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AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

D-Glucopyranose, oligomeric, 2-ethylhexyl glycosides

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals Act 2019 (the IC Act) and Industrial Chemicals (General) Rules 2019 (the IC Rules) by following the Industrial Chemicals (Consequential Amendments and Transitional Provisions) Act 2019 (the Transitional Act) and Industrial Chemicals (Consequential Amendments and Transitional Provisions) Rules 2019 (the Transitional Rules)*. The legislations are Acts of the Commonwealth of Australia. The Australian Industrial Chemicals Introduction Scheme (AICIS) is administered by the Department of Health, and conducts the risk assessment for human health. The assessment of environmental risk is conducted by the Department of Agriculture, Water and the Environment.

This Public Report is available for viewing and downloading from the AICIS website. For enquiries please contact AICIS at:

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Executive Director
AICIS

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SUMMARY

The following details will be published on our website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1716	Nouryon Chemicals Australia Pty Ltd & Volkswagen Group Australia Pty Ltd	D-Glucopyranose, oligomeric, 2-ethylhexyl glycosides	Yes	< 150 tonnes per annum	Component of household, automotive, and industrial cleaning products, metal working fluids, drilling fluids and soil wetting agents

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Serious Eye Damage/Eye Irritation (Category 1)	H318 – Causes serious eye damage

Human Health Risk Assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the assessed chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental Risk Assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the assessed chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemical should be classified as follows:
  – Serious Eye Damage/Eye Irritation (Category 1): H318 – Causes serious eye damage

  In the absence of eye irritation data for end-use products, concentrations of the assessed chemical at ≥ 3% in end-use products warrant classification as causing serious eye damage (Category 1), according to the GHS criteria.

  Concentrations of the assessed chemical at ≥ 1% but < 3% in end-use products warrant classification as eye irritant (Category 2), according to the GHS criteria.

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

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the assessed chemical during reformulation:
  - Enclosed/automated processes
  - Adequate general ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the assessed chemical:
  - Avoid contact with skin and eyes
  - Use in a well ventilated area

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the assessed chemical:
  - Safety glasses or goggles
  - Impervious gloves
  - Protective clothing
  - Respiratory protection if inhalation exposure may occur

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

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

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

Public Health

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the assessed chemical for listing on the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP).
- Formulators should take into account the potential for the assessed chemical to cause serious eye damage, when manufacturing consumer products containing the assessed chemical for spray application.
- Products available to consumers containing the assessed chemical at or above concentrations causing eye effects should be labelled with warnings on potential adverse effects from exposure to the eyes.

Emergency procedures

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

Disposal

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

Regulatory Obligations

Specific Requirements to Provide Information
This risk assessment is based on the information available at the time of the application. The Executive Director may initiate an evaluation of the chemical based on changes in certain circumstances. Under Section 101 of the IC Act the applicant of the assessed chemical has post-assessment regulatory obligations to provide information to AICIS when any of these circumstances change. These obligations apply even when the assessed chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory).

Therefore, the Executive Director of AICIS must be notified in writing within 20 working days by the applicant or other introducers if:

- the final use concentration of the assessed chemical exceeds 15% in products available to the public;
- the function or use of the chemical has changed from a component of household, automotive, and industrial cleaning products, metal working fluids, drilling fluids and soil wetting agents;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on human health, or the environment.

The Executive Director will then decide whether an evaluation of the introduction is required.

Safety Data Sheet
The SDS of the products containing the assessed chemical provided by the applicant was reviewed by AICIS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND APPLICATION DETAILS

APPLICANT(S)
Nouryon Chemicals Australia Pty Ltd (ABN: 64 621 806 273)
44 Lakeview Drive
SCORESBY VIC 3179

Volkswagen Group Australia Pty Ltd (ABN: 14 093 117 876)
24 Muir Road
CHULLORA NSW 2190

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details taken to be protected information include: specific other names, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, use details, import volume, site of reformulation, identity of manufacturer/recipients, identity of analogues and identity of test facilities.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for hydrolysis as a function of pH, particle size and acute inhalation toxicity.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
EU (ELINCS, 2009)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
AG 6202 (product containing the assessed chemical at 60 – 70% concentration)

CAS NUMBER
161074-93-7

CHEMICAL NAME
D-Glucopyranose, oligomeric, 2-ethylhexyl glycosides

OTHER NAME(S)
2-Ethylhexyl glucoside
DFE-731

MOLECULAR WEIGHT
< 500 g/mol (UVCB)

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

3. COMPOSITION

DEGREE OF PURITY
> 97%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Crystalline paste (contains 84% assessed chemical, 16% water), dark brown liquid (AG 6202, contains 60 – 70% assessed chemical in water)

Property	***Value***	***Data Source/Justification***
Freezing Point	-5 °C	Measured*
Boiling Point	> 300 °C at 101.3 kPa	Analogue data
Density	1,182.9 kg/m^3 at 20 °C	Measured*
Vapour Pressure	5 × 10^{-8} kPa at 25 °C	Measured*
Water Solubility	≥ 790 g/L at 20 °C	Measured*
Hydrolysis as a Function of pH	Not determined	Cannot be estimated (QSAR, 2019ab)
Partition Coefficient (n-octanol/water)	log Pow = 1.1 at 20 °C	Measured*
Adsorption/Desorption	K_{oc} = 5 L/kg at 20 °C	Measured^
	K_{oc} = 2.49 L/kg at 20 °C	Analogue 1, calculated, QSAR (2019a)
	K_{oc} = 0.069 L/kg at 20 °C	Analogue 2, calculated, QSAR (2019b)
Dissociation Constant	Not determined	Does not contain dissociable functions
Surface Tension	30.2 mN/m at 23 °C	Measured*
Flash Point	> 110 °C at 101.7 kPa	Measured*
Flammability	Not flammable	Measured*
Autoignition Temperature	Not expected to autoignite	Measured*
Explosive Properties	Not explosive	Calculated
Oxidising Properties	Not oxidising	Calculated

* Conducted on a sample containing 84% assessed chemical and 16% water.
^ Conducted on a sample containing 63.5% assessed chemical and 36.5% water.

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

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

Physical Hazard Classification
Based on the submitted physico-chemical data depicted in the above table, the assessed chemical is not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
Nouryon
The assessed chemical will not be manufactured in Australia. It will be imported at 60 – 70% concentration for reformulation into varied domestic and industrial products at ≤ 15% concentration.

Volkswagen
The assessed chemical will not be manufactured or reformulated in Australia. It will be imported at < 5% concentration as finished cleaning products.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 150	< 150	< 150	< 150	< 150

PORT OF ENTRY
Sydney, Melbourne (by both applicants), Perth (by Volkswagen only)

TRANSPORTATION AND PACKAGING

Nouryon

The assessed chemical at 60 – 70% concentration will be introduced into Australia by sea in 20 kg HPDE plastic containers. These containers will be shipped on pallets with multiple pallets per container. The assessed chemical will be transported by road to warehouses for storage.

Volkswagen

The products containing the assessed chemical at < 5% concentration will be introduced into Australia by sea in bottles of 100 mL or 250 mL. These bottles will be transported in cardboard boxes or grid boxes to warehouses for storage.

USE

Nouryon

The assessed chemical will be used as a component (at concentrations ≤ 15%) of domestic and industrial cleaning products and industrial products such as metalworking fluids, drilling fluids, and soil-wetting agents.

Volkswagen

The assessed chemical will be used as a component (at concentrations < 5%) of automotive cleaning products.

OPERATION DESCRIPTION

Reformulation

The assessed chemical will not be manufactured in Australia. The formulations containing the assessed chemical (at 60 – 70% concentration) will be reformulated with additional components to form the finished end-use products at ≤ 15% concentration. Reformulation procedures are expected to vary depending on the nature of the products being made, and may involve both automated and manual transfer steps.

In general, it is expected that the local reformulation processes will typically involve transport of the assessed chemical to a raw material store. A chemist will sample the ingredient for Quality Assurance (QA) purposes. The compounder will subsequently weigh the appropriate amount of the ingredient into a blending tank. The mixing process is expected to be carried out in a closed system with fireproof mixers and pumps designed not to create aerosols or a dust hazard, and earthed for static discharges. The finished products containing the assessed chemical will be filled into retail containers of various sizes. Samples may be collected at various stages of blending process for QA testing.

End-use as cleaning products

Industrial, automotive, and domestic cleaning products containing the assessed chemical at ≤ 15% concentration may be used by consumers and professional cleaners. The cleaning products may be diluted with water prior to application. Industrial and professional cleaners may be used in either closed systems, such as automatic washing machines, or manually by rolling, brushing, spraying and dipping. There will be some application of the cleaning products on industrial equipment such as for cleaning photochemical plates. Domestic products containing the assessed chemical are expected to include hand and automatic dishwashing detergents, laundry detergents, and general and hard surface cleaners. Users of such products may apply them with dispensers, scoops, cloths, sponges, mops or brushes, or by spray followed by wiping. There will be some products intended for vehicle cleaning. The cleaning products will be completely discharged into sewerage systems after use.

