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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Methyl glycine diacetic acid, trisodium salt

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Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888.
Website:	www.nicnas.gov.au

Director
Chemicals Notification and Assessment

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Methyl glycine diacetic acid, trisodium salt

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
BASF Australia Ltd (ABN 62 008 437 867) of 500 Princes Highway, Noble Park, Melbourne, 3174

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:
Chemical Name
Other Names (selected)
CAS Number
Spectral Data
Purity
Identity of Impurities

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
No

NOTIFICATION IN OTHER COUNTRIES
The notified chemical has been submitted for notification in the USA, Canada, Japan and Europe.

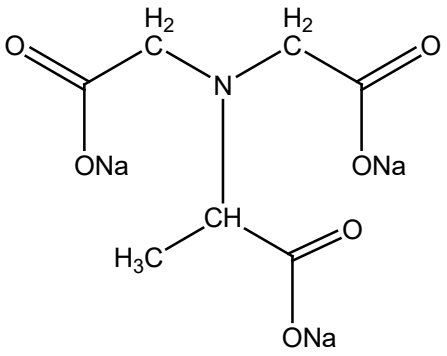
2. IDENTITY OF CHEMICAL

OTHER NAME(S)
Methyl glycine diacetic acid, trisodium salt, Na_3MGDA

MARKETING NAME(S)
Trilon ES 9964 Powder, Trilon M Liquid (approximately 40% aqueous solution of the notified chemical)

MOLECULAR FORMULA
$C_7H_{11}NO_6$. 3Na

STRUCTURAL FORMULA



MOLECULAR WEIGHT
271.11

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD	^{1}H NMR, IR and UV Spectroscopy.
Remarks	Spectra provided consistent with the structural formula.
TEST FACILITY	BASF (1995a)

3. COMPOSITION

DEGREE OF PURITY
>75%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
All hazardous impurities or residual monomers are present at below the relevant cut offs for classification of the notified polymer as introduced (i.e. Trilon M Liquid) as a hazardous substance.

ADDITIVES/ADJUVANTS
For Trilon M Liquid only.

Chemical Name	Water		
CAS No.	7732-18-5	*Weight %*	56-58

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia by sea. Although the notified chemical is available as a powder (Trilon ES Powder) and in aqueous solution (Trilon M Liquid), the notifier intends only to import the notified chemical in the form of an aqueous solution.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	12-20	12-20	12-20	12-20	12-20

USE
Component of an industrial detergent, degreaser or cleaning agent. The technical bulletin for Trilon M liquid indicates that the notified chemical has a wide variety of potential uses.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY
Predominantly Melbourne although Sydney, Perth and Brisbane may be used

IDENTITY OF MANUFACTURER/RECIPIENTS
When imported into Melbourne the notified chemical will be stored at Patricks Intermodal Warehouse, North Laverton, Victoria, prior to distribution to customers. When other ports of entry are used the notified chemical will be stored at nearby Patricks Intermodal Warehouses.

TRANSPORTATION AND PACKAGING
The notified chemical in the form of an aqueous solution will be transported by road in 250kg polyethylene drums (with a non removable head) and 1200kg polyethylene tanks with an outer metal cage.

5.2. Operation Description

Reformulation
The notified chemical in the form of an aqueous solution is transferred to the production or blend facility where it is mixed with other additives. Two main techniques are employed to deliver the correct quantity of notified chemical to the blend vessel. For amounts over 10 litres the process involves placing the drum on a scale, inserting a dip pipe and pumping the correct quantity to the blend

vessel. The transfer hose and dip pipe are then washed to effluent. For smaller quantities the tap that is fitted to the closed head drum is used. The drum is placed on a drum stand and small amounts are decanted into a bucket.

The mixing process is typically done in a closed vessel, ranging in size from 1 to 5 tonnes. Final formulated product will contain between 1 and 10% (usually around 5%) notified chemical. Following quality control of the formulated product, the bottom valve of the blend vessel is connected to a hose for the filling of packages via either gravity feed or via feed to manifold for pumping into packages. Small containers (<20 litres) are typically filled using an automated pump and manifold operation, whereas larger containers are filled manually using either a calibrated measurement or relying on the operator to shut off the fill mechanism when the required weigh level is reached.

Empty drums will be collected by a licensed drum recycler.

End –Use
Depending on their purpose, the final industrial cleaning products will be applied by a number of methods. Typical application methods are described below:

Floor Cleaner: 250mL of formulated cleaning product is added to 10L of water. This is applied to the floor using a mop.

Surface cleaner: The formulated product is diluted with water at a ratio of 1:40 and added to a pump spray bottle. The solution is applied to the bench surface via the pump spray bottle and wiped off using a sponge/cloth. In some cases the diluted formulated product will be poured directly onto a surface or sponge/cloth.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Transport workers	1-2		
Warehouse workers	2-3		
Plant technicians	2	5 hour/week	48 weeks
Laboratory technicians	2	30 min/day	48 weeks
Drum recyclers	1	10 min/week	48 weeks
End Use	500-10000	5 day/week	48 weeks

Exposure Details
Transport and Storage:
Transport and storage workers should not be exposed to the notified polymer except in the case of an accidental spill.

