burning of a cellulose/test substance mixture to a cellulose/barium nitrate mixture.

No significant protocol deviations.

TEST FACILITY RCC Notox (1991l)

Appendix B: Toxicological investigations

The robust summaries of the toxicological studies analysed for the assessment of the notified chemical as a new chemical are presented here.

B.1 Animal toxicological data

B.1.1 Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (45% active ingredient)
METHOD	OECD TG 401 Acute Oral Toxicity EC Directive 84/449/EEC B.1 Acute Toxicity - Oral
Species/Strain	Rat/HanIbm: WIST (SPF)
Vehicle	Water (with one drop of Tween 80)
Remarks - Method	No significant protocol deviations; administration by gavage, dose expressed as mg/kg bw of active ingredient

RESULTS

Group	*Number and sex of animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 males, 5 females	900	0
2	5 males, 5 females	2000	0

LD50	> 2000 mg/kg bw
Signs of toxicity	In the low dose group, three animals appeared slightly sedated and one had slightly ruffled fur. In the high dose group, two animals appeared slightly sedated. One female from the high dose group showed moderate loss of weight. Body weight gain of two other females in the high dose group, and one from the low dose group, were also retarded.
Effects in organs	None – gross abnormalities were observed on day 15.
Remarks - Results	No deaths occurred.
CONCLUSION	The notified chemical is of very low acute oral toxicity in rats
TEST FACILITY	RCC (1991a)

B.1.2 Acute toxicity - dermal

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	EC Directive 84/449/EEC B.3 Acute Toxicity - Dermal
Species/Strain	Rabbit/New Zealand White
Vehicle	Water
Types of dressing	Semi-occlusive
Remarks - Method	Dose level 2000 mg/kg bw; test material moistened with water; 24 hour exposure. No significant protocol deviations.

RESULTS

Group	Number and sex of animals	Dose (mg/kg bw)	Mortality
1	5 male, 5 female	2000	0

LD50	> 2000 mg/kg bw
Signs of toxicity - Local	Well defined erythema and well defined oedema (slight in one case) were observed following removal of dressings; necrotic foci at the dose site for 3 animals. The reactions were maintained, often accompanied by hyperkeratinisation, throughout the study, or developed to necrosis with well-defined oedema (6 animals). Focal scabbing or scabs at the treatment site for 2 males and 2 females. Necrotic reactions were still present in 3 animals at study termination.
Signs of toxicity - Systemic	None
Effects in organs	Pale kidneys and congested lungs for 1 male, congestion at the tips of the papillae for both kidneys for 1 female.
Remarks - Results	No deaths occurred.
CONCLUSION	The notified chemical is of very low acute dermal toxicity in rabbits
TEST FACILITY	HRC (1991a)

B.1.3 Irritation – skin

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion

	EC Directive 84/449/EEC B.4 Acute Toxicity – Skin Irritation
Species/Strain	Rabbit/ChbbIbm: NZW (SPF)
Number of animals	3
Vehicle	Water
Observation period	14 days
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean score* Animal No.*			*Maximum value*	*Maximum duration of any effect*	*Maximum value at end of observation period*
	1	2	3			
Erythema/Eschar	1	1	1	1	7 days	0
Oedema	1	0	0.67	1	7 days	0

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

Remarks - Results	A single dose of 0.5 g E4194.01 (moistened in distilled water) was applied semi-occlusively to the abraded and intact skin of 3 New Zealand White rabbits. Very slight erythema and oedema persisted to day 7 and was reversible by day 14.
CONCLUSION	The notified chemical is a slight irritant to the skin of rabbits.
TEST FACILITY	RCC (1991b)

B.1.4 Irritation - eye

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	Proctor and Gamble Protocol No. C2B-E
Species/Strain	Rabbit/New Zealand White
Number of animals	3

Observation period	35 days
Remarks - Method	Method similar to OECD TG 405 except the quantity instilled in the eye was <10 mg, rather than 100 mg as recommended in the Guideline.

RESULTS

Lesion	*Mean score** *Animal No.*			*Maximum value*	*Maximum duration of any effect*	*Maximum value at end of observation period*
	1	2	3			
Conjunctiva: redness	2	2	1	2	28 days	0
Conjunctiva: chemosis	1.7	2	1	2	7 days	0
Conjunctiva: discharge	1.7	2	0.3	2	7 days	0
Corneal opacity	1.3	1.3	0	2	35 days	1
Iridial inflammation	0.3	1	0	1	4 days	0

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

Remarks - Results	A single dose of E4194.01 (6.3 mg for animals 1 and 2, and 4.6 mg for animal 3) was applied directly onto the corneal surface of one eye. In animal 3, all effects cleared by 7 days; in animal 1 all effects cleared by 14 days; in animal 2, vascularisation of the cornea was observed at 14 days, and corneal opacity persisted to day 35; conjunctival effects cleared by day 35.
CONCLUSION	The notified chemical is severely irritating to the eyes of rabbits based on the persistence of the effects.
TEST FACILITY	HRC (1991b)

B.1.5 Skin sensitisation

TEST SUBSTANCE	Notified chemical (45% active ingredient)
METHOD	OECD TG 406 Skin Sensitisation – Buehler EC Directive 84/449/EEC B.6 Acute Toxicity – Skin

	Sensitisation
Species/Strain	Guinea pig/Ibm: GOHI (SPF)
Vehicle	Water
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 5%
MAIN STUDY	
Number of animals	Test Group: 10/sex Control Group: 5/sex
INDUCTION PHASE	Induction Concentration: topical: 20%
Signs of irritation	Slight erythema was observed in one animal after the initial application.
CHALLENGE PHASE	Challenge Concentration:
1st challenge	topical: 10%
2nd challenge	topical: 10%
Remarks - Method	No significant protocol deviations.

RESULTS

Animal	*Challenge concentration*	*Number of animals showing skin reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	48 h	24 h	48 h
Test group	10%	0	0	0	0
Control group	10%	0	0	0	0

Remarks - Results	No skin reactions were observed at 24 hours or 48 hours in the treated or control groups. The positive control (formaldehyde) confirmed the sensitivity of the test system.
CONCLUSION	The notified chemical is not sensitising to the skin of guinea pigs.
TEST FACILITY	RCC (1991c)

B.1.6 Repeat dose oral toxicity - 28 days

TEST SUBSTANCE	Notified chemical (45% active ingredient)
METHOD	OECD TG 407 Repeated Dose 28-Day Oral Toxicity Study in Rodents
Species/Strain	Rat/Crl: CDF F344
Route of administration	Oral – gavage
Exposure information	Total exposure: 28 days
Vehicle	Water
Remarks - Method	

RESULTS

Dose (mg/kg bw/day)	*Number and sex of animals*	*Mortality*
0	10 male, 10 female	5
10	10 male, 10 female	5
100	10 male, 10 female	5
500	10 male, 10 female	5
1000	10 male, 10 female	12

Mortality and time to death

5 males and 7 females receiving 1000 mg/kg bw/day died or were sacrificed in extremis due to treatment related causes during the first two weeks of the study. 5 animals across all treated groups died during blood collection on days 27 and 28, but these deaths were not considered treatment related.