End-use as metalworking fluids

Products containing the assessed chemical at ≤ 15% concentration may be used by industrial workers as metalworking fluids. The products are expected to be diluted with water prior to use, to a concentration of typically ≤ 1.8%. The metalworking fluid will be applied onto machinery during use to lubricate the metal surface, act as a sealant against foreign particles, or provide corrosion resistance. The metalworking fluid will be collected for disposal at the end of its service lifetime.

End-use as drilling fluids

Water-based drilling fluid products (also known as drilling muds) containing the assessed chemical at ≤ 15% are expected to be used at onshore and offshore drilling sites, where they will be used to aid the drilling of boreholes.

End-use as soil-wetting agents
The assessed chemical may be used in agriculture as a soil-wetting agent. These type of products would wet the soil to reduce the surface tension of water, and allow applied liquids such as pesticides to penetrate into the soil for enhancing delivery. The assessed chemical will be a component in these products at a concentration of 0.1 to 4%, or preferably 0.2 to 2%. The expected method of application would be by spraying the wetting agent onto the soil surface into the crop furrows by press wheels, typically at a cost effective rate of 0.5 – 2 L/ha. Alternatively, the soil wetting agent may be boom sprayed onto the soil at a rate of 20 – 50 L/ha.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	4	12
Professional compounder	8	12
Chemist	3	12
Packaging staff	8	12
Store persons	4	12
Cleaning products end-users	8	365
Metalworking fluid users	8	220
Metalworking fluid blending	8	100
Photochemical workers	8	30
Agricultural workers	4	20
Drillers	8	220

EXPOSURE DETAILS

Transport and storage
Transport, storage and warehouse workers may come into contact with the assessed chemical at 60 – 70% concentration only in the event of accidental breaching of containers.

Reformulation
During reformulation, dermal and ocular exposure of workers to the assessed chemical at 60 – 70% concentration may occur during pouring from containers, during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. It is expected that exposure will be minimised through the use of enclosed systems, and workers wearing personal protective equipment (PPE) such as protective clothing, eye protection and impervious gloves, as stated by the applicant. Inhalation exposure is not expected given the low vapour pressure of the assessed chemical and the use of adequate ventilation during the process.

End-use as cleaning products
Exposure to the assessed chemical in end-use products (at ≤ 15% concentration) may occur in professions where the services provided involve in the use of cleaning products. The principal route of exposure will be dermal, while incidental ocular and inhalation exposure are also possible. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place when using the assessed chemical.

End-use in industrial products
Workers may be exposed to products containing the assessed chemical at ≤ 15% concentration during various industrial processes. Dermal, ocular and possible inhalation exposure to the assessed chemical (at ≤ 15% concentration) may occur during the liquid mixing process, the transfer of the liquid into equipment, and during maintenance and servicing of equipment. In an industrial setting, it is likely that appropriate personal protection equipment such as gloves, safety glasses/googles and protective coveralls will be worn. Inhalation exposure is expected to be minimised by the use of local exhaust ventilation in areas around machinery where appropriate.

6.1.2. Public Exposure

There will be repeated exposure of the public to the assessed chemical at up to 15% concentration through the use of household cleaning products. The main route of exposure will be dermal, while ocular and inhalation exposure are also possible, particularly if products are applied by spray/applicators.

Data on typical use patterns of household cleaning product categories (SCCS, 2012; Cadby et al., 2002; ACI, 2010) in which the assessed chemical may be used are shown in the following tables. For the purposes of the exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed for the assessed chemical (ECHA, 2017). A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Household products (Indirect dermal exposure - from wearing clothes):

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	15	0.95	10	0.5121
Total					**0.5121**

C = maximum intended concentration of assessed chemical
Daily systemic exposure = (Amount × C × PR × PT × DA)/BW

Household products (Direct dermal exposure):

Product type	Frequency (use/day)	C (%)	Contact Area (cm^2)	Product Use C (g/cm^3)	Film Thickness (cm)	Time Scale Factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	15	1980	0.01	0.01	0.007	0.0046
Dishwashing liquid	3	15	1980	0.009	0.01	0.03	0.0376
All-purpose cleaner	1	15	1980	1	0.01	0.007	0.3248
Total							**0.3671**

C = maximum intended concentration of assessed chemical
Daily systemic exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale Factor × DA)/BW where C = concentration, DA = Dermal absorption rate, BW = Average bodyweight

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 assessed chemical at the maximum intended concentrations specified by the applicant in various product types. This would result in a combined internal dose of 0.8792 mg/kg bw/day for the assessed chemical.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – rat	LD50 > 5,000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity*
Skin irritation – rabbit	Non-irritating
Eye irritation – rabbit	Severely irritating
Eye irritation – rabbit (truncated study)	Likely to be severely irritating
Skin sensitisation – guinea pig, maximisation test (1993)	Inadequate evidence of sensitisation†
Skin sensitisation – guinea pig, maximisation test (2012)	Inadequate evidence of sensitisation^
Skin sensitisation – Buehler test (1992)	No evidence of sensitisation†
Repeat dose oral toxicity – rat, 28 days	NOAEL = 750 mg/kg bw/day*
Repeat dose oral toxicity – rat, 90 days	NOAEL = 150 mg/kg bw/day^
Mutagenicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – *in vitro* chromosome aberration test	Non genotoxic*
Genotoxicity – *in vitro* gene mutation test	Non genotoxic^

Endpoint	*Result and Assessment Conclusion*
Reproductive and developmental toxicity – rat	NOAEL (parental) = 150 mg/kg bw/day^ NOAEL (reproductive/developmental) = 750 mg/kg bw/day^

***** Conducted on a sample containing 84% assessed chemical and 16% water.
^ Conducted on a sample containing 63.5% assessed chemical and 36.5% water.
† Conducted on a sample containing 50% assessed chemical and 50% water.

Toxicokinetics, Metabolism and Distribution
No toxicokinetic data were provided for the assessed chemical. For dermal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption (ECHA, 2017). Additionally Log P values between 1 and 4 favour dermal absorption particularly if water solubility is high (ECHA, 2017). The assessed chemical has a molecular weight of 292 - 617 g/mol, very high water solubility (> 790 g/L) and a log Pow of 1.1 at 20 °C, indicating potential for absorption.

Following dermal exposure, alkyl glucosides such as the assessed chemical are expected to be metabolised by glucoside hydrolases in the skin into the separate glucoside and fatty alcohol components (Fiume *et al*., 2013). An expected metabolite (1-hexanol, 2-ethyl-) of the assessed chemical is a potential developmental toxicant, with a NOAEL of 130 mg/kg bw/day (NICNAS).

Acute Toxicity
The assessed chemical had low acute toxicity to rats in studies via the oral and dermal routes. There is no information available on the acute inhalation toxicity of the assessed chemical.

Irritation
The assessed chemical was found to be non-irritating to the skin of rabbits.

Two eye irritation test were conducted on rabbits using the assessed chemical and similar protocols. One rabbit only was used in each study, as adverse effects such as cornea opacity, iridial inflammation, and conjunctival irritation were evident. In the first study, some of these effects persisted through the full duration of the study to 21 days. The second study showed similar effects initially, but was terminated after 48 h for humane reasons. The assessed chemical was considered to be severely irritating to eyes.

Sensitisation
In a guinea pig maximisation test (GPMT) on a commercial solution of the assessed chemical using 50% topical induction concentration, dermal reactions were observed in some test animals following challenge. The dermal responses were seen in 5/30 test animals and to a lesser extent in 4/30 test animals. The result of this study is considered equivocal. A second guinea pig maximisation test on a similar commercial solution was conducted using 100% topical induction. In the challenge test, 14/20 and 8/20 treated animals (70% and 40%, respectively) showed discrete, patchy to moderate, confluent erythema at 24 and 48 hours after the challenge at 50% (corresponding to 31% active ingredient in the formulation). However, 7/10 and 3/10 control animals (70% and 30%, respectively) also showed skin reactions at 24 and 48 hours after the same challenge treatment. The cause of the skin reactions in control animals was not clear and the study was considered inconclusive.

An earlier guinea pig Buehler test was conducted using a test substance containing 50% concentration of the assessed chemical, and showed no evidence of skin sensitisation.

Alkyl glucosides are a class of chemicals that are commonly used in cosmetic and household products, and have recently been investigated for their sensitisation potential as there have been multiple reports of allergy contact dermatitis caused by some alkyl glucosides (Alfalah et. al, 2017, Monteiro et al., 2019). However, the exact mechanism of sensitisation caused by these chemicals is not well understood (Loranger et al., 2017). The assessed chemical is comprised of D-glucopyranoside (primarily mono- or di-) and 1-hexanol, 2-ethyl-. The chemical 1-hexanol, 2-ethyl- is not expected to be a skin sensitiser (NICNAS), and the D-glucopyranosides that are used for the synthesis of the assessed chemical are predominantly glucose and maltose which are not reported as being dermal sensitisers.