Reformulation

Larger volumes of Trilon M liquid (containing the notified chemical at a concentration of approximately 40%) are transferred by dip pipe. There is the potential for dermal and ocular exposure from drips and splashes when adding or removing the dip pipe. Smaller volumes are transferred to the blend vessel via a bucket. There is the potential for dermal and ocular exposure from drips and splashes when filling and emptying the bucket.

After the blending process the notified chemical is present at a concentration of 1-10 % (usually 5%). Incidental skin contact may occur during connection of the pipe to the bottom valve of the blending vessel. The filling of smaller packages is automated therefore exposure is expected to be negligible except in the event of machine malfunction. Larger packages are filled via gravity with manual intervention required when the correct level has been reached. There is the potential for dermal and ocular exposure during this process.

Workers handling the notified chemical are instructed to follow procedures for personal protective equipment (PPE) as shown on batch sheets. These typically include coveralls, chemical goggles/safety glasses, boots, face shield, apron and impervious gloves. Local exhaust ventilation would be employed in areas where natural ventilation is considered inadequate and inhalation may occur.

Laboratory Staff & maintenance Workers:

Laboratory staff will take samples of the finished formulation containing the notified chemical at a concentration of 1-10% for analysis. There is the potential for dermal exposure.

Empty drums are collected by drum recyclers for cleaning. There is the potential for incidental skin contact during this process.

End Use
Typically the notified chemical is diluted before final application. The concentration of the notified chemical after dilution will be 0.025% to 0.25% (usually 0.125%). Incidental dermal exposure to the notified chemical (concentration of 1-10%) may occur during the addition to water. During end application dermal exposure is the most likely route, although inhalation of aerosols is also possible when using a spray-on pump. Workers will usually wear rubber gloves.

5.4. Release

RELEASE OF CHEMICAL AT SITE
At the customer's reformulation facility, a dip pipe and transfer hose are used to transfer required volumes of the notified chemical from storage drums into the blend vessel. The pipe and hose are then washed out with an estimated maximum of 0.5%, or up to 100 kg/y, of the notified chemical going to sewer. An estimated similar amount will be retained in "empty" containers which will be disposed of to sewers also at about 100 kg/y by drum recyclers. Any incidental spills or wastes from normal operating procedures will be contained and soaked up with absorbent material before being transported off-site to an approved industrial facility for disposal by incineration or landfill by approved operators.

RELEASE OF CHEMICAL FROM USE
The release of the notified chemical to the environment due to its use in floor and surface cleaning is considered to be high. It is expected that nearly all of the product will eventually be used in an application resulting in disposal to sewers, whether this is from wash outs of blend vessels and transfer equipment during blending or dilute solutions of floor cleaning products being put to drain.

5.5. Disposal

The recommended method for disposal of liquid wastes containing the notified chemical is in secure landfill at an approved site in accordance with local regulations.

5.6. Public exposure

The formulated products containing the notified chemical are to be supplied for industrial use only and therefore would not be available to the public. However, the formulated products are likely to be used in public places, e.g. hotels, schools etc, although cleaning is usually carried out during times when the public are not present.

6. PHYSICAL AND CHEMICAL PROPERTIES

The physico-chemical properties have been supplied for either the notified chemical or its 40% aqueous solution (Trilon M Liquid)

Appearance at 20ºC and 101.3 kPa	Notified chemical: white powder Trilon M Liquid: Yellowish liquid with product specific odour
Melting Point	> 390ºC (notified chemical)

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Two experiments were performed. In both, no melting was observed up to 390 ˚C. An exothermic process could be observed between 75 ºC and 320 ºC.
TEST FACILITY BASF (1995b)

Density 1464 kg/m^3 at 20ºC (notified chemical)

METHOD OECD TG 109 Density of Liquids and Solids.
TEST FACILITY BASF (1996a)

Vapour Pressure Not determined

Remarks As the melting point for the notified chemical is >390 ºC, the vapour pressure is not determinable.

Water Solubility >500 g/L at 24ºC (notified chemical)

METHOD In house method containing the procedure for determination of the water solubility according to EC Directive 92/69/EEC A.6 Water Solubility, as well as the determination of the water solubility of substances that are water-miscible.
Remarks On addition of the test substance to water, a turbid solution was produced, however, the test substance continued to dissolve on further addition. The turbidity therefore was considered to stem from by-products. For concentrations >500 g/L, the consistency of the test item/water mixture passes via viscous to pasty to solid.
TEST FACILITY BASF (1995b)

Hydrolysis as a Function of pH Not determined.

Remarks The notified chemical is stable at all pH levels. There are no hydrolysable groups.