Clinical observations

In the 500 mg/kg bw/day animals, wheezing, salivation, urine stains and bloody crust on the nose were observed. In the 1000 mg/kg/day animals, dyspnoea and languid appearance were noted in addition to the above clinical signs. No treatment related clinical signs were seen in animals of the lower dose groups.

Ophthalmic examination revealed no treatment related findings.

Food consumption and bodyweight

Reduced food consumption and corresponding decreases in body weight gain were seen in the animals of the 500 mg/kg bw/day and 1000 mg/kg bw/day groups.

Laboratory findings - clinical chemistry, haematology, urinalysis

A wide range of clinical chemistry parameters was significantly changed in the 500 mg/kg bw/day and 1000 mg/kg bw/day groups compared with the controls. These changes were considered to be generally secondary to the poor nutrition of these animals. A statistically significant decrease in triglycerides in 100 mg/kg bw/day males was not considered biologically relevant due to the small magnitude of the change and the lack of consistency between the sexes at this dose.

Mild anaemia was observed in the 1000 mg/kg/day animals. Incidental changes in white blood cell counts were considered consistent with an inflammatory response.

Urinalysis parameters were generally unchanged from controls, except for the observation of lower pH in the 1000 mg/kg bw/day males. This was considered consistent with the nutritional deficiencies which have been previously noted.

Gross Pathology

Thickened or roughened mucosa of the non-glandular region of the stomach was observed in the 500 and 1000 mg/kg bw/day groups. Thickened or dark mucosa of the glandular region of the stomach was also observed for the 1000 mg/kg bw/day group. The stomach weight was observed to be increased relative to the body and brain, and the thymus weight decreased relative to the body and brain in the 500 and 1000 mg/kg bw/day groups

Histopathology

A number of histopathological changes in the stomach were observed in the 500 and 1000 mg/kg bw/day groups. These included ballooning degeneration of the surface epithelium, acanthosis, parakeratosis, erosion, ulceration, focal haemorrhage and inflammation of the non-glandular region and an increased number of goblet cells and/or mucous on the mucosal surface in the glandular region. These changes are suggestive of test substance related irritation to the non-glandular region of the stomach.

Necrosis and/or lymphoid depletion of the thymus were observed in the 1000 mg/kg bw/day animals and to a lesser effect in the 500 mg/kg bw/day animals; the study authors considered these changes to be stress-related.

Remarks - Results

Administration of the test substance at 500 and 1000 mg/kg bw/day resulted in increased mortality (at 1000 mg/kg bw/day), decreased bodyweight and food consumption, altered clinical pathology values consistent with inflammation and nutritional deficits, and histological changes to the stomach.

Alterations to clinical chemistry parameters were not associated with microscopic changes in specific organs and tissues.

CONCLUSION

Based on the findings at 500 and 1000 mg/kg bw/day, a NOAEL of 100 mg/kg bw/day was established in this study

TEST FACILITY Hazelton (1991a)

B.1.7 Repeat dose oral toxicity – 90 days

TEST SUBSTANCE	Notified chemical (45% active ingredient)
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents EC Directive 84/449/EEC B.7 Subacute Toxicity – Oral
Species/Strain	Rat/HanIbm: WIST (SPF)
Route of administration	Oral – gavage
Exposure information	Total exposure: 90 days
Vehicle	Water

RESULTS

Dose (mg/kg bw/day)*	*Number and sex of animals*	*Mortality*
0	10 male, 10 female	0
10	10 male, 10 female	1
50	10 male, 10 female	0
200	10 male, 10 female	1
500	10 male, 10 female	8

Active ingredient

Mortality and time to death

4 males and 2 females receiving 500 mg/kg bw/day died or were sacrificed in extremis due to treatment related causes between days 27 and 91 of the study. Dosing errors led to deaths of 4 other animals across several treated groups (1 male and 1 female at 500 mg/kg bw/day, 1 male at 200 mg/kg bw/day and 1 male at 10 mg/kg bw/day).

Clinical observations

There was a dose related increase in incidence and severity of respiratory problems, primarily noisy breathing, in the 50 (males only), 200 and 500 mg/kg bw/day groups; dyspnoea and laboured respiration were observed in some 500 mg/kg bw/day animals. Noisy breathing persisted in three out of ten recovery group females at the end of 28 treatment free days.

Slight to moderate sedation and emaciation was found in some animals and ruffled fur was found in all animals treated with 500 mg/kg bw/day.

Ophthalmic examination revealed no treatment related findings.

Food consumption and bodyweight

Food consumption was significantly reduced in the 500 mg/kg bw/day animals, during weeks 1 and 2, and 5 to 10 in males, and during weeks 1 and 2, 7 and 8 and 12 and 13 in females. Reductions in body weight gain were noted in the males of the two highest dose groups, during weeks 9, 12 and 13 for the 200 mg/kg bw/day group (6.1-6.9%) and during weeks 2 to 14 in the 500 mg/kg bw/day group (6.5-13.3%). The body weight for the latter group returned to normal following one treatment free week.

Laboratory findings - Clinical chemistry, haematology, urinalysis

Among the animals treated with 500 mg/kg bw/day, there were a number of significant clinical chemistry findings. For both sexes there was a decrease in chloride concentration. For the males a slight increase in alanine aminotransferase and alkaline phosphatase was observed, and for the females a slight increase in uric acid and triglyceride concentration was observed.

A slight decrease in calcium concentration was seen for the males of all treated groups, and a slight increase in total protein and globulin concentration and a decrease in albumin to globulin (A/G) ratio was seen for the females treated with 50 mg/kg bw/day and above.

All findings with the exception of the chloride concentration for both sexes at 500 mg/kg bw/day and the A/G ratio for the 500 mg/kg bw/day females were reversed after 28 treatment free days. The study authors concluded that the findings are likely to reflect metabolic adaptation due to an increased functional load on the liver.