Based on all studies and the information available, the assessed chemical is not classified for skin sensitisation. However, the potential to cause skin sensitisation cannot be ruled out.

Repeated Dose Toxicity
A 28 day repeated dose oral toxicity study in rats was conducted on the assessed chemical with dose levels of 0, 15, 150, and 750 mg/kg bw/day. The No Observed Adverse Effect Level NOAEL was established as 750 mg/kg bw/day, based on the absence of toxicologically relevant adverse effects up to this dose level.

A 90 day repeated dose oral toxicity study in rats was conducted on the assessed chemical with dose levels of 0, 50, 150 and 450 mg/kg bw/day. Under the conditions of this study, the NOAEL was established at 150 mg/kg bw/day, based on effects observed at 450 mg/kg/day.

Mutagenicity/Genotoxicity
The assessed chemical was negative in a bacterial reverse mutation assay, in an *in vitro* mammalian chromosome aberration test using cultured human lymphocytes cells and in an *in vitro* mammalian cell gene mutation test using mouse lymphoma/L5178Y cells.

Toxicity for Reproduction
A one generation reproductive and developmental toxicity study in rats was conducted on the assessed chemical with dose levels of 0, 15, 150, and 750 mg/kg bw/day. The NOAEL for parental systemic toxicity was established at 150 mg/kg bw/day, based on deaths and signs of systemic toxicity observed in both males and females in the high dose group. The NOAEL for reproductive and developmental toxicity was established as 750 mg/kg bw/day in this study, based on the absence of toxicologically relevant adverse effects at this dose. It is noted that there was a dose related reduction in the fertility and conception indices at the mid and high doses that was not statistically significant. This variation was stated to be within the historical control values and thus considered by the study authors to be a normal biological variation. Although also not statistically significant, the number of live pups at the first litter check was slightly lower and post-natal loss for days 1-4 was slightly higher at the high dose.

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

Hazard Classification	*Hazard Statement*
Serious Eye Damage/Eye Irritation (Category 1)	H318 – Causes serious eye damage

6.3. Human Health Risk Characterisation
Based on the studies provided, the assessed chemical is severely irritating to eyes. A skin sensitisation potential of the assessed chemical cannot be ruled out.

6.3.1. Occupational Health and Safety
Workers at reformulation sites may be exposed to the assessed chemical at 60 – 70% concentration during sampling, mixing and packaging. Dermal and possible ocular exposure to the assessed chemical is expected to be minimised by the use of safe work practices and workers wearing personal protective equipment (PPE) including impervious gloves, coveralls and goggles. Inhalation exposure is expected to be minimised by use of a closed reformulation system, local exhaust ventilation and respirator equipment if ventilation is inadequate.

During use as a metal-working fluid, drilling fluid, soil wetting agent fluid or other industrial product, exposure to the assessed chemical in end-use products (at ≤ 15% concentration) may occur during the blending and mixing processes, during use or service and maintenance of equipment. Exposure to the assessed chemical at lower concentrations may occur when applying the products to substrates with roller, brush or dipping. Inhalation exposure may be possible if formation of aerosols and mists occur during application. Dermal and ocular exposure to workers would be mitigated through the use of personal protective equipment (PPE) including protective coveralls, impervious gloves and goggles. Inhalation exposure will be minimised by the use of local exhaust ventilation in areas around machinery.

Workers involved in professions where the services provided involve the use of cleaning products, may come into contact to the assessed chemical at ≤ 15% concentration. Products containing the chemical at ≥ 3% are classified as severe eye irritants according to the GHS criteria. Personal protective equipment, such as safety glasses/goggles/face shields, aprons/coveralls and protective gloves will minimise exposure through spills and splashes. Respiratory protection is not normally used during cleaning, but respirators may be used if ventilation is inadequate. Safe work practices such as collection and containment of small spills, and availability of personal washing facilities is expected to minimise exposure to the assessed chemical. Overall, the exposure and risk to

workers who regularly use these products is expected to be of a similar or higher extent than that experienced by consumers using products containing the assessed chemical (for details of the public health risk assessment, see Section 6.3.2).

Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described), the assessed chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health

Various types of household cleaning products containing the assessed chemical at ≤ 15% concentration will be available to the public. The main route of exposure is expected to be dermal with some potential for inhalation if used as a spray. The use of spray products is likely to increase accidental ocular and oral exposure.

The assessed chemical is a severe eye irritant and is classified as causing severe eye irritation at concentrations ≥ 3% according to the GHS criteria. In the absence of eye irritation data for products containing the assessed chemical at ≤ 15% concentration (i.e. at concentrations ≥ 3% GHS classification cut-off), eye irritation effects from accidental ocular exposure to products is considered possible. The risk to the public would be mitigated by safe use instructions and warnings on products.

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MOE) of the assessed chemical using the worst case exposure scenario from use of multiple products containing the assessed chemical as 0.8792 mg/kg bw/day (see Section 6.1.2). Using the NOAEL of 150 mg/kg bw/day, as determined in a 90-day repeated dose toxicity study, a MOE of 171 was estimated. A MOE value ≥ 100 is considered acceptable to account for intra- and inter-species differences, and to account for long-term exposure; therefore, the MOE is considered to be acceptable.

When used at a maximum concentration of 15% in household cleaning products, with warnings on product labels for potential eye effects and safety directions for use, the assessed chemical is not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The assessed chemical is not manufactured in Australia, but is introduced in finished products or reformulated into domestic, institutional and industrial products including metal working fluids, drilling water based muds, agricultural wetting agents and other cleaning based products. During any formulation and mixing, release of the assessed chemical to the environment is expected to be negligible as these processes occur in closed systems in industrial settings. Any accidental spills are to be collected and disposed of in accordance with local government regulations. Wash waters from equipment cleaning, containing the assessed chemical are expected to be collected and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The assessed chemical will be used for a wide variety of uses. Uses such as metal working fluids will result in minimal environmental exposure, from disposal of spent fluids to licensed waste management facilities. Uses as automotive cleaners will result in a wide dispersive environmental exposure. Agricultural uses will result in direct release to soil. For agricultural uses the application rate will be up to 2000 g/ha (4% w/v × 50 L/ha). Use in water based drilling muds will result in direct release to the ocean. However, the majority of the assessed chemical is expected to be washed into sewer waters as a part of its various uses including cleaning products where it will be treated in sewage treatment plants nationwide before being released into surface waters.

RELEASE OF CHEMICAL FROM DISPOSAL

A small proportion of the assessed chemical is expected to remain as residues in empty product containers. These containers are expected to be either recycled or disposed of to domestic landfill.

7.1.2. Environmental Fate

Following its use particularly in cleaning products, the assessed chemical is expected to be primarily released into the sewer system and treated at sewage treatment plants before release to surface waters nationwide.

The assessed chemical is readily biodegradable (90% biodegradation after 28 days). For details, refer to Appendix C. The assessed chemical is not expected to bioaccumulate due to its low log Pow (log Pow = 1.1). Some of the assessed chemical may remain in the end use and bulk containers, which are either recycled or disposed of to landfill. In surface waters and landfill, the assessed chemical is expected to degrade into water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The use pattern will result in most of the assessed chemical being washed into the sewer. The predicted environmental concentration (PEC) has been calculated based on the realistic scenario with 100% release of the assessed chemical into sewer systems nationwide over 365 days per annum. The extent to which the assessed chemical is removed from the effluent in STP processes is based on the physico-chemical properties and its ready biodegradability, modelled by SimpleTreat 3.0 (Struijs, 1996) and is estimated as 67%. The PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	100,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	100,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	273.97	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	24,386	million
Removal within STP	67	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River:	18.54	µg/L
PEC – Ocean:	1.85	µg/L

For the terrestrial environment the assessed chemical will be applied at a rate of up to 2000 g/ha (≡ 0.2 g/m^2). As the chemical is mobile it is expected to disperse in the top 10 cm of soil. The concentration in soil is calculated based on the application rate per volume of soil in the top 10 cm for each hectare, which is 1000 m^3, (100 m × 100 m × 0.1 m), resulting in a concentration of 2 g/m^3 [2000 g/ha ÷ (100 m × 100 m × 0.1 m)]. On a mass basis, the concentration is calculated based on the default density of soil of 1500 kg/m^3. This will result in a concentration 1.33 mg/kg = [(2000 g ÷ 1000 m^3) ÷ 1500 kg/m^3] × 1000 mg/g.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 310 mg/L 28 d NOEC = 20 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L 21 d NOEC = 18.2 mg/L	Not harmful to daphnia
Algal Toxicity	72 h EC50 > 100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	EC50 > 200 mg/L	Not inhibition to microorganisms
Terrestrial plants	EC50 > 100 mg/kg	Not harmful to terrestrial plants
Earthworms 14 d	LC50 = 748 mg/kg	Slightly toxic to earthworms

The two chronic studies were conducted under semi-static or flow-through conditions. These also demonstrated low toxicity to aquatic species. However, the endpoints have not been directly used in a quantitative risk assessment as the endpoints are based on semi-static or flow-through conditions, while the assessed chemical is expected to rapidly degrade. This would lead to an overestimate of the toxicity of the assessed chemical.