Partition Coefficient (n-octanol/water) log Pow < -4 at 25±2ºC (notified chemical)

METHOD EC Directive 92/69/EEC, Shake Flask Method
Remarks The notified chemical was added to 25 mL of water and octanol (mutually saturated with each other) in triplicate and shaken for an unspecified time. After centrifugation, the aqueous phase was diluted by 100 times before analysis by ion exclusion chromatography.
TEST FACILITY BASF (1995b)

Adsorption/Desorption log K_{oc} < 1.5 at 25°C (notified chemical)

METHOD Draft OECD TG on the estimation of the adsorption coefficient (Koc) on soil and on sewage sludge using high-performance liquid chromatography (HPLC). December 1998.
Remarks The log Koc was determined using an HPLC screening method based on a separation done with a cyanopropyl stationary phase under isocratic conditions. As only log Koc values ranging from 1.5-5 can be determined using this method and the log Koc of the notified chemical eluted in the dead time with no peak evaluable, the log Koc was <1.5.
TEST FACILITY BASF (2001a)

Dissociation Constant $pK_1 = 1.6$, $pK_2 = 2.5$, $pK_3 = 10.5$

Remarks The stepwise dissociation constants are for the free acid. This information was taken from the Technical Data Sheet. No study provided.

Particle Size Not determined

Remarks The notified chemical will only be imported as an aqueous solution.

Flash Point Not determined

Flammability Limits Not determined.

Remarks The notified chemical is imported as an aqueous solution. The notified chemical does not react with water.

Autoignition Temperature 338ºC (notified chemical)

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
TEST FACILITY BASF (1996b)

Explosive Properties Not predicted to be explosive

Remarks There are no chemical groups that would imply explosive properties, therefore the result has been predicted to be negative

Reactivity

Remarks Trilon M Liquid is very stable. It does not degrade or decompose under normal conditions of use. It is however, corrosive to aluminium.

ADDITIONAL TESTS

Viscosity	Approximately 30 mPa·s at 23ºC (Trilon M Liquid)
Remarks	From Technical Data Sheet. No study provided.
Surface Tension	71.5 mN/m at 20ºC (notified chemical)
METHOD	EC Directive 92/69/EEC A.5 Surface Tension: OECD ring method using an automatic tensiometer.
Remarks	Concentration: 1g/L A time dependent variation of the surface tension could not be observed.
TEST FACILITY	BASF (1995b)

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral	low toxicity, LD50 >2000 mg/kg bw
Rat, acute dermal	low toxicity, LD50 >2000 mg/kg bw
Rat, acute inhalation	not determined
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation - adjuvant test	no evidence of sensitisation.
Rat, oral repeat dose toxicity – 90 days	NOAEL 170 mg/kg bw/day in males, 207 mg/kg bw/day in females
Rat, oral repeat dose toxicity – 28 days	NOAEL 82 mg/kg bw/day
Genotoxicity - bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosomal aberration test	genotoxic
Genotoxicity – in vitro gene mutation test	non genotoxic
Genotoxicity – in vivo mouse micronucleus test	non genotoxic
Toxicokinetic studies – Absorption and excretion study	Rapid but incomplete absorption (17 – 33%) Rapid urinary excretion, half life 3 –6 hours

7.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: EC Directive 92/69/EEC B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.

- Species/Strain: Rat/Wistar
- Vehicle: Aqua Bidest
- Remarks - Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3/female	2000	0
II	3/male	2000	0

- LD50: >2000 mg/kg bw
- Signs of Toxicity: An impaired general state, dyspnoea, staggering and piloerection were observed in all female animals. Apathy was observed in one female. All animals appeared normal 5 days after application. No signs of toxicity were observed in the male animals.
- Effects in Organs: No abnormalities noted.
- Remarks - Results:

CONCLUSION: The notified chemical is of low toxicity via the oral route.

TEST FACILITY: BASF (1995c)

7.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.

- Species/Strain: Rat/Wistar
- Vehicle: Aqua Bidest
- Type of dressing: Semi-occlusive.
- Remarks - Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5/male	2000	0
II	5/female	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Very slight to well defined erythema and scaling was observed during the study. There were no signs of skin reaction at the end of the observation period.
Signs of Toxicity - Systemic	No signs of toxicity were observed in any animals.
Effects in Organs	No abnormalities noted.
Remarks - Results	

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

TEST FACILITY BASF (1995d)

7.3. Acute toxicity - inhalation

Not submitted

7.4. Irritation – skin

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Test substance moistened with Aqua Bidest.
Observation Period	72 hours animal 1. 15 days animal 2 & 3
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	0	1.3	1.7	2	15 days	1
Oedema	0	0	0	0	N/A	0

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

Remarks - Results Mechanical skin lesions due to the adhesive test substance could be observed in two animals during the observation period. This skin reaction was not reversible in one animal within 15 days after removal of the patch.

CONCLUSION The notified chemical is slightly irritating to skin.