A number of haematology parameters were significantly changed for the animals treated at 200 and 500 mg/kg bw/day. These included a slight increase in erythrocyte count for both sexes at 500 mg/kg/day, slightly increased haemoglobin concentration for the 500 mg/kg bw/day males, slightly increased methaemoglobin concentration for the 500 mg/kg/day females, slightly increased haematocrit for males at 200 mg/kg bw/day and both sexes at 500 mg/kg/ bwday and slightly decreased mean corpuscular haemoglobin concentration for females at 200 mg/kg bw/day and both sexes at 500 mg/kg bw/day.

The study authors concluded that the changes reflect slight haemoconcentration and suggest changes in basal fluidity, and do not consider them to be of toxicological significance. The changes were found to be reversible after 28 treatment-free days.

The only change in urinalysis parameters which was reported was a slight increase in overnight urinary output for both sexes at 500 mg/kg bw/day during week 13. This was considered to be due to increased fluid intake.

Gross Pathology

No treatment related abnormalities were observed at necropsy.

The liver weights for females at 500 mg/kg bw/day, as well as the liver weight relative to body weight for females and males at 500 mg/kg bw/day, were significantly increased at the end of the treatment period; no significant increase was seen after the recovery period.

Histopathology

Four premature decedents showed lung changes indicative of an accident in dosing; no histopathological indication of the cause of death was noted for the animals which died of

treatment related causes.

Inflammatory changes were observed in the nasal cavity (exudate) and lungs of some animals in the 200 mg/kg bw/day and 500 mg/kg bw/day groups. These changes were considered by the study authors to be related to the general poor condition of the animals and consistent with reflux of irritating material.

Thymic atrophy (cortical) and focal haemorrhage was noted in some animals at 500 mg/kg bw/day and was considered by the study authors to be due to the poor condition of the animals. Thymic changes were also seen in the 28 day study in animals receiving 500 and 1000 mg/kg bw/day.

Stomach changes observed in the 28 day study were not seen in the 13 week study.

Remarks - Results

Administration of the test substance at 500 mg/kg bw/day resulted in increased mortality and biochemical and histopathological changes that were considered due to the poor general condition of the animals. No microscopic indication of the mechanism of toxicity was observed.

On the basis of mortality and morbidity (the only observed indicators of toxicity) at 500 mg/kg bw/day, the NOAEL is determined at 200 mg/kg bw/day. Based on clinical signs and the slight, transient effects on body weight gain, a NOEL of 50 mg/kg bw/day was established.

CONCLUSION

A NOAEL of 200 mg/kg bw/day and a NOEL of 50 mg/kg bw/day were established in this study.

TEST FACILITY	RCC (1991d)

B.1.8 Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (45% purity; batches MA# T9570 and MA# TA010)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation procedure
Species/Strain	*Salmonella typhimurium*: TA98, TA100, TA1535, TA1537, TA1538 *Escherichia coli*: WP2uvrA
Metabolic activation system	S9 microsomal fraction of male rat liver induced with Aroclor1254
Concentration range in main test	0, 0.5, 1.5, 4.5, 15, 45, 150, 450, 1500, 2250 µg/plate (active ingredient)

Vehicle	Water
Remarks - Method	Concentrations were tested in triplicate, in the presence and absence of metabolic activation; each batch was tested in two repeat experiments. Appropriate strain specific positive control reference substances were used

RESULTS

Metabolic activation	*Test substance concentration (µg/plate) resulting in:*			
	Cytotoxicity in preliminary test	*Cytotoxicity in main test*	*Precipitation*	*Genotoxic effect*
Absent				
Test 1	≥ 1500	≥ 450	> 2250	Negative
Test 2		> 150	> 150	Negative
Present				
Test 1	≥ 1500	≥ 450	> 2250	Negative
Test 2		> 150	> 150	Negative

Remarks - Results	Two independent reports were generated on the two separate batches of test article (MA# T9570, MA# TA010). MA# T9570: in the first experiment with concentrations of 45 µg/plate and above, toxicity as indicated by a moderate reduction in background lawn became apparent for all *Salmonella* strains at 450 µg/plate with or without S9; repeat experiments on this sample using lower concentration ranges were performed. MA# TA010: maximum concentrations of 450 µg/plate were used for the *Salmonella* strains. No substantial increase in the number of revertant colonies or indication of clear dose response was observed for either sample; due to a dosing error, only one of two assays was evaluated.
CONCLUSION	The notified chemical was not considered mutagenic in the bacterial strains tested in the absence or presence of metabolic activation provided by rat liver S9 fraction.
TEST FACILITY	MA (1991a; 1991b)

B.1.9 Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (45 % active ingredient)

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test.

Cell Type/Cell Line Mouse lymphoma L5178Y

Metabolic Activation System S9 fraction from animals pretreated with Aroclor 1254 and Arochlor 1242 (2:1 mixture)

Vehicle Water

Remarks - Method Cell culture was treated with test material in the presence or absence of metabolic activation for 4 hours; the cells were washed and resuspended in fresh medium; a fixed number of cells was then suspended in selection medium to selectively recover only TK-/- mutants; they were then seeded into dishes and colonies allowed to grow for 10 to 12 days. Positive controls were ethyl methanesulphonate 0.5, 0.25 µL/mL (for cells treated without metabolic activation) and 7,12-dimethylbenz(a)anthrene 2.5, 5.0 µg/mL (for cells treated with metabolic activation).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 2.3, 4.5, 14, 23, 29, 36	4 h	24-48 h	10-12 days
Test 2	0, 2.3, 4.5, 9.0, 13, 18, 22, 27, 32	4 h	24-48 h	10-12 days
Present				
Test 1	0, 2.3, 4.5, 14, 23, 29, 36, 43, 50	4 h	24-48 h	10-12 days
Test 2	0, 13, 18, 22, 27, 32, 36, 38, 41, 43, 45	4 h	24-48 h	10-12 days

RESULTS

Metabolic Activation	Test Substance Concentration (µg/mL) resulting in:			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 450	≥ 43	none	Negative
Test 2	-	≥ 36	none	Negative
Present				
Test 1	≥ 450	≥ 56	none	Negative
Test 2	-	≥ 50	none	Negative

Remarks - Results In an initial range-finding test, 100 % toxicity was observed at and above 450 µg/mL. The mutant frequencies observed at the TK locus were below the minimum criteria with and without

	metabolic activation and the compound was considered to be non-mutagenic. The solvent and positive controls fulfilled the requirements for a valid test.
CONCLUSION	The notified chemical did not induce forward mutations in mouse lymphoma L5178Y cells in vitro with or without metabolic activation.
TEST FACILITY	MA (1991c)