Based on the above ecotoxicological endpoints, the assessed chemicals are not expected to be acutely harmful to aquatic life. The assessed chemical is readily biodegradable, therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the assessed chemical is not formally classified for toxicity to aquatic life.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) was not calculated as the assessed chemical is not considered toxic to aquatic species (acute (L)EC >100 mg/L). Although the assessed chemical has some chronic effects, these are low and are an overestimate of the toxicity.

The Predicted No-Effect Concentration (PNEC) was calculated based on the most sensitive terrestrial species (LC50 earthworm) and an assessment factor of 1000 as there is data for only one endpoint.

Predicted No-Effect Concentration (PNEC) for the Terrestrial Compartment			
Earthworms LC50	748		mg/kg
Assessment Factor	1000		
Mitigation Factor		1.00	
PNEC	748		µg/kg

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) was not calculated for the aquatic environment as the assessed chemical is not toxic to aquatic organisms.

The Risk Quotient (Q = PEC/PNEC) for the terrestrial environment was calculated as follows.

Risk Assessment	PEC (µg/kg)	PNEC (µg/kg)	Q
Q – soil	1330	794	1.67
Q – soil (TWA 14-d)	790	794	0.99

TWA = Time weighted average.

The assessed chemical may reach concentrations of 1330 µg/kg, with a resultant risk quotient of 1.67, just above the level considered not to pose an unreasonable risk to the terrestrial environment. However, the assessed chemical rapidly degrades and the actual exposure to soil organisms is better represented by a time-weighted-average concentration. This may be calculated by $PEC_{TWA} = \frac{1-e^{-kt}}{kt} \times PEC$ (EFSA 2009).

Wherein k is the rate constant in days, and t is the time of the exposure of the study (14 d).

The rate constant is calculated from the usual formula $DT50 = \frac{ln2}{k}$, or in generalised form $\left(100 - \frac{100}{x}\right) = \frac{\ln x}{k}$
For the assessed chemical 90% degraded after 28 days in a ready biodegradability test. Assuming similar degradation rates in soil, k is calculated from $DT90 = \frac{ln10}{k}$ or rearranged and substituted $k = \frac{ln10}{28\ days}$.
The resulting rate constant is 0.0822 day^{-1}.

Therefore the PEC_{TWA} for 14-d, is 790 µg/kg with a corresponding Q value of 0.99. This indicates a risk just below what is considered to not be unreasonable. However, this needs to be taken in context as the preferred concentration for use as a wetting agent is 0.2-2% which when applied at rates of between 20 and 50 L/ha, results in PEC_{TWA} of between 15.8 and 395 µg/kg, with corresponding Q values of between 0.02 and 0.50. Therefore on the basis of the low aquatic hazard and the terrestrial PEC/PNEC ratio, the assessed chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point -5 °C

Method EC Directive 84/449/EEC A.1 Melting/Freezing Temperature
Remarks A standard crystallising point apparatus was used. The sample did not possess a conventional freezing point, but showed signs of solidification at -5 °C.
Test Facility Huntingdon (1993a)

Boiling Point > 300 °C at 101.3 kPa

Method Method AB46-1215, "Determination of melting point, boiling point and enthalpy using Differential Scanning Calorimetry"
Remarks Differential Scanning Calorimetry was used. Conducted on Analogue 3. No definite boiling point was noted up to 300 °C.
Test Facility Akzo Nobel (2009)

Density 1,182.9 kg/m^3 at 20 °C

Method EC Directive 84/449/EEC A.3 Relative Density
Remarks The pycnometer method was used.
Test Facility Huntingdon (1993a)

Vapour Pressure $\leq 5 \times 10^{-8}$ kPa at 25 °C

Method EC Directive 84/449/EEC A.4 Vapour Pressure
Remarks A vapour pressure balance was used. Condensation was noted during the study, which was thought to affect the accuracy of the results. Therefore a maximum vapour pressure was estimated.
Test Facility Huntingdon (1993a)

Water Solubility ≥ 790 g/L at 20 °C

Method OECD TG 105 Water Solubility
EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks Flask Method/Column Elution Method
Test Facility Huntingdon (1993a)

Partition Coefficient (n-octanol/water) log Pow = 1.1.at 20 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method/Flask Method
Test Facility Huntingdon (1993a)

Surface Tension 30.2 mN/m at 23 °C

Method EC Directive 84/449/EEC A.5 Surface Tension
Remarks Concentration: 1% (w/v). The OECD harmonised ring method was used.
Test Facility Huntingdon (1993a)

Adsorption/Desorption – screening test Mean K_{oc} = 5 mL/g

Method OECD TG 106 Adsorption – Desorption Using a Batch Equilibrium Method

Soil Type	*Organic Carbon Content (%)*	*pH*	*Koc (mL/g)*
Sandy loam	1.64	5.38	2
Loam	1,28	6.78	7

Clay loam	4.19	6.97	7
Silt loam	1.86	5.32	2
Clay	2.95	6.49	5

Remarks Due to the low adsorption of the test item to soil and its extremely rapid degradation on contact with soil, neither a desorption nor an advanced test were performed.

Test Facility RCC (2006)

Flash Point > 110 °C at 101.7 kPa

Method EC Directive 84/449/EEC A.9 Flash Point
Remarks Pensky-Martens closed cup method was used. The test substance started boiling at 105 °C, with the omission of white fumes.
Test Facility Huntingdon (1993a)

Solid Flammability Not flammable

Method EC Directive 84/449/EEC A.10 Flammability (Solids)
Remarks The test substance melted to a black liquid with evolution of smokebut did not ignite.
Test Facility Huntingdon (1993a)

Autoignition Temperature Not expected to autoignite

Method EC Directive 67/548/EEC A.16 Relative Self-Ignition Temperature for Solids
Remarks The test substance was heated to 450 °C with no indication of ignition. None of the test substance remained at the end of the test.
Test Facility Huntingdon (1993a)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The chemical does not have functional groups associated with explosive properties. The oxygen balance was calculated to be -197% to -169%.
Test Facility NOTOX (2010)

Oxidizing Properties Not oxidising

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids)
EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks The test substance does not contain groups that act as an oxidizing agent. All oxygen atoms present are chemically bonded to carbon or hydrogen.
Test Facility NOTOX (2010)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Assessed chemical
METHOD	EC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84). Part B, Method B.1. Acute Toxicity (oral)
Species/Strain	Rat/Crl CD (SD) BR VAF plus
Vehicle	Distilled water
Remarks – Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 per sex	5,000	4/10
2	5 per sex	2,000	0/10

LD50	> 5,000 mg/kg bw
Signs of Toxicity	A total of four rats (two male and two female) died when dosed at 5,000 mg/kg bw. Pilo-erection, hunched posture, waddling, lethargy, decreased respiratory rate and pallor of the extremities were observed in all animals dosed at either 2,000 or 5,000 mg/kg bw. Ptosis, ataxia and prostration were also observed but only in rats dosed at 5,000 mg/kg bw. Recovery of surviving rats was observed by Day 3 for groups treated at 2,000 mg/kg bw, Day 4 for male rats treated at 5,000 mg/kg bw and Day 5 for female rats treated at 5,000 mg/kg bw.
Effects in Organs	Congestion of the blood vessels of the small and large intestines was noted in animals that died during the study. No abnormalities were noted at the macroscopic examination on Day 15 for animals that survived until the end of the study.
Remarks – Results	Body weight loss (11.3%) was observed in one female treated at 2,000 mg/kg bw and slightly low bodyweight gains were observed on Day 8 on two males treated at 2,000 mg/kg bw and one at 5,000 mg/kg bw. These rats reached the expected gains on Day15 and the rest of rats throughout the study.
CONCLUSION	The assessed chemical is of low acute toxicity via the oral route.
TEST FACILITY	Confidential (1992a)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Assessed chemical at 84% concentration
METHOD	EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. 19.09.84), Part B, Method B.3. Acute Toxicity (dermal)
Species/Strain	Rat/Hsd/Ola SD(CD)
Vehicle	Water
Type of dressing	Occlusive.
Remarks – Method	Similar to EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal).