TEST FACILITY BASF (1995e)

7.5. Irritation - eye

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Observation Period 8 days
Remarks - Method No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.3	1.7	1.7	2	72 hours	0
Conjunctiva: chemosis	0.3	0.7	0.3	2	48 hours	0
Conjunctiva: discharge	1.0	0.3	0.3	2	72 hours	0
Corneal opacity	0	0	0	0	N/A	0
Iridial inflammation	0	0	0	0	N/A	0

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

Remarks - Results Minimal to moderate conjunctival irritation was noted in all treated eyes and persisted up to 72 hours. Treated eyes appeared normal 8 days after treatment.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY BASF (1995f)

7.6. Skin sensitisation

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 406 Skin Sensitisation – Magnusson & Kligman
Species/Strain Guinea pig
PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal: no data supplied
topical: 75% notified chemical
MAIN STUDY
Number of Animals Test Group: 10 Control Group: 5 per group – 2 groups.
INDUCTION PHASE Induction Concentration:
intradermal: 5% in 0.9% aqueous NaCl-solution or in Freunds adjuvant/0.9% aqueous NaCl solution
topical: test substance as supplied (i.e. undiluted)
Signs of Irritation Intradermal induction
Well defined erythema was observed at sites receiving 5% test substance in the NaCl aqueous solution. Well defined erythema and slight oedema were observed in sites receiving 5% test substance in Freunds adjuvant/0.9% aqueous NaCl solution and in control and test animals at sites receiving Freunds adjuvant/0.9% aqueous NaCl solution. No signs of skin reaction were noted in control animals at sites receiving 0.9% aqueous NaCl solution.

Topical induction
Incrustation, partially open (caused by the intradermal induction) could be observed in addition to well-defined erythema and slight oedema.

CHALLENGE PHASE

1st challenge	topical: test substance as supplied (i.e. undiluted)
Remarks - Method	No challenge dose was applied to control group 2. No significant deviations from protocol A positive control (reliability check) with a known sensitiser is not included in this study. A separate study is performed twice a year; a summary of the latest study was included. 1-chloro-2,4-dinitrobenzene is used as the positive control

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	100%	0	0	N/A	N/A
Control Group 1	100%	0	0	N/A	N/A

Remarks - Results

CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	BASF (1995g)

7.7.1. 90 day Repeat dose oral toxicity in rats

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents. EC Directive 87/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.
Species/Strain	Rat/Wistar
Route of Administration	Oral –diet.
Exposure Information	Total exposure days: 90 days; Dose regimen: 7 days per week;
Vehicle	
Remarks - Method	Deviations from protocol Sensory reactivity to stimuli not tested

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Mortality*
		Nominal (ppm)	*Mean Actual (mg/kg bw/day)*	
I (control)	10 male/10 female	0	0	0
II (low dose)	10 male/10 female	2400	170(m)/204(f)	0
III (mid dose 1)	10 male/10 female	12000	874(m)/1056(f)	0
IV (mid dose 2)	10 male/10 female	18000	1325(m)/1588(f)	0
V (high dose)	10 male/10 female	24000	1774(m)/2097(f)	0

Mortality and Time to Death

No mortality was observed during the study.

Clinical Observations

Discoloration of urine was seen in one high dose male and one female. Dark discolouration of faeces was seen in all high dose animals.

Body weight change was impaired in high dose males with statistical significance from days 7-63 and on day 84, and in males of group IV with statistical significance on days 7 and 21-49. Water consumption was dose-dependently increased in test groups III, IV and V, with males slightly more affected than females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

There were no treatment-related changes in the serum enzyme activities. Creatinine concentrations in blood were significantly reduced for high dose animals. Magnesium levels were lower for high dose animals and for group IV females. This was not significant for high dose males.

Haematology

Mean corpuscular volume was significantly reduced in high dose males and in the females of all test groups. In addition, decreased mean corpuscular haemoglobin was measured in the high dose male animals.

Urinalysis

Significant increased haemoglobin levels and numbers of erythrocytes were found in the urine specimens of the high dose males. Due to the occurrence of increased amounts of red blood cells in urine specimens, the urine colour was orange/brown and appeared cloudy. Three of the high dose females also had increased amounts and erythrocytes. Dose related increases in zinc levels were observed.

Pathology

Organ Weight

The mean kidney weight was significantly increased in males of dose groups III to V and females of groups IV and V. The mean liver weight was significantly increased in females of groups IV and V. The mean weights of the testes, epididymides, heart and the brain were significantly increased in high dose males. This is related to the decreased mean terminal body weight.

Gross Pathology

No significant findings.

Histopathology

Focal vacuolisation of tubular epithelia in the kidneys was noted in two high dose males, one high dose female and one group III male. The graded severity was minimal in the male animals and moderate in the female animal. In all cases, the lesion was present only unilateral. Unilateral and bilateral focal hyperplasia of the urothelium in the renal pelvis was noted in four high dose, one group III and one group IV male animals and three high dose and one group IV female animals. Zonal fatty infiltration of the liver cells was seen most often in control and group II animals, especially when taking the graded severity into account. In dose groups III to V the average fat content of the liver was lower than in the control group and group II, being lowest in dose group V in males and comparably low in groups IV and V in females.

Remarks – Results

Clinical Observations

The impairment of body weight and increase in water consumption may be indicative of kidney toxicity. The discoloration of urine observed might also be due to the impairment of renal function.

Clinical Chemistry

The decrease in creatinine in the high dose animals is possibly associated with the reduction of bodyweights. Reduced Magnesium concentrations may be related to the chelating ability of the notified chemical.