B.1.10 Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (45 % active ingredient)
METHOD	OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.
Cell Type	Chinese Hamster Ovary (CHO)
Metabolic Activation System	Rat liver S9 fraction from animals pretreated with Aroclor 1254
Vehicle	Water
Remarks - Method	Where metabolic activation was used, test material or positive controls were added to cell cultures in serum free medium for 6-hour incubation with S9 mix. The cells were then washed and incubated in fresh complete medium for an additional 18 hours incubation time. A similar procedure was carried out in the absence of S9; in the absence of metabolic activation, cells were also exposed continuously for 24 and 48 hours with colcemid was added two hours before harvest to arrest cells in metaphase.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1A	1.7, 3.3, 6.5, 13*, 25*, 50*, 100	6	24
Test 1B	1.7, 3.3, 6.5*, 13*, 25*, 50, 100	24/48	24/48
Test 2A	1.7, 3.3, 6.5, 13*, 25*, 50*, 100	6	24
Test 2B	1.7, 3.3, 6.5*, 13*, 25*, 50, 100	24/48	24/48
Present			
Test 1A	1.7, 3.3, 6.5, 13, 25, 50, 100	6	24

Test 2A	1.7, 3.3, 6.5, 13*, 25*, 50*, 100	6	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1A	> 50	100	none	Positive
Test 1B	> 50	≥ 50	none	Positive
Test 2A	> 50	100	none	Negative
Test 2B	> 25	≥ 50	none	Negative
Present				
Test 1A	> 50	100	none	Negative
Test 2A	> 50	100	none	Negative

Remarks - Results

No precipitation occurred for any of the test concentrations used. Excessive cytotoxicity (growth inhibition), as indicated by < 25 % confluency, was observed at the highest dose used in all cases. Concentrations of 13, 25 and 50 µg/mL were used for scoring except for the 48-hour exposure, where an insufficient yield of metaphase cells was found for 50 µg/mL, therefore the 6.5 µg/mL dose was used for scoring.

In the initial assay, cytotoxicity (mitotic inhibition) was approximately 72 % and 38 % at the highest dose evaluated in the 24 and 48-hour continuous treatment studies. An increase in polyploid cells was seen at 50 µg/mL in the 6-hour non-activated treatment however no statistically significant increase in chromosome aberrations was observed with and without S9 in the 6 and 24-hour treatments. The percentage of cells with structural aberrations was significantly increased for the 25 and 50 µg/mL 48-hour treatments and a positive dose response trend was found. In the presence of S9, no significant increase in the percentage of cells with structural and numerical aberrations was observed

In the repeat assay, cytotoxicity (mitotic inhibition) was approximately 51 % and 80 % at the highest dose evaluated in the 24 and 48-hour continuous treatment studies: an increase in polyploid cells was seen for all doses in the 48-hour non-activated treatment. No statistically significant increase in chromosome aberrations was observed with and without S9 in any of the treatments and no dose response trend was observed.

CONCLUSION	The notified chemical was found to induce chromosome aberrations in the absence of metabolic activation under the conditions of the test. The test authors did not consider the result to be biologically significant because the increases in structural and numerical aberrations were within the historic control range, and the results for the highest 48-hour dose in the initial experiment varied widely between flasks, indicating excessive toxicity, and the results were not reproduced in a repeat experiment. No statistically significant increase in chromosome aberrations was observed in the presence of metabolic activation.
TEST FACILITY	MA (1991d)

B.1.11 Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical (45 % active ingredient)
METHOD	OECD TG Rat Bone Marrow In Vivo Cytogenicity Study
Species/Strain	Rat/Sprague Dawley
Method of administration	Gavage; single dose; dose volume 20 mL/kg test material
Vehicle	Water
Remarks - Method	Harvest times of 8 and 12 hours (5 animals per time point) were used based on the results of the cell cycle kinetics test

Dose (mg/kg bw)	*Number and sex of animals*	*Sacrifice time (hours)*
0 (cell cycle kinetics test)	3 male	24
1800	3 male	24
0 (cytogenetic assay)	10 male, 10 female	8, 12
180	10 male	8, 12
210	10 female	8, 12
600	10 male	8, 12
700	10 female	8, 12
1800	10 male	8, 12
2100	10 female	8, 12

20 (Positive control, CP)	5 male, 5 female	12

CP = cyclophosphamide

RESULTS

Doses producing toxicity	During the cell cycle kinetics test, one animal exhibited lethargy and diarrhoea, another exhibited breathing difficulties. During the cytogenetic assay, no clinical signs of toxicity were observed.
Genotoxic effects	No significant change in mitotic index was observed; no significant increases in percentage of cells containing one or more aberrations or the mean aberrations per cell per animal were observed; no evidence of dose response was observed.
Remarks - Results	Clear positive results were obtained with the positive control, indicating that the test system responded appropriately.
CONCLUSION	The notified chemical was negative in the acute cytogenetic assay using male and female rats.
TEST FACILITY	MA (1991e)

B.1.12 Developmental toxicity

TEST SUBSTANCE	Notified chemical (45% active ingredient)
METHOD	OECD TG 414 Teratogenicity, adopted May 1981; Health Effects Test Guidelines (TSCA), issued September 1985 and Series 83-3 of the Environmental protection Agency Pesticide Assessment Guidelines (FIFRA), issued November 1984.
Species/Strain	rat/Crl:CD VAF/Plus
Route of Administration	Oral-gavage
Exposure Information	Test material administered as a single dose on days 6 through 15 of gestation.
Vehicle	Deionised water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number of Animals*	*Dose** *(mg/kg bw/day)*	*Mortality*

I	25 females	0	0
II	25 females	15	0
III	25 females	150	0
IV	25 females	363	0

*Active ingredient

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases.

Effects on Dams

Increased salivation was observed in all animals in the 363 mg/kg bw/day group and, at low incidence, in the 150 mg/kg bw/day group.

Decreased activity was observed in two animals in the 363 mg/kg bw/day group and material around the mouth was observed in 4 animals of this group and one animal in the 150 mg/kg bw/day group.

A significant decrease in bodyweight gain was observed in the 363 mg/kg bw/day group during the overall gestation time (days 0 to 20), particularly during the early treatment period (days 6 to 9).

No significant treatment-related effects were observed at necropsy.