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5M	2,380 mg/kg bw	0/5

2	5F	2,380 mg/kg bw	0/5

LD50	> 2,380 mg/kg bw
Signs of Toxicity – Local	No irritation, erythema, oedema or other dermal changes were observed on any animals.
Signs of Toxicity – Systemic	Slightly low bodyweight gains were noted.
Effects in Organs	No abnormalities were noted at the macroscopic examination.
Remarks – Results	No mortality occurred in both groups treated at 2,380 mg/kg bw.

CONCLUSION The assessed chemical is of low acute toxicity via the dermal route.

TEST FACILITY Confidential (1993a)

B.3. Skin Irritation – Rabbit

TEST SUBSTANCE Assessed chemical

METHOD EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.4 Acute Toxicity (Skin Irritation)

Species/Strain	Rabbit/New Zealand White
Number of Animals	Three
Vehicle	None
Observation Period	4 days
Type of Dressing	Semi-occlusive
Remarks – Method	Similar to EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation)

RESULTS

Remarks – Results No signs of toxicity in any rabbit during the observation period were noted. No dermal reaction to treatment was observed in any rabbit during the observation period.

CONCLUSION The assessed chemical is non-irritating to the skin.

TEST FACILITY Confidential (1992b)

B.4. Eye Irritation – Rabbit

TEST SUBSTANCE Assessed chemical

METHOD EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.5. Acute toxicity (eye irritation)

Species/Strain	Rabbit/New Zealand White
Number of Animals	One male
Observation Period	21 days
Remarks – Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva – Redness	2	2	21 days	1
Conjunctiva – Chemosis	2	2	7 days	1
Conjunctiva – Discharge	N/A	N/A	N/A	N/A
Corneal Opacity	2	3	21 days	3
Iridial Inflammation	1	1	3 days	1

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

Remarks – Results No signs of systemic toxicity were noted.

Cornea dulling was observed one hour after instillation followed by development of corneal opacity. This persisted after 21 days with neo-vascularisation also present. Iridial inflammation persisted until Day 3. Conjunctival irritation persisted for 21 days.

CONCLUSION The assessed chemical is severely irritating to the eye.

TEST FACILITY Confidential (1992c)

B.5. Eye Irritation – Rabbit

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion (1987)
EC Commission Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation)
Species/Strain Rabbit/New Zealand White
Number of Animals 1
Observation Period 48 hours
Remarks – Method No significant protocol deviations.
The mean score was calculated on the basis of the scores at 24 and 48 hours, due to the early termination of the study after the 48 h observation.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva – Redness	2.5	3	> 48 hours	3
Conjunctiva – Chemosis	2.5	3	> 48 hours	3
Conjunctiva – Discharge	3	3	> 48 hours	3
Corneal Opacity	1.5	2	> 48 hours	2
Iridial Inflammation	1	1	> 48 hours	1

* Calculated on the basis of the scores at 24 and 48 hours

Remarks – Results Cornea dulling was observed one hour after instillation followed by development of corneal opacity. Iridial inflammation and conjunctival effects were seen at all observations until the study was terminated.

The animal was euthanized after 48 hours due to signs of intense pain and distress, and no further animals were tested. Based on calculation of the results according to the modified Kay and Calandra method, the test substance was considered to be at least a severe eye irritant.

CONCLUSION The assessed chemical is severely irritating to the eye.

TEST FACILITY Confidential (1996)

B.6. Skin Sensitisation – Guinea Pig Buehler Test

TEST SUBSTANCE Assessed chemical at 50% concentration

METHOD Similar to OECD TG 406 Skin Sensitisation – Guinea Pig Buehler Test
Species/Strain Guinea pig/Dunkin-Hartley Albino
PRELIMINARY STUDY Maximum non-irritating concentration: 100%
Intradermal: None
Topical: 100%, 50%, 25%, 10%
MAIN STUDY
Number of Animals Test Group: 20 F Control Group: 19 F
Vehicle Distilled water
Positive Control Not conducted in parallel with the test substance

INDUCTION PHASE	Induction concentration: Topical: 100%
Signs of Irritation	Slight irritation was observed in 11/20 of the treated animals.
CHALLENGE PHASE	
1st Challenge	Topical: 50%
2nd Challenge	Topical: None
Remarks – Method	No GLP Compliance Statement. No positive control used. One control animal had died prior to commencement of the study.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	50%	0/20	0/20
Negative Control Group	50%	0/19	0/19

Remarks – Results	No skin reactions were observed in any of the animals in either the test group or the control group. All treated animals showed expected body weight gain comparable to the controls.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the assessed chemical under the conditions of the test.
TEST FACILITY	Confidential (1992d)

B.7. Skin Sensitisation – Guinea Pig - Maximisation Test (GMPT)

TEST SUBSTANCE	Assessed chemical at 50% concentration
METHOD	EC Directive 84/449/EEC B.6 Skin Sensitisation – Maximisation test
Species/Strain	Guinea pig/Dunkin Hartley
PRELIMINARY STUDY	Maximum non-irritating concentration: 10% intradermal Intradermal: 0.1% to 10% v/v in water Topical: 2.5% to 70% in water
MAIN STUDY	
Number of Animals	Test Group: 30 Control Group: 10
Vehicle	Distilled water
Positive Control	Formalin (not conducted in parallel with the test substance).
INDUCTION PHASE	Induction concentration: Intradermal: 0.5% v/v in water Topical: 50% v/v in distilled water
Signs of Irritation	Necrosis was observed at intradermal injection sites that received the test substance along with Freund's Complete Adjuvant (50%) in water. Slight irritation was seen at intradermal injection sites where the test substance was diluted with only water. Slight to moderate erythema was seen at the topical induction sites.
CHALLENGE PHASE	
Challenge	Intradermal: None Topical: 10% v/v in distilled water (Anterior site of the animal) Topical: 5% v/v in distilled water (Posterior site of the animal)
Remarks – Method	No significant protocol deviations, except for concentration error mentioned below. A table of positive control data using formalin was included in the study report.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: Challenge*		
		24 h	*48 h*	*72 h*
Test Group	10%	9/30	5/30	2/30
	5%	0/30	0/30	0/30
Control Group	10%	0/9	0/9	0/9
	5%	0/9	0/9	0/9

Remarks – Results

No signs of toxicity were observed in the treated animals.

One control animal died following topical application, with the cause of death not determined. A post mortem showed no macroscopic abnormalities.

Slight to well defined erythema was seen in 9/30 test animals at the anterior site (10% challenge) at the 24 hour observation, with the irritant effects reducing over time. No erythema was seen at the posterior sites (5% challenge). No erythema was seen in control animals.

The study authors noted that 5 of the animals had dermal reactions that were more marked than those of the controls (erythema persisting to 48 h or 72 h), whilst in the other 4 animals there was a lower level of dermal reactions (slight erythema at 24 h only) and that these responses were inconclusive. Using these parameters to judge when a positive response had been obtained, the study authors concluded that the test substance produced evidence of skin sensitisation in only 5/30 animals.

A second challenge was not performed.

CONCLUSION

There was limited evidence of reactions indicative of skin sensitisation to the assessed chemical in less than 30% of the treated animals under the conditions of the test. The GHS criteria for a chemical to be considered as a skin sensitiser in GPMT- Freunds Complete Adjuvant – test, a response rate of at least 30% of the animals should be positive. Therefore the chemical cannot be classified as a skin sensitiser.

TEST FACILITY

Confidential (1993b)

B.8. Skin Sensitisation – Guinea Pig - Maximisation Test (GMPT)

TEST SUBSTANCE

Assessed chemical at 62% concentration

METHOD

OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test
EC Council Regulation No 440/2008 B.6 Skin Sensitisation – Guinea Pig Maximisation Test

Species/Strain

Guinea pig/Dunkin-Hartley Albino

PRELIMINARY STUDY

Maximum non-irritating concentration: 100%
Intradermal: 10%, 50%, 75%
Topical: 10%, 50%, 75%, 100%

MAIN STUDY

Number of Animals

Test Group: 20 M Control Group: 10 M

Vehicle

Water

Positive Control

Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.