Haematology

The decrease in mean corpuscular volume and mean corpuscular haemoglobin may not be treatment related as the changes are only marginal and no dose response relationship was noted in the reduction of mean corpuscular volume in the females.

Urinalysis

Increases in haemoglobin and erythrocytes are indicative of renal dysfunction or kidney damage, respectively. The notified chemical is a metal chelating agent which is predominantly excreted in the urine as a complex with zinc.

Pathology
The increase in kidney and liver weight are considered to be due to water influx resulting in the 'cloudy swelling' of the cytoplasm. This alteration is known to reverse within a short time after the withdrawal of treatment and therefore not of toxicological importance. The incidence of focal vacuolisation and hyperplasia observed microscopically in the kidney are considered as treatment related.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 2400ppm (170 mg/kg bw/day in males, 204 mg/kg bw/day in females) under the conditions of this study, based on treated related findings observed in animals dosed at 12000ppm and above including, increased water consumption, increased mean kidney weight, decreased fat content in the liver and presence of focal vacuolisation of tubular epithelia in the kidneys and focal hyperplasia of the urothelium in the renal pelvis. Besides an increase in urinary zinc concentrations, no substance related findings were observed at 2400ppm.

TEST FACILITY BASF (1998a)

7.7.2. 28 day Repeat dose oral toxicity in rats (with a 14 day recovery period)

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain Rat/Wistar
Route of Administration Oral – drinking water.
Exposure Information Total exposure days: 28 days;
Dose regimen: 7 days per week;
Post-exposure observation period: 2 weeks (control and high dose groups)
Vehicle Drinking water
Remarks - Method Deviations from protocol
Sensory reactivity to stimuli not tested.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Mortality*
		Nominal (ppm)	*Mean Actual (mg/kg bw/day)*	
I (control)	5 male/5 female	0	0	0
II (low dose)	5 male/5 female	750	82	0
III (mid dose)	5 male/5 female	3000	347	0
IV (high dose)	5 male/5 female	12000	1409	0
V (control recovery)	5 male/5 female	0	0	0
VI (high dose recovery)	5 male/5 female	12000	1409	0

Mortality and Time to Death
No mortality was observed during the study.

Clinical Observations
Food consumption was statistically significantly decreased in high and mid dose males on Day 28. During the treatment-free period, recovery was observed. In high dose males, body weights and body weight gain were statistically significantly impaired. Recovery was observed during the treatment-free period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Clinical Chemistry
Statistically significant decreased magnesium concentrations were found in the sera of high dose males and high and low dose females. Statistically significant increased urea concentrations were found in the sera of

high dose males. All treatment-related changes normalised in the course of the recovery period.

No treatment related changes to the urinalytical and haematological parameters were observed.

Pathology

Organ weight

The mean kidney weight of high dose group animals was significantly increased. The mean weight of the adrenal glands in the mid dose group males and the spleen in mid dose group females were significantly increased. In the recovery groups, there were no significant changes in the mean organ weights.

No gross lesions were recorded in groups I – IV. One male control recovery rat revealed a mass in the colon.

Histopathology

Focal or multifocal vacuolisation of the tubular epithelia in the renal cortex was observed in one high dose male and all high dose females. No remarkable microscopic findings, especially no tubular vacuolisation were obtained in the kidneys of male and female rats of the recovery group. In the liver of male and female animals of groups II-IV, zonal fatty infiltration and focal lymphoid cell infiltration were observed. There was no clear indication of a treatment related effect in relation to either the incidences or graded severity of the lesions.

Remarks – Results

The food consumption and body weight findings were considered to be substance related.

The slight increase in sera urea is possibly indicative of mild impairment of kidney function. Reduced magnesium concentrations may be related to the chelating ability of the notified chemical After withdrawal of the test substance all changes normalised.

Pathology revealed substance related findings in the kidneys in the high dose animals and the mid dose females, indicating the kidney as the target organ.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 750ppm (82 mg/kg bw/day) in this study, based on the signs of renal toxicity observed in the high and mid dose animals.

TEST FACILITY	BASF (1995h)

7.8. Genotoxicity - bacteria

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure (Test 1) Pre incubation procedure (Test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2 uvrA
Metabolic Activation System	Aroclor 1254 induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 0-7500 µg/plate. b) Without metabolic activation: 0-7500 µg/plate.
Vehicle	Purified Water
Remarks - Method	Deviations from protocol 2-aminoanthracene used as sole positive control is presence of metabolic activation N-methyl-N'-nitro-N-nitroso-guanidine used as positive control for TA100 and TA1535 in the absence of metabolic activation 4-nitro-o-phenylendiamine used as positive control for TA98 in the absence of metabolic activation

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	N/A	>7500	>7500	negative
Test 2	N/A	2500	>7500	negative
Present				
Test 1	N/A	>7500	>7500	negative
Test 2	N/A	7500	>7500	negative

Remarks - Results: No significant increase in the frequency of revertant colonies was recorded for any bacterial strain used with any dose of the test material in two separate experiments either with or without metabolic activation

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

TEST FACILITY: BASF (1995i)

7.9.1. Genotoxicity – in vitro Chromosomal Aberration Assay

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 473 In vitro Mammalian Chromosomal Aberration Test.