No treatment-related differences were observed in the caesarian section parameters.

Effects on Foetus

No statistically significant treatment related differences were observed between the incidence of foetal malformations between the treated groups and the control groups.

Developmental variations were generally comparable between treated and control groups although a slight increase in unossified sternebrae #5 and #6 was observed in the 150 mg/kg bw/day and 363 mg/kg bw/day groups; no dose related trend was observed and the variations were assigned to normal biological variability.

Remarks - Results

Oral administration of the notified chemical as an aqueous solution on days 6 through 15 of gestation at a dose level of 363 mg/kg bw/day produced maternal toxicity indicated by salivation, decreased activity and material around the mouth and significant depression in maternal body weight gain during the treatment period. Increased salivation was also observed at low incidence at 150 mg/kg bw/day. No significant adverse effects on selected reproductive parameters were observed in the treated animals.

CONCLUSION

The notified chemical does not appear to be a selective developmental toxicant at dose levels producing maternal toxicity; a NOEL for developmental toxicity of 363 mg/kg bw/day was established in this study; a NOAEL for maternal toxicity of 150 mg/kg bw/day was established on the basis of depression of bodyweight gain at the higher dose.

Test Facility IR&DC (1991)

B.1.13 Pharmacokinetic/toxicokinetic – oral (C12- and C18 components)

TEST SUBSTANCE	C12 Glucose amide, C18 Glucose amide, ^{14}C labelled
METHOD	Oral uptake, as per predetermined protocol
Species/Strain	Rat/Sprague Dawley
Number/Sex of animals	12 males per compound
Observation period	8 hours; sacrifices at 2 hour intervals
Administration method	Gavage, dose levels 1736 mg/kg (C_{12}) and 3830 mg/kg (C_{18})
STUDY DESIGN	Designed to provide information about the absorption, distribution and elimination of the test substance in blood, plasma, liver, kidney, testes and bone marrow following oral administration.
RESULTS	
Gross Pathology	The stomachs were distended with food, a white precipitate and gas for the duration of the study. At 2 hours post dose, the entire small intestine was filled with a pale yellow fluid with a small amount of white material in the upper quarter. At 4 hours the white material was present along the full length of the small intestine and had entered the caecum in the C_{18} dosed animals. By the 8 hour observation the small intestines appeared normal and white material was present in the caeca; no formed faeces in the large intestine appeared to contain white material.
Radioanalytical	Absorption of radioactivity increased through the experiment with the highest tissue radioactivity levels being found at 8 hour after dosing All tissues sampled with the exception of the testes showed parallel increases in radioactivity, with consistently higher levels than whole blood. The liver showed the highest level, followed by kidney, plasma and bone marrow; the bone marrow had a 2 fold increase over whole blood for C_{12}, and a 3.5 fold increase for C_{18}. The testes showed consistently lower levels than whole

	blood. No radioactivity balance was performed in this study.
CONCLUSION	The C12 Glucose amide and C18 Glucose amide were absorbed from the digestive system and these chemicals or metabolites were widely distributed throughout the tissues after 8 hours.
TEST FACILITY	P&G (1991a)

B.1.14 Pharmacokinetic/toxicokinetic – dermal (C12 component)

TEST SUBSTANCE	C12 Glucose amide, ^{14}C labelled
METHOD	Dermal uptake, as per predetermined protocol
Species/Strain	Rat/Sprague Dawley
Number/Sex of animals	4/male
Observation period	72 hours
Administration method	Semi-occluded dose cell applied for 72 hours, test material dissolved in absolute ethanol; dose level 9.9 mg/kg; skin area 7.63 cm^2
Remarks - Method	The dose cell for one animal became unattached by 72 hours. Based on the differences in radioactive distribution for this animal, it was concluded that this animal had ingested test material. The radiochemical data for another animal indicated that seepage from the dose cell and subsequent ingestion had occurred. The results were therefore based on the remaining two animals.
STUDY DESIGN	Designed to provide information about the absorption, distribution and elimination of the test substance following dermal administration.
RESULTS	
Radioanalytical	A radioactive material balance of 95 % (+/-5 %) was found at the end of 72 hours, 94.4 % of the dosed radioactivity was found in the dose cell and skin wash, 0.27 % was found in the urine and cage wash, 0.19 % was found in the examined tissues and the carcass, 0.1 % was found in the faeces and gastrointestinal tract wash and 0.02 % was found in the expired carbon dioxide. Very low levels of radioactivity were found in all tissues examined at 72 hours; the highest level was in the femur, followed by the carcass, whole blood, adipose tissue and bone marrow.

CONCLUSION	The C12 glucose amide was absorbed through the skin to the extent of 0.5 % of the applied dose during 72 hours; the principal route of elimination was through urine.
TEST FACILITY	P&G (1991b)

B.2 Human toxicological data

B.2.1 Skin irritation – human volunteers

TEST SUBSTANCE	Notified chemical (36.5% active ingredient)
METHOD	Three application patch test as per predetermined protocol provided by the sponsor.
STUDY DESIGN	The test substance (0.5 mL, 0.25 % w/v) was applied under semi-occlusive dressing to the upper arm for 24 hours on days 1, 4 and 6. A number of other chemicals were tested simultaneously. The skin reaction grading was performed by an experienced assessor using the scoring scale outlined in the note to the Results table.
Study Group	Pilot study: 4 volunteers; age and sex not specified; Main study: 12 volunteers; age and sex not specified.
Vehicle	The test substance was administered in distilled water.
Remarks - Method	No significant deviation to the study protocol. One panellist failed to complete the study.

RESULTS

	Panelist No. and Irritation Score										
Day	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***
4	0*	0	1	1	0.5	0	1	1	1	0.5	0.5
6	0.5	0.5	1	1	1	0.5	1	1	1	0.5	0.5
8	0.5	0.5	1	1	1	1	1	1	1	1.5	1

* the grading scale used was:
0 no apparent cutaneous involvement
1 faint but definite erythema, no eruptions or broken skin, **or** no erythema but definite dryness; may have epidermal fissuring
2 moderate erythema, may have a few papules or deep fissures, moderate to severe erythema in the cracks
3 severe erythema (beet redness), may have generalised papules **or** moderate to severe erythema with slight oedema (edges well defined by raising)
4 generalised vesicles or eschar formation **or** moderate to severe erythema **or** oedema extending beyond the area of the patch

Remarks - Results	All subjects out of the total study group showed evidence of irritation at two or three time points. Of the 11 panellists completing the study, 9 showed at most a faint erythema or no erythema but definite dryness at the application site. The average irritation score was determined to be 0.76.
CONCLUSION	The notified chemical was mildly irritating under the conditions of the test.
TEST FACILITY	I S Consultancy (1992)

B.2.2 Skin irritation – human volunteers (C12 component)

TEST SUBSTANCE	C12 glucose amide (88% active ingredient)
METHOD	Three application patch test as per predetermined protocol provided by the sponsor.
STUDY DESIGN	The test substance (0.5 mL; 0.01%, 0.1%, 1.0% w/v) was applied under semi-occlusive dressing to the upper arm for 24 hours on days 1, 4 and 6. The skin reaction grading was performed by an experienced assessor using the scoring scale outlined in the B.4 Result table.
Study Group	12 volunteers: 9 females, 3 males; 18-55 years of age.
Vehicle	The test substance was administered in distilled water.
Remarks - Method	No significant deviation to the study protocol.