INDUCTION PHASE

Induction concentration:
Intradermal: 25%
Topical: 100%

Signs of Irritation	After intradermal induction, the tested animals showed signs of irritation including erythema, oedema, necrotising dermatitis, encrustation and exfoliation of encrustation. This result was likely caused by the dermal application of Freund's Complete Adjuvant (FCA) in saline for the purpose of causing local irritation. Discrete, patchy erythema was seen at the topical induction sites on all animals after 24 hours, and in 15 animals after 48 hours.
CHALLENGE PHASE	
1st Challenge	Topical: 50%
2nd Challenge	Not performed
Remarks – Method	GLP Compliance Statement. In the preliminary study, the concentrations that were intended for topical use were 25%, 50%, 75% and 100%, but 10% concentration was applied instead of 25% due to an error.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	50%	14/20	8/20
Negative Control Group	50%	7/10	3/10

Remarks – Results	At challenge in the test group, discrete, patchy to moderate, confluent erythema was observed in 14 animals after 24 hours and discrete, patchy erythema persisted in 8 animals after 48 hours (70% and 40%, respectively). Scaling was observed in one animal after 48 hours. At challenge, 7/10 control animals after 24 h and 3/10 control animals after 48 h showed similar skin reactions to the test group (70% and 30%, respectively). This is despite no skin reactions being observed in the preliminary test, when 50% was used as the highest non-irritating concentration. A second challenge was not performed. The study authors believed that the local reactions in the control group were due to irritation, and therefore concluded that the results observed in the tested animals were questionable. The highest observed irritation effects in the control group and the test group were at the same grade and incidence was similar.
CONCLUSION	There was inadequate evidence of reactions indicative of skin sensitisation to the assessed chemical under the conditions of the test. Therefore the study authors concluded that the chemical cannot be classified as a skin sensitiser.
TEST FACILITY	Confidential (2012)

B.9. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Assessed chemical at 84% concentration
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B7. Subacute toxicity (oral)
Species/Strain	Rat/Sprague Dawley (Crl:CD BR VAF Plus)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week
Vehicle	Distilled water

Remarks – Method
No significant protocol deviations
Doses were selected based on a preliminary seven day study at doses of 250, 500 and 750 mg/kg bw/day.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5M, 5F	0	0/10
Low Dose	5M, 5F	15	0/10
Mid Dose	5M, 5F	150	0/10
High Dose	5M, 5F	750	0/10

Mortality and Time to Death
All animals survived the scheduled treatment and were killed and examined macroscopically on Day 29.

Clinical Observations
Increased salivation was noted in all rats treated at 750 mg/kg bw/day of the test substance. Three female rats treated at 750 mg/kg bw/day had a thin looking appearance in week 3 of treatment. There were no clinical signs noted for all rats treated at 150 or 15 mg/kg bw/day.

There were no statistically significant changes in food consumption or body weight between treated and control rats. However, overall bodyweight gain for females treated at 750 mg/kg bw/day was statistically significantly lower (20%) than the control group. Bodyweight gains for all treated male rats were comparable to those of the control groups throughout the study period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Mean corpuscular volume showed a slight but statistically significant decrease in all three treated groups of male animals. There were no other statistically significant changes noted in haematological parameters measured.

Total protein was decreased in male and female animals at 150 and 750 mg/kg bw/day and also in male animals at 15 mg/kg bw/day. Globulin levels were also decreased in both male and female animals in the 150 and 750 mg/kg bw/day dose groups and also in females dosed at 15 mg/kg bw/day. The albumin/globulin ratio showed a statistically significant increase for females in all three treatment groups in comparison to the controls. Chloride and sodium levels showed a slight but statistically significant increase in male animals dosed at 150 and 750 mg/kg bw/day, and chloride in male animals dosed at 15 mg/kg bw/day.

Effects in Organs
Male rats treated at 750 mg/kg bw/day showed higher relative liver weights than control groups, however, this finding was not associated with histopathological or biochemical changes.

All treated male rats showed a statistically significantly lower adrenal weight than control groups. However, individual values for treated rats were within the expected range for rats of this age and strain and most of the individual values for control groups were high. Therefore, this finding was not treatment related. No other statistically significant differences in organ weight between treated and control animal groups were noted.

Macroscopic and microscopic effects in the organs noted in the treated animals were at a similar level and frequency to those seen in the control groups

Remarks – Results
Test substance-related adverse effects observed included lower food consumption and lower mean body weight gain for female rats treated at the high dose. However, the final bodyweights of the animals were comparable to control animals. In addition, the high liver weights in the high dose males may possibly be adaptive in nature and not considered to be of toxicological importance in the absence of histopathological or biochemical changes.

CONCLUSION
The No Observed Adverse Effect Level NOAEL was established at the highest dose of 750 mg/kg bw/day in this study, based on no toxicologically relevant adverse effects at this dose level.

TEST FACILITY	Confidential (1994)

B.10. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Assessed chemical at 63.5% concentration
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (1998) EC Directive 67/548/EEC, B Repeated Dose (90 days) Toxicity (oral) (2001)
Species/Strain	Rat/ Wistar Crl:(WI) BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: 28 days
Vehicle	Water (Milli-U)
Remarks – Method	No significant protocol deviations. Dosage was adjusted to account for the purity of the test substance.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M, 10F	0	0/20
Low Dose	10M, 10F	50	0/20
Mid Dose	10M, 10F	150	0/20
High Dose	10M, 10F	450	1/20
Control Recovery	10M, 10F	0	0
High Dose Recovery	10M, 10F	450	1/20

Mortality and Time to Death

One male in the 450 mg/kg bw/day recovery group and one female in the 450 mg/kg bw/day main group died on days 29 and 21, respectively. Clinical signs in the deceased animals consisted of laboured respiration, hunched posture and piloerection. Observations from the necropsy consisted of severe necrosis in addition to an exudation of the tracheal mucosa, autolysis and red foci on the lungs, and red discolouration of the mesenteric lymph nodes. The pathology report noted the cause of the deaths as gavage errors.

Clinical Observations

Clinical signs in the animals dosed at 450 mg/kg bw/day included rales (4M, 8F), laboured respiration (1M, 3F), hunched posture (2M, 3F), gasping (1F), and piloerection (1M, 1F) and lethargy (2M). All animals treated at high dose showed salivation during both main and recovery tests. Incidental findings were also observed such as a purple colouration of the toes or ear (noted in two control males and one male treated at 50 mg/kg bw/day), alopecia, scabs, swelling of the ears, a wound on the mouth, focal erythema of the ear, and brown staining of the fur. These observations were considered by the study authors as signs of no toxicological significance as these findings were often noted in rats of this age and strain. One female animal dosed at 150 mg/kg bw/day was reported to have rales.

There were no treatment related changes in motor activity or functional observation parameters when compared to the controls.

There were no differences in food or water consumption, or changes in bodyweight that were related to treatment.

No ophthalmoscopic findings in treated animals were observed when compared to controls during the study period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

There were statistically significant increases (80%) in the level of neutrophils in male animals dosed at 150 and 450 mg/kg bw/day. This was not seen in the recovery group or in female animals. Mean corpuscular

haemoglobin showed a statistically significant decreases of 3.1% and 2.8% in female animals dosed at 150 and 450 mg/kg bw/day respectively. This was not seen in male animals or in the recovery group. All other statistically significant changes in haematology parameters showed no dose response relationship or were only present in the recovery group.

Clinical chemistry and Urinalysis
Total protein values were statistically significantly higher (4.2%) in male animals treated at 450 mg/kg bw/day compared to controls. The increase in total protein was not observed in females or males in the recovery group. Female animals dosed at 50, 150 and 450 mg/kg bw/day showed decreased alanine aminotransferase and aspartate aminotransferase with increased phosphorus levels. Female animals dosed at 150 and 450 mg/kg bw/day had decreased glucose levels and increased potassium levels. None of the statistically significant changes seen at the highest doses in the female test groups were present in the female recovery group. All other statistically significant changes in clinical chemistry and urinalysis parameters showed no dose response relationship or were only present in the recovery group.

Effects in Organs
Except for the two dead animals, the incidence and severity of gross and microscopic lesions observed were similar in both treated animals and control animals.

The absolute liver weights of females at 150 and 450 mg/kg bw/day showed a statistically significant decrease. No statistically significant decrease in absolute or relative liver weights was observed in the recovery group females or in male animals. There were no histopathological changes. All other statistically significant changes in organ weights showed no dose response relationship or were only present in the recovery group.

Remarks – Results
No adverse treatment related changes were noted in animals dosed at 50 or 150 mg/kg bw/day.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 150 mg/kg bw/day, based on the effects observed at 450 mg/kg bw/day.

TEST FACILITY	Confidential (2003a)

B.11. Genotoxicity – Bacteria

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test EEC Directive 79/831/EEC, Annex V (Directive 84/449/EEC), Method B.14: *Salmonella typhimurium* – Reverse Mutation Assay Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1538, TA1535, TA1537, TA98, TA100
Metabolic Activation System	Liver preparation from Aroclor 1254-induced rats
Concentration Range in Main Test	a) With metabolic activation: 0-5,000 µg/plate b) Without metabolic activation: 0-5,000 µg/plate
Vehicle	DMSO
Remarks – Method	No significant protocol deviations. Positive controls used: *In the absence of S9-Mix:* *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine for strains TA 1535 and TA 100 9-aminoacridine for strain TA 1537 2-nitrofluorene for strains TA 1538 and TA 98 *In the presence of S9-Mix:* 2-aminoanthracene for all tested strains

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	-	Negative
Test 2	-	> 5,000	-	Negative
Present				
Test 1	> 5,000	> 5,000	-	Negative
Test 2	-	> 5,000	-	Negative

Remarks – Results: There was no evidence of mutagenic activity that was seen at any concentration level of the test substance in either mutation test.