EC Directive 92/69/EEC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test

Species/Strain: Chinese Hamster
Cell Type/Cell Line: V79
Metabolic Activation System: Aroclor 1254 induced rat liver S9 fraction
Vehicle: Minimal Essential Medium (MEM)
Remarks - Method: No significant protocol deviations.
Test 2 was carried out without S-9 mix only but with closer doses than test 1 to demonstrate a possible dose-response relationship.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	900*, 1800*, 2700*[a]	4	18
Test 2	1800*, 2250*, 2700*[b]	4	18
Present			
Test 1	900*, 1800*, 2700*	4	18
Test 2	Not performed		

*) Cultures selected for metaphase analysis

a) Due to a clear increase in chromosomally damaged cells, the number of metaphases was reduced from the intended 200 mitoses to 50 cells

b) Due to a clear increase in chromosomally damaged cells, the number of metaphases was reduced from the intended 200 mitoses to 100 cells

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	3000			
Test 1		2700	>2700	2700
Test 2		2700	>2700	2700

Present	None			
Test 1		>2700	>2700	2700 (weak)

Remarks - Results

Test 1
Without S-9 mix an evident increase in the number of aberrant metaphases with a high proportion of exchanges was observed but only at the highest dose.
With metabolic activation there was only a slight increase in chromosomally damaged cells at the top dose.

Test 2
There was a clear increase in the number of chromosomal damaged cells at 2700 µg/mL. The aberration rate at 2250 µg/mL is within historical values and therefore not considered to be an indication of clastogenic activity.

CONCLUSION

The notified chemical was clastogenic to Chinese Hamster V79 cells treated in vitro under the conditions of the test. However, it cannot be ruled out that these findings are due to the chelating properties of the test substance which might interfere with cellular cationic pools.

TEST FACILITY

BASF (1995j)

7.9.2. Genotoxicity – in vitro cell gene mutation Assay

TEST SUBSTANCE

Notified chemical

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	Chinese Hamster
Cell Type/Cell Line	Ovary, CHO substrain K1
Metabolic Activation System	Aroclor 1254 induced rat liver S9 fraction
Vehicle	Ham's F12 Medium
Remarks - Method	HPRT test. No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 109.38, 218.75, 437.5, 875, 1750	4 hours	7-9 days	7 days
Test 2	0, 109.38, 218.75, 437.5, 875, 1750	4 hours	7-9 days	7 days
Present				
Test 1	0, 218.75, 437.5, 875, 1750, 3500	4 hours	7-9 days	7 days
Test 2	0, 218.75, 437.5, 875, 1750, 3500	4 hours	7-9 days	7 days

All 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	1750			
Test 1		1750	>1750	negative
Test 2		1750	>1750	negative
Present	>3500			
Test 1		>3500	>3500	negative
Test 2		>3500	>3500	negative

Remarks - Results	The test substance did not cause any increase in mutant frequencies either with or without metabolic activation, over the levels observed in the vehicle control. Both positive controls led to the expected increase in the frequencies of forward mutations.
CONCLUSION	The notified chemical was not clastogenic to Chinese Hamster Ovary (CHO) cells treated in vitro under the conditions of the test.
TEST FACILITY	BASF (2001b)

7.10 Genotoxicity – in vivo Mouse Micronucleus Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test. EC Directive 2000/32/EC B.12 Mutagenicity Mammalian Erythrocyte Micronucleus Test.
Species/Strain	Mouse/NMRI
Route of Administration	Oral – gavage
Vehicle	Purified Water
Remarks - Method	No significant protocol deviations

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 male/5 female	0	24
II (vehicle control)	5 male/5 female	0	48
III (low dose)	5 male/5 female	500	24
IV (mid dose)	5 male/5 female	1000	24
V (high dose)	5 male/5 female	2000	24
VI (high dose)	5 male/5 female	2000	48
VII (positive control, CP)	2 male/3 female	20	24
VIII (positive control, VCR)	3 male/2 female	0.15	24

CP=cyclophosphamide. VCR=vincristine

RESULTS

Doses Producing Toxicity	The high dose (2000 mg/kg bw) reached the limit dose for a non-toxic test substance. There were no premature deaths or clinical signs of toxicity observed in any of the dose groups
Genotoxic Effects	The test substance did not lead to any increase in the rate of micronuclei. The number of normochromatic (NCE) or polychromatic (PCE) erythrocytes containing small nuclei did not deviate from the vehicle control. The ratio of PCE/NCE was in the same range as that of the vehicle control values in all dose groups. Results from the vehicle and positive control demonstrated that the test method was operating satisfactorily. Therefore, the test substance is considered negative in this micronucleus assay
Remarks - Results	
CONCLUSION	The notified chemical was not genotoxic in this in vivo mouse micronucleus assay under the conditions of the test.
TEST FACILITY	BASF (1997a)