RESULTS

		Panelist No. and Irritation Score											
Conc	*Day*	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***
0.01%	4	0*	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0		0	0	0	0	0	0	0
0.1%	4	0	1	0	0	2	0.5	0	0.5	0.5	0.5	0	0
	6	0.5	1	0	0	1.5	1	0	0	1	0.5	0.5	0
	8	0.5	1	0	0	0.5	1	0.5	0.5	1	1	0.5	0.5
1.0%	4	0.5	1	0	0	2.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5
	6	0.5	1	1	0	2	1	0.5	0	1	0.5	0.5	2
	8	1	1.5	2.5	2	1	1.5	1.5	0.5	2	2.5	1	1.5

* grading scale as described in the not to B.4 Results table.

Remarks - Results	The average irritation scores were determined to be 0.0 (0.01%), 0.56 (0.1%) and 1.07 (1.0%). With one exception, all subjects showed evidence of irritation following at least two (of three) applications at the highest concentration. The C12 glucose amide was found to be non-irritant at 0.01 %, mildly irritating at 0.1 % and slightly irritating at 1.0%.
CONCLUSION	The C12 glucose amide was slightly irritating at the highest concentration tested in this patch test.
TEST FACILITY	P&G (1990)

B.2.3 Skin sensitisation – human volunteers (C12 component)

TEST SUBSTANCE	C12 glucose amide (88% active ingredient)
METHOD	Human repeat insult patch test (in house test method).
STUDY DESIGN	The study was conducted in two phases, a pilot study and main study. The main study would be initiated only if there was no indication of contact sensitisation in the pilot study.
Study Group	88 females, 30 males; age range 28 to 84 (107 at completion of study)
Vehicle	Distilled water
Induction Procedure	Nine repeat, 24-hour applications of 0.5 mL of the test substance (diluted to 0.05% in vehicle) under semi occluded patch conditions at three applications per week for 3 weeks, to the same skin area of the upper arm.
Rest Period	17 days
Challenge Procedure	Challenge patches were applied to both the original and the alternate arm for 24 hours. Challenge sites were examined for dermal reactions 48 and 72 or 96 hours post-application.
Remarks - Method	One subject did not receive a patch on the alternate arm due to a surgical procedure. Seven subjects began the study three days after the main cohort. These deviations did not invalidate the results of the study.
RESULTS	During the induction phase, 37 subjects exhibited responses of grade 1 and one subject a response of grade 2. During the challenge phase, five subjects exhibited responses of grade 1 with the response patterns concluded to be indicative of primary

	irritation. There were no reactions indicative of sensitisation to the test substance following the challenge exposures.
Remarks - Results	11 volunteers did not complete the study for reasons unrelated to treatment.
CONCLUSION	The C12 glucose amide was non-sensitising under the conditions of the repeat insult patch test.
TEST FACILITY	Harris (1990)

Appendix C: Environmental fate and ecotoxicological investigations

The robust summaries of the ecotoxicological studies analysed for the assessment of the notified chemical/polymer as a new chemical are presented here.

C.1 Environmental fate

C.1.1 Ready biodegradability

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	OECD TG 301B Ready Biodegradability – Modified Sturm Test EC Directive 84/449/EEC C.5 Biotic Degradation – Modified Sturm Test
Inoculum	Sewage microorganisms obtained from a sewage treatment plant.
Exposure period	34 days
Auxiliary solvent	$Ba(OH)_2$
Analytical Monitoring	The amount of CO_2 produced was determined by titration of the remaining amount of $Ba(OH)_2$ with standardized HCl solution.
Reference Substance	Diethylene glycol
Remarks - Method	No significant protocol deviations. The degradation of the test substance was assessed by determination of carbon dioxide produced (% of Th CO_2) at various time periods. Dissolved organic carbon (DOC) was also measured after 34 days, but is not reported here.

RESULTS

Test substance (20 mg/L)		*Diethylene glycol (20 mg/L)*	
Day	*% Degradation**	*Day*	*% Degradation**
5	21	5	3
12	46	12	7
21	81	21	39
28	83	28	71

34	89	24	91

* Based on CO_2 evolution

Remarks - Results	The ready biodegradability of the notified chemical was determined at two different concentrations: 20 mg/L (as shown in the above table) and 10 mg/L, with the lower concentration showing a degradation of 86% after 34 days. Although the results indicate the test substance is inherently biodegradable, it cannot be classified as being ready biodegradable as 60% degradation was only achieved after 14 days. The reference substance showed a degradation of 91% within 34-day period, but 60% degradation was not achieved until 26 days. Thus the reference substance also did not satisfy the criteria for ready biodegradability.
CONCLUSION	The notified chemical is biodegradable under aerobic conditions, but cannot be considered ready biodegradable.
TEST FACILITY	LISEC (1991)

C.2 Ecotoxicological investigations

C.2.1 Acute toxicity to fish

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	EC Directive 79/831/EEC C.2 Acute toxicity for Daphnia
Species	*Brachydanio rerio* (zebra fish)
Exposure period	96 h
Auxiliary solvent	None
Water hardness	210 mg $CaCO_3$/L
Analytical monitoring	Not applicable
Remarks - Method	No significant protocol deviations.