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

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

TEST FACILITY: Confidential (1992e)

B.12. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE: Assessed chemical at 84% concentration

METHOD: OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
EEC Methods for Determination of Toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.10. In vitro Mammalian Cytogenetic Test

Species/Strain: Human
Cell Type/Cell Line: Lymphocytes
Metabolic Activation System: S9 fraction from Aroclor 1254-induced rat liver
Vehicle: Water
Remarks – Method: No significant protocol deviations.
Positive controls used were: ethylmethanesulphonate in the absence of metabolic activation, and cyclophosphamide in the presence of metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 9.8, 19.5, 39.1, 78.1, 156.3*, 312.5, 625*, 1,250*, 2,500, 5,000	3 h	18 h
Test 2	0*, 8.2, 16.4, 32.8, 65.6, 131.3*, 262.5, 525*, 1,050*, 2,100, 4,200	3 h	32 h
Present			
Test 1	0*, 9.8, 19.5, 39.1, 78.1, 156.3, 312.5, 625*, 1,250, 2,500* and 5,000*	3 h	18 h
Test 2	0*, 9.8, 19.5, 39.1, 78.1, 156.3, 312.5*, 625, 1,250*, 2,500*, 5,000	3 h	32 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	≥ 2,500	≥ 1,250	Negative

Test 2	-	≥ 2,100	≥ 2,100	Negative
Present				
Test 1	-	> 5,000	> 5000	Negative
Test 2	-	> 5,000	> 5000	Negative

Remarks – Results

In the presence of metabolic activation and after 18 h harvest, cells dosed at 5,000 µg/mL showed a statistically significant increase (6%) in the mean percentage of chromosomal aberrations including gaps. This is within the historical control range (0 – 6.5%) and subsequently was not considered to be indicative of clastogenic activity.

In the absence of metabolic activation and after a 32 h harvest, cells dosed at 525 and 1,050 µg/mL showed a statistically significant increase in the mean percentage of chromosomal aberrations (4.0% (both including and excluding gaps) at 525 µg/mL or 2.5% (including gaps only) at 1,050 µg/mL). These values are within the historical control ranges of 0 – 5.25% and 0 – 6.5% for excluding and including gaps, respectively, and subsequently was not considered to be indicative of clastogenic activity.

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

CONCLUSION

The assessed chemical was not clastogenic to cultured human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY

Confidential (1993c)

B.13. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE

Assessed chemical at 63.5% concentration

METHOD

OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test
EC Directive 2000/32/EC B.17 Mutagenicity – *In vitro* Mammalian Cell Gene Mutation Test

Species/Strain: Mouse
Cell Type/Cell Line: Lymphoma/L5178Y
Metabolic Activation System: S9 fraction from Aroclor 1254-induced rat liver
Vehicle: DMSO (dimethyl sulfoxide)
Remarks – Method: No significant protocol deviations.
Positive controls used were: Ethylmethanesulphonate (EMS) in the absence of metabolic activation, and Dimethylnitrosamine (DMN) in the presence of metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0*, 5, 10, 25*, 50*, 100*, 175*, 225*, 300*, 375*, 500*, 750, 1000	3h	3 days	9-11 days
Test 2	0, 10, 25, 100, 175, 225, 300*, 375*, 500*, 750*, 875*, 1,000*, 1,125*, 1,250*,	24h	2 days	9-11 days
Present				
Test 1	0*, 50*, 100*, 250*, 500*, 750*, 1,000*, 1,250*, 1,500*, 1,750, 2,000, 2,250	3h	3 days	9-11 days
Test 2	0*, 100*, 250*, 500*, 750*, 1,000*, 1,200*, 1,300*, 1,400*, 1,500, 1,600	3h	3 days	9-11 days
Test 3	0*, 1,000*, 1,200*, 1,400*, 1,500*, 1,550*, 1,600*, 1,651*, 1,700	3h	3 days	9-11 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 994 (3h Treatment)	≥ 500	> 1,000	Negative
Test 2	≥ 994 (24h Treatment)	≥ 1,125	> 1,250	Negative
Present				
Test 1	≥ 3,313 (3h Treatment)	≥ 1,250	> 2,250	Negative
Test 2	-	> 1,400	> 1,600	Negative
Test 3	-	> 1,500	> 1,700	Negative

Remarks – Results

The maximum concentration level used was limited by the test substance induced cytotoxicity. Cytotoxicity was observed at all dose levels in the absence and presence of S9-mix in all experiments.

The test substance did not induce significant increases in the mutant frequency in the absence or in the presence of S9 metabolic activation in independent repeated experiments.

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

CONCLUSION

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

TEST FACILITY

Confidential (2001)

B.14. Reproductive Toxicity – Rat One Generation Study

TEST SUBSTANCE

Assessed chemical at 63.5% concentration

METHOD

OECD TG 415 *In vitro* One-Generation Reproductive Toxicity Study (1983)
OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test (2000)
EEC Directive 87/302/EEC Part B: Methods for the determination of Toxicology – One-Generation Reproductive Toxicity Test

Species/Strain: Rat/ Wistar Crl:(WI) BR

Route of Administration: Oral – gavage

Exposure Information: Exposure period – female: 2 weeks pre-mating, mating, pregnancy, and lactation
Exposure period – male: 10 weeks pre-mating, until confirmation of mating

Vehicle: Purified water

Remarks – Method: A number of varied protocol deviations and errors in the procedures were identified and their possible effects evaluated by the study authors, who concluded that they were unlikely to affect the study outcomes.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	24 F, 24 M	0	0/48
Low Dose	24 F, 24 M	15	0/48
Mid Dose	24 F, 24 M	150	0/48
High Dose	24 F, 24 M	750	9/48

Mortality and Time to Death

In the high dose group, there were 9 animals (5 females and 4 males) found dead, cannibalised, or were euthanized during the study period. Most of these deaths occurred from Days 5 – 17 of treatment. Clinical signs exhibited by deceased animals included hunched posture, piloerection, rales, laboured respiration, and gastrointestinal tracts distended with gas. Seven (4 females and 3 males) of the nine premature deaths were considered by the study authors to be treatment-related. The remaining deaths (1 male and 1 female) were considered unrelated to treatment.

No unscheduled deaths occurred in the other groups.

Effects on Parental (P) animals:

Animals in the high dose group showed an increased incidence in rales, hunched posture, and brown staining of several body parts during treatment. Females were more frequently affected by clinical symptoms than males. Laboured respiration was also observed in females from the high dose group.

Body weight, body weight gain and food consumption were affected in the high dose group, with statistically significant decreases in these parameters at the earlier stages of treatment.

No treatment-related macroscopic or microscopic changes were observed in parental animals. Absolute and relative organ weights were unchanged by treatment, except that relative epididymides weights were statistically significantly increased at the high dose. As related macroscopic and microscopic changes were not seen, this was considered not to be a sign of toxicity by the study authors.

No statistically significant changes between groups were seen in the reproductive parameters, except for an increase in the low dose group in implantation sites and number of pups at birth. This finding was not considered to be toxicologically relevant as it was not dose related. Although not statistically significant, there was a dose related reduction in the fertility and conception indices at the mid and high doses (number of pregnant females in relation to number paired and mated). This variation was stated to be within the historical control values and thus considered by the study authors to be a normal biological variation. The gestation index (number of females bearing live pups in relation to number of pregnant females) was unchanged by treatment.

Although small changes were seen in some of the breeding parameters, none were statistically significant. The number of live pups at the first litter check was slightly lower at the high dose. Post-natal loss for days 1-4 was slightly higher at the high dose, leading to a slightly lower viability index. The mean duration of gestation, sex ratio of the pups and post-natal loss from days 5-21 were unaffected by treatment.

Effects on 1st Filial Generation (F1)

Initial pup weights and development up to weaning were similar between control and treated groups. Increased mean pup weight in the mid dose group at Day 7 was considered incidental as it was not dose related. Macroscopic effects seen in some pups included a small appearance, lack of milk, cannibalism, emaciation, bruises and wounds on the body. Some effects observed on organs include a constricted spleen, a black soft mass at the lower back, cyst at left kidney, dilated pelvis of the kidney, and red foci on the lungs. However, these changes were not dose related and were considered to be within normal biological variation.

Remarks – Results

The study authors concluded that no adverse reproductive or developmental effects were identified at any dose level.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for parental toxicity was established as 150 mg/kg bw/day, and the NOAEL for reproductive/developmental toxicity was established as 750 mg/kg bw/day.