ADDITIONAL INVESTIGATIONS

7.18T. Pharmacokinetic/toxicokinetic

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 417 Toxicokinetics – Absorption and Excretion study
Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Vehicle	0.5% Tylose CB 30.000 in aqua bidest
Remarks - Method	No significant protocol deviations

STUDY DESIGN

Experiment	*Number and Sex of Animals*	*Dose mg/kg bw*		*Dosing Regimen*
		Nominal	Average actual	
1	4 male	25	24	Single administration
2	4 male	500	465	Single administration
3	4 male	500	476	Daily, 7 days

Based on theoretical considerations and published data on structurally related substances, it was assumed that the absorbed portion of the dose is exclusively excreted via urine and no metabolism of the test substance occurs. The portion of the dose of test substance that is found in the urine, thus, directly gives the bioavailability of the test substance. Urine samples were collected for the following time intervals after the last administration: 0-6h, 6-12h, 12-24h, 24-48h, 48-72h and in 24h intervals during dosing in experiment 3.

RESULTS

Experiment 1

Within 72 hours after single oral administration, 16.98% of the applied dose was found in the urine and hence, based on the assumption mentioned above, the bioavailability if the test substance corresponded to 16.98%. Of the systematically available test substance 100% was excreted in urine within 12 hours after application which indicates rapid absorption and rapid renal excretion. From the time course of the amount of test substance in the urine, the urinary excretion half-life was calculated to be 2.5 hours. Kinetic analysis revealed a mean residence time of 4 hours.

Experiment 2

Within 72 hours after single oral administration, 20.94% of the applied dose was found in the urine and hence, based on the assumption mentioned above, the bioavailability if the test substance corresponded to 20.94%. Of the systematically available test substance 80% was excreted in urine within 12 hours after application which indicates rapid absorption and rapid renal excretion. From the time course of the amount of test substance in the urine, the urinary excretion half-life was calculated to be 6.1 hours. Kinetic analysis revealed a mean residence time of 10.2 hours.

Experiment 3

During the treatment period, the bodyweight of the animals decreased continuously and increased again during the 72 hour observation period after the last administration. Of the total dose applied 32.94% could be found in the urine collected during the dosing period and up to 72 hours after the last administration and hence, based on the assumption mentioned above, the bioavailability if the test substance corresponded to 32.94%. On a daily basis there was some variation in the bioavailability ranging from 24.35% to 44.95%. From the time course of the amount of test substance in the urine, the urinary excretion half-life was calculated to be 6.3 hours. Kinetic analysis revealed a mean residence time of 17.5 hours. All these data do not give an indication that induction or saturation of urinary excretion of the test substance occurs after repeated oral administration.

CONCLUSION

After single and repeated oral the test substance was rapidly absorbed from the gastrointestinal tract. Absorption, however, was incomplete amounting to 17 – 33 % of the dose applied. The excretion of the test substance was rapid with a urinary excretion half life of about 3-6 hours.

TEST FACILITY	BASF (1997b)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

Several short reports were provided on the biodegradability of the notified chemical with most conducted to OECD Good Laboratory Practice standards.

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 E Ready Biodegradability: Modified OECD Screening Test. Directive 92/69/EEC, July 1992.
Inoculum	River water of the Rhine downstream of the BASF waste water treatment plant
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	DOC analyser
Remarks - Method	A test concentration of 103 mg/L (equivalent to 20 mg/L DOC) was incubated and aerated at room temperature for 28 d with regular sampling to determine the DOC concentration. CO_2 formation was not measured.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
10	5	3	84
14	50	7	100
17	67		
21	91		
28	97		

Remarks - Results	The sodium benzoate reference substance was completely degraded after 7 d while the inhibition control experienced 98% degradation in 28 d. At the end of the exposure, a mean of 97% degradation occurred in the two treatment replicates, as measured by the disappearance of DOC, and was within the 10-d window required by the Test Guideline to be considered readily biodegradable.
CONCLUSION	The test substance was readily biodegradable under the conditions of this test.
TEST FACILITY	BASF (1992a)

8.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 E Ready Biodegradability: Modified OECD Screening Test. Directive 92/69/EEC, July 1992.
Inoculum	Effluent of the municipal waste water treatment plant of Lambsheim.
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	DOC analyser
Remarks - Method	A test concentration of 103 mg/L (equivalent to 20 mg/L DOC) was incubated and aerated at room temperature for 28 d with regular sampling to determine the DOC concentration. CO_2 formation was not measured.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*

3	3	3	94
7	10	7	100
14	16		
17	96		
28	96		

Remarks - Results: After 28 d incubation, a mean of 96% degradation occurred in the two treatment replicates, as measured by the disappearance of DOC, and was within the 10-d window required by the Test Guideline to be considered readily biodegradable. The sodium benzoate reference substance was completely degraded after 7 d while the inhibition control was 98% degraded in 28 d.

CONCLUSION: The test substance was readily biodegraded in this test.

TEST FACILITY: BASF (1992b)

8.1.3. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 301 E Ready Biodegradability: Modified OECD Screening Test. Directive 92/69/EEC, July 1992.