RESULTS

Nominal concentration (mg/L)	*Number of fish*	Mortality					*% Mortality (96 h)*
		2 h	*24 h*	*48 h*	*72 h*	*96 h*	

Control	10	0	0	0	0	0	0
3.2	10	0	0	0	0	0	0
5.6	10	0	0	0	0	0	0
10	10	0	10	0	0	0	10%
18	10	10	0	0	0	0	100%
32	10	10	0	0	0	0	100%

LC50	>5.6, <10 mg/ at 96 hours
NOEC	5.6 mg/L at 96 hours
Remarks - Results	The results were based on nominal concentrations as the actual concentration of the test substance was not determined during the fish toxicity test. No mortality or aberrant behaviour was observed for test concentrations below 5.6 mg/L over the full 96-hour test period. However, after 24 hours exposure to a nominal concentration of 10 mg/L, all fish (in both duplicate tests) had died, while no fish mortality was observed in either of the duplicate control vessels. The 96-hour LC50 value was estimated as 7.6 mg/L using a parametric analytical method. The steepness of the effect curve precluded probit analysis, but it appears that the 96 hour LC50 value would be between 5.6 and 10 mg/L.
CONCLUSION	The notified chemical is moderately toxic to fish.
TEST FACILITY	TNO (1991a)

C.2.2 Acute toxicity to fish (C14 component)

TEST SUBSTANCE	C14 linear glucose amide
METHOD	US EPA (1975) Methods for Acute toxicity tests with fish, macroinvertebrates and Amphibians (EPA-660/3-75-009)
Species	*Pimephales promelas* (fathead minnow)
Exposure period	96 h
Auxiliary solvent	None
Water hardness	34 mg $CaCO_3$/L

Analytical monitoring	Not applicable
Remarks – Method	The study protocol required dissolved oxygen concentration at > 40% saturation. For the 3.3 mg/L vessel at 48 h exposure time the dissolved oxygen ranged from 32-39% before aeration was started to raise it to > 40%. The study authors stated that, based on the clear dose-response observed for this group, the decrease in oxygen concentration at 48 h did not adversely affect the exposed fish.

RESULTS

Nominal concentration (mg/L)	*Number of fish*	*Mortality (%)*				
		0 h	*24 h*	*48 h*	*72 h*	*96 h*
Control	2 x 5	0	0	0	0	0
1.2	2 x 5	0	0	0	0	0
2.0	2 x 5	0	0	0	10	10
3.3	2 x 5	0	40	50	60	60
5.5	2 x 5	0	100	100	100	100
9.2	2 x 5	0	100	100	100	100

LC50	2.9 mg/L at 96 hours
NOEC	1.2 mg/L at 96 hours
Remarks – Results	The results were based on nominal concentrations as the actual concentration of the test substance was not determined. No mortality or other effects were observed in the fish over the 96-hour test period for 1.2 mg/L, but 10 % of the fish had died after 48 hours exposure to the 2.0 mg/L solution. All the fish exposed to the 5.5 mg/L solution were dead after 24 hours. The data was analysed using probit analysis to give the 96-hour LC50 value of 2.9 mg/L (95 % confidence interval 2.4-3.7 mg/L). Sub-lethal effects such as darkening of pigmentation and loss of equilibrium were also observed in fish exposed to ≥ 2.0 mg/L and greater which had not died.
CONCLUSION	The C14 component of the notified chemical is moderately toxic to this species of fish.
TEST FACILITY	Springborn (1992a)

C.2.3 Chronic toxicity to fish

TEST SUBSTANCE	Notified chemical (44.7% purity)
METHOD	US EPA TSCA – 40CFR 797-1600 (1987) – Protocol For Conducting an Early Stage Toxicity Test with Fathead Minnow (*Pimephales promelas*)
Species	*Pimephales promelas (*fathead minnow)
Exposure period	35 days
Auxiliary solvent	None
Water hardness	24–56 mg $CaCO_3$/L
Analytical monitoring	HPLC
Remarks - Method	No significant protocol deviations. A preliminary range finding test resulted in 0% survival after 13 days of exposure to 10 mg/L. The definitive test involved 35 days of continuous exposure, which included a 5 day hatching period and a 30 day post-hatching period.

RESULTS

Concentration (mg/L)		*Number of embryos*	*% Embryo hatching success*	*% Larval survival at termination*
Nominal	*Actual**			
Control	Control	60	81	98
0.63	0.69	60	78	94
1.3	1.5	60	88	95
2.5	2.5	60	80	94
5.0	4.8	60	89	93
10.0	10.0	60	0	0

* Mean measured concentrations

LOEC	10 mg/L at 35 days
NOEC	4.8. mg/L at 35 days
Remarks - Results	At 10 mg/L of the test substance, no animals survived the hatching period. Those organisms exposed to concentrations of the test substance at a nominal concentration of 5 mg/L (measured 4.8 mg/L) and below had a survival rate of 93 to 95

	%, not statistically different to the 98 % survival rate of the control organisms. The weight and length of the larvae after the 30 day post hatch period were also very similar at all test concentrations below the (nominal) 5 mg/L to those of the controls, and were within 95 % of the control values. The results of this test give a LOEC of 10 mg/L (measured and nominal) and a NOEC of 4.8 mg/L (nominally 5 mg/L).
CONCLUSION	The notified chemical is toxic to fish but not with long lasting effects.
TEST FACILITY	Springborn (1992b)

C.2.4 Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	EC Directive 79/831/EEC C.2 Acute toxicity for Daphnia
Species	*Daphnia magna*
Exposure period	48 hours
Auxiliary solvent	None
Water hardness	217 mg $CaCO_3$/L
Analytical monitoring	Not applicable
Remarks - Method	No significant protocol deviations.

RESULTS

Nominal concentration (mg/L)	*Number of D. magna*	*Number immobilised*	
		24 h	*48 h*
Control	20	0	0
3.2	20	0	0
5.6	20	1	1
10	20	0	1
18	20	12	7

32	20	-	-

EC50	18 mg/L at 48 hours
NOEC	10 mg/L at 48 hours
Remarks - Results	It was noted that some flocculant was present in the vessel containing the highest test concentration of 32 mg/L, but that all other test media were clear. No statistically significant mortality or sublethal effects were observed over the 48 hour test period for the test concentrations of 10 mg/L and lower, but after 24 hours exposure at 18 mg/L two of the test animals were immobile, and 7 had become immobile after 48 hours exposure. All 20 animals were dead after 24 hours exposure to the 32 mg/L test solution.
CONCLUSION	The notified chemical is slightly toxic to aquatic invertebrates.
TEST FACILITY	TNO (1991b)

C.2.5 Acute toxicity to aquatic invertebrates (C14 component)

TEST SUBSTANCE	P2704.01 (C14 component)
METHOD	US EPA (1975) Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Committee on Methods for Toxicity Tests with Aquatic Organisms (EPA-3-75-009)
Species	*Daphnia magna*
Exposure period	48 hours
Auxiliary solvent	None
Water hardness	160 mg $CaCO_3$/L
Analytical monitoring	Not applicable
Remarks – Method	A total of 20 daphnids (5 daphnids/replicate across 4 replicates) were used. No significant deviations to the test protocol were reported.