TEST FACILITY Confidential (2003b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical Oxygen Demand (BOD)
Remarks – Method	Sodium benzoate was used as a reference substance. A toxicity test was also conducted; however, the percentage of degradation was not calculated.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	54	5	81
15	83	15	84
28	90	28	106

Remarks – Results	All validity criteria were met. The difference in extremes between replicate plateaus was < 10%. The reference substance reached the pass level by day 5. The oxygen depletion in the inoculum blank was 0.15 mgO_2/L after 28 days and the residual oxygen did not fall below 0.5 mgO_2/L.
CONCLUSION	Test substance is readily biodegradable.
TEST FACILITY	Huntingdon (1992)

C.2. Ecotoxicological Investigations

C.2.1. Toxicity to Fish

ACUTE FISH TOXICITY

TEST SUBSTANCE	Solution containing 84% of the assessed chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Semi static
Species	*Orcorhynchus mykiss*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	171 ± 12 mg $CaCO_3/L$
Analytical Monitoring	HPLC
Remarks – Method	The test concentrations were prepared by directly dissolving a measured amount of the test substance in water.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	-	10	0	0	0	0	0
32	31	10	0	0	0	0	0
56	54	10	0	0	0	0	0

10	94	10	0	0	0	0	0
180	170	10	0	0	1	2	2
320	310	10	0	0	0	1	3

LC50	> 310 mg/L at 96 hours
NOEC (or LOEC)	54 mg/L at 96 hours
Remarks – Results	All validity criteria were met. The dissolved oxygen content was maintained at > 60% of the air saturation value and the concentration of the test substance was analysed. LC50 values were calculated based on the measured test concentrations.
CONCLUSION	Test substance is not harmful to fish.
TEST FACILITY	Confidential (1993d)
CHRONIC FISH TOXICITY	
TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 215 Fish, Chronic Toxicity Test – Flow-through
Species	*Orcorhynchus mykiss*
Exposure Period	28 days
Auxiliary Solvent	None
Water Hardness	None
Analytical Monitoring	HPLC
Remarks – Method	Stock solution of 10 g/L (6.4 g ac/L) was prepared in purified water. Sixteen fish were exposed to five treatment levels (5.6, 10, 18 32, 56 mg/L) and control. Specific growth rate based on body weight was measured after 14 and 28 days. A reference test using pentachlorophenol was used to test the sensitivity of the test.
RESULTS	The LC50 after 28 days was > 37 mg/L active ingredient. The EC50 after 28 days for juvenile growth was > 37 mg/L active ingredient. The NOEC from juvenile growth was 20 mg/L assessed chemical. Results are expressed as mean measured concentrations. No significant harmful effect was observed between control and the tested levels.
CONCLUSION	The test substance is not harmful to fish.
TESTING FACILITY	Confidential (2003c)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Solution containing 84% of the assessed chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Analytical Monitoring	HPLC
Remarks – Method	A limit test only was conducted. The test concentration was prepared by directly dissolving a measured amount of the test substance in water.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	-	20	0	0
100	100	40	0	0

LC50	> 100 mg/L at 48 hours
NOEC (or LOEC)	> 100 mg/L at 48 hours
Remarks – Results	All validity criteria were met. Dissolved oxygen was maintained between 7.7 – 8.6 mg/L, pH was maintained between 7.7 and 8.1 and temperature was maintained at 20°C ± 1°C.

CONCLUSION Test substance is not harmful to aquatic invertebrates.

TEST FACILITY Huntington (1993c)

C.2.3. Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Solution containing 63.5% of the assessed chemical

METHOD OECD TG 211 Daphnia sp. Acute Immobilisation Test and Reproduction test – Semi static

Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	None
Water Hardness	Not recorded
Analytical Monitoring	HPLC
Remarks – Method	A stock solution was prepared in M7 growth medium and diluted to get the target concentrations (10, 18, 32, 56 and 100 mg/L). This is corresponding to measured concentrations of 6.4, 11, 20, 36 and 64 mg assessed chemical/L.
Remarks – Results	Reproductive capacity was not affected up to 28.6 mg/L (18.2 mg assessed chemical/L). A significant decrease in reproductive capacity was found at 51.9 and 94.8 mg/L corresponding to 33.6 and 60.2 mg ac/L. At 94.8 mg/L (60.2 mg assessed chemical/L), the length of daphnids was significantly reduced. EC50 for reproduction was determined at 21 days to 73.0 mg/L assessed chemical and NOEC for reproduction at 21 days to 18.2 mg/L assessed chemical. Results are expressed as mean measured concentrations.

CONCLUSION The test substance is not harmful to daphnia.

TEST FACILITY NOTOX (2003)

C.2.4. Algal Growth Inhibition Test

TEST SUBSTANCE Solution containing 84% of the assessed chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test (1981)

Species	*Selenastrum capriconutum*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 98 mg/L
Auxiliary Solvent	None
Analytical Monitoring	HPLC

Remarks – Method	A limit test only was conducted. The test concentration was prepared by directly dissolving a measured amount of the test substance in water.

RESULTS

Growth rate		*Yield*	
ErC50 (mg/L)	*NOEC (mg/L)*	*EyC50 (mg/L)*	*NOEC (mg/L)*
> 100	≥ 100	> 100	≥ 100

Remarks – Results	All validity criteria (OECD TG 201 1981). The control groups exhibited a logarithmic growth phase resulting in cell concentrations ≥ 4.56×10^6, the test concentration was maintained at > 98 mg/L and the test concentration did not show a significant decrease in growth rate compared to the control group.
CONCLUSION	Test substance is not harmful to algal growth.
TEST FACILITY	Huntington (1993d)

C.2.5. Inhibition of Microbial Activity

TEST SUBSTANCE	A solution containing 65 ± 2% of the assessed chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge from a sewage treatment plant processing mainly domestic waste.
Exposure Period	3 hours
Concentration Range	Nominal: 10, 20, 50 100 200 mg/L Actual: Not measured
Remarks – Method	No deviations were recorded. 3,5-dichlorophenol at a concentration of 10 mg/L was used as a reference substance, and an abiotic control was run.
RESULTS	
IC50	> 200 mg/L
NOEC	≥ 200 mg/L
Remarks – Results	All validity criteria were met. The oxygen uptake rate in the blank controls was < 16.6 mg/L. The coefficient of variation of oxygen uptake rate in control replicates was 2%. No abiotic degradation was observed. The inhibition of the rate of respiration from the reference substance was 61% and demonstrates that the inoculum is sufficiently sensitive. The percentage inhibition at all concentrations was ≤ 13% and was not dose responsive. The inhibition of respiration is not considered significant.
CONCLUSION	The assessed chemical is not inhibitory to the respiration of activated sludge at the concentrations tested.
TEST FACILITY	VKI (1994)

C.2.6. Acute Toxicity to Earthworms

TEST SUBSTANCE	Assessed chemical
METHOD	OECD 207
Species	*Eisenia foetida*
Duration	14 days
Concentration range	100 – 1000 mg/kg (dry wt.)

Remarks – Method Based on a range finding test, five concentrations of the assessed chemical were prepared by dissolving it in purified water and adding directly to soil. A control was run and a reference substance (chloracetamide) was run prior to the definitive study as part of a regular quality assurance program.

RESULTS

Nominal Concentration (mg/kg dry weight)	*Total number of test earthworms*	*Exposure duration*	
		7 d *Cumulative mortality (%)*	*14 d* *Cumulative mortality (%)*
Control	40	0	0
100	40	0	0
180	40	0	0
320	40	0	0
560	40	0	0*
1000	40	92.5	100

*Does not include one missing earthworm

Remarks – Results All validity criteria were met. The LC50 for the reference substance was 16.9 mg/kg (dry wt), which is slightly lower than the expected range. However this indicates that earthworms used in this batch were more sensitive and that subsequent studies using the batch will underestimate the toxicity. The LC50 was calculated from the geometric mean of the LC0 and LC100 and estimated to be 748 mg/kg dry wt.

CONCLUSION The assessed chemical is slightly toxic to earthworms.

TEST FACILITY NOTOX (2001)

C.2.7. Toxicity to Terrestrial Plants

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 208, Toxicity to terrestrial plants

Species Three species of terrestrial plants (*Avena sativa, Brassica pekinensis* and *Lactuca sativa*).

Exposure Period 3 weeks

Concentration Range Nominal: 1.0, 10 and 100 mg assessed chemical/L

Auxiliary Solvent None

Remarks – Method Seeds of each plant species were sown in standard soil (Speyer 2.3 mixed with approximately 5% sand). Four treatment levels including and one control, 4 replicates and 10 seeds per concentration per replicate were used in the test. Seedling emergence, growth inhibition and phytotoxicity were recorded over three weeks.

RESULTS

Remarks – Results No adverse effects were observed at the highest treatment level. For all three terrestrial plants, NOEC was100 mg active ingredients/kg dry soil, EC50 > 100 mg active ingredients/kg dry soil for growth inhibition and LC50 > 100 mg active ingredients/kg dry soil for the emergence.

CONCLUSION Test substance is not harmful to terrestrial plants.

TEST FACILITY NOTOX (2002)

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