RESULTS

Nominal concentration	*Number of D. magna*	*Cumulative immobilized (%)*

(mg/L)		*24 h*	*48 h*
Control	20	0	0
1.2	20	0	0
2.0	20	0	0
3.3	20	0	0
5.5	20	0	65
9.2	20	100	100

EC50	5.0 mg/L at 48 hours
NOEC	3.3 mg/L at 48 hours
Remarks – Results	It was noted that the 9.2 mg/L test solution was slightly cloudy (probably reflecting the expected lower solubility of the C14 component compared with the C12 component of the notified chemical), but that all other test media were clear. No immobilisation of the test animals was observed for the test concentration at 3.3 mg/L and lower, but after 48 hours exposure to the 5.5 mg/L solution, 65 % of the *Daphnia* had been immobilised. All test animals were dead after 24 hours exposure to the 9.2 mg/L solution. The results were analysed using probit analysis to give the 48 hour LC50 of 5.0 mg/L (95 % confidence interval 3.3-9.2 mg/L). A NOEC of 3.3 mg/L at 48 hours was determined.
CONCLUSION	The C14 component of the notified chemical is moderately toxic to *Daphnia magna*.
TEST FACILITY	Springborn (1992c)

C.2.6 Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	*Daphnid* chronic toxicity test - 40 CFR 797.1330
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	None

Water Hardness	160-180 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	The definitive test was conducted at the nominal concentrations of 0.63, 1.3, 2.5, 5.0, and 10 mg/L of the test substance, which corresponds to 0.56, 0.97, 2.2, 4.3, and 8.9 mg/L of the active components. A total of 40 daphnids (10 daphnids/replicate across 4 replicates per test concentration) were used. No significant deviations to the test protocol were reported.

RESULTS

Mean Measured Test Concentration (mg/L)

	Control	*0.56*	*0.97*	*2.2*	*4.3*	*8.9*
Cumulative no. of offspring produced per female	212 ± 10	220 ± 7	211 ± 14	225 ± 16	162 ± 103	5 ± 3
Survival (%)	90	90	98	98	60	13

NOEC	4.3 mg/L at 21 days
Remarks - Results	All validity criteria for the test were satisfied. The test solutions had a 90% replacement rate of approximately every 9 hours during the 21 d test period. The actual concentrations of the test substance were measured at 0, 7, 14 and 21 days during the 21 d test period. The survival and reproductive output was significantly reduced at the highest concentration of the test substance as compared to the control. Throughout the 21 d period, adults were observed to be small, to be swimming and to be exhibiting other behavioural abnormalities at the two highest test concentrations. The 21 d EC50 and NOEC were determined to be 6.8 mg/L (using non-linear interpolation) and 4.3 mg/L respectively based on actual concentrations of the active components.
CONCLUSION	Under the conditions of the study, the notified chemical is not considered to be harmful to aquatic invertebrates on a chronic basis.
TEST FACILITY	Springborn (1992d)

C.2.7 Algal growth inhibition test

TEST SUBSTANCE	Notified chemical (98.8% purity)
METHOD	OECD TG 201 Algal Growth Inhibition Test
Species	*Selenastrum capricornutum (*green alga)
Exposure period	92 hours
Concentration range	Nominal: 0, 3.2, 5.6, 10, 18, 32 and 56 mg/L
Auxiliary solvent	None
Water hardness	Not reported
Analytical monitoring	Not applicable
Remarks - Method	No significant protocol deviations.

RESULTS

*Biomass**		*Growth**	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
(mg/L at 92 h)	*(mg/L)*	*(mg/L 0-92 h)*	*(mg/L)*
14	5.6	30	5.6

Remarks - Results	Actual concentrations were not determined. Inhibition of algal growth was apparent after about 30 hours, particularly at the higher test concentrations, and the data was analysed using parametric models. The EC50 with respect to the area under the curve (EbC50) was found to be 14 mg/L (in the range 10–18 mg/L) and the EC50 with respect to growth rate (ErC50) was 30 mg/L with a 95% confidence interval of 26–34 mg/L. The NOEC was estimated to be 5.6 mg/L by visual comparison of the measured and calculated growth curves to the teat substance with those of the algal controls. Microscopic examination of the algae revealed some distorted cells at the higher exposures.
CONCLUSION	The notified chemical is slightly toxic algae.
TEST FACILITY	TNO (1991c)

C.2.8 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: 5, 10, 20, 40, 80 mg/L Actual: Not determined
Remarks – Method	No significant deviation in protocol. Two controls containing no test substance. 3,5-dichlorophenol was used as the reference control. The rate of oxygen uptake was determined after a 3-hour incubation period.
RESULTS	
IC50	~115 mg/L
NOEC	~ 10 mg/L
Remarks – Results	All validity criteria for the test were satisfied. A 14% inhibition of respiration rate over the controls was observed at 10 mg/L, which increased to 31% inhibition for the 80 mg/L test. The 3 h EC50 was determined to be approximately 115 mg/L based on the nominal concentration.
CONCLUSION	The notified chemical exhibits some inhibition of respiration in sewage treatment bacteria.
TEST FACILITY	LISEC (1991)

C.2.9 Acute Toxicity to Earthworm

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 207 Earthworm, Acute Toxicity Tests
Species	*Eisenia fetida*
Exposure Period	14 days
Concentration	Nominal: 1,000 mg per kg of dry soil

	Actual: Not determined
Remarks – Method	No significant deviation in protocol. The test material was homogeneously distributed through artificial soil (moisture content = 52.2 %) at a level of 1,000 mg per kg of dry soil. Ten worms were placed in each of two test containers containing the soil and the notified chemical, and a further ten placed in two "control" containers. The containers were maintained at a temperature of 20±2°C, and the general appearance and behaviour of the worms was monitored over a 14-day period.
RESULTS	
LC50	> 1,000 mg/kg dry soil at 14 days
NOEC	1,000 mg/kg dry soil at 14 days
Remarks – Results	All validity criteria for the test were satisfied. At the test concentration, no effects on mortality, weight increase, behaviour or appearance were observed over the 14 day test period.
CONCLUSION	The notified chemical is not toxic to earthworms.
TEST FACILITY	TNO (1994)

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