Inoculum: Soil suspension of filtered rich humus garden mould.
Exposure Period: 28 d
Auxiliary Solvent: None
Analytical Monitoring: DOC analyser
Remarks - Method: A test concentration of 103 mg/L (equivalent to 20 mg/L DOC) was incubated and aerated at room temperature for 28 d with regular sampling to determine the DOC concentration. CO_2 formation was not measured.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
3	3	3	92
10	7	7	100
14	98		
28	98		

Remarks - Results: After 28 d incubation, a mean of 98% degradation occurred in the two treatment replicates, as measured by the disappearance of DOC. This was within the 10-d window required by the Test Guideline to be considered readily biodegradable The sodium benzoate reference substance was completely degraded after 7 d while the inhibition control was 98% degraded in 28 d.

CONCLUSION: The test substance was readily biodegraded in this test.

TEST FACILITY: BASF (1992c)

8.1.4. Inherent biodegradability

TEST SUBSTANCE: Notified chemical (35.3% purity)

METHOD: OECD TG 302 B Zahn-Wellens/EMPA Test. Directive 88/302/EEC, 1987. International Standard ISO 7827:1984.

Inoculum: Activated sludge from waste water treatment plant, 1 g/L dry substance
Exposure Period: 10 d

Auxiliary Solvent	None
Analytical Monitoring	DOC analyser after centrifugation.
Remarks - Method	A test concentration of 390 mg/L (equivalent to 50 mg/L DOC) was incubated and aerated at room temperature for 10 d. Regular samples were taken to measure the DOC concentration but no CO_2 measurements were made.

RESULTS

Test substance		*Diethylene glycol*	
Day	*% degradation*	*Day*	*% degradation*
1	21	1	15
3	18	3	6
5	9	5	-1
7	12	7	46
9	98	9	78
10	97	10	91

Remarks - Results	Despite some variability in the disappearance of DOC in the first 10 d, the notified chemical achieved 97% removal of DOC after this time, compared to 91% for the reference substance diethylene glycol. As the reference compound was degraded by ≥70% within 14 d, the test was considered valid.

CONCLUSION The test substance was inherently biodegradable in this test.

TEST FACILITY BASF (1995k)

8.1.5. Ready biodegradability

TEST SUBSTANCE Notified chemical

METHOD OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test. Directive 92/69/EEC. International Standard ISO 9408:1991.

Inoculum	Activated sludge from laboratory waste water treatment plants with municipal and synthetic sewage.
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	Respirometer measuring oxygen consumed.
Remarks - Method	A test concentration of 450 mg/L was incubated and aerated at room temperature for 28 d in a respirometer measuring biochemical oxygen demand. The OECD TG stipulates 100 mg/L should be tested. Six replicates were run but one was considered an outlier and not considered in the results.

RESULTS

Test substance		*Aniline*	
Day	*% degradation relative to ThOD (%)*	*Day*	*% degradation*
7	8.6	7	46
14	71.6	14	84
21	97.2	21	87
28	110	28	89

Remarks - Results	The notified chemical was biodegraded by 110% of the theoretical oxygen demand by 28 d, satisfying the conditions for ready biodegradability (>60% within 28 d and within a 10-d window).

CONCLUSION The notified chemical was readily biodegradable in this test.

TEST FACILITY BASF (1995l)

8.1.6. Ready biodegradability

TEST SUBSTANCE Notified chemical (~75% purity)

METHOD OECD TG 301 A Ready Biodegradability: DOC Die-Away Test. Directive 92/69/EEC. International Standard ISO 7827:1994.

Inoculum	Activated sludge from the laboratory waste water treatment plants which run with municipal and synthetic waste water 4:1.
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	DOC analysis after centrifugation.
Remarks - Method	The test concentration of 20 mg/L was incubated aerobically at room temperature for 28 d with regular sampling for DOC determination.

RESULTS

Test substance		*Aniline*	
Day	*% degradation*	*Day*	*% degradation*
7	11	1	3
10	86	3	9
13	92	5	100
14	98		

Remarks - Results The notified chemical was almost completely degraded (98%) by 14 d as measured by the loss of DOC. This was achieved within the 10-d window of reaching 10% DOC loss, thus classifying the notified chemical as readily biodegradable. The reference substance aniline was completely degraded by 5 d which fulfilled the validity criteria.

CONCLUSION The notified chemical was readily biodegradable in this test.

TEST FACILITY BASF (1995m)

8.1.7. Ready biodegradability

TEST SUBSTANCE Notified chemical

METHOD OECD TG 301 C Ready Biodegradability: Modified MITI Test (I). Directive 92/69/EEC.

Inoculum	Different inoculum prepared on conformity with OECD TG 301.
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	Respirometer measuring oxygen consumed.
Remarks - Method	The test concentration of 450 mg/L was aerobically incubated in a respirometer at room temperature, originally for 28 d.

RESULTS

Remarks - Results The exposure was increased to 37 d, presumably because two of six replicates only showed ≤17% degradation at 28 d. One of these two replicates plus another deviated by >20% and were not considered valid by the authors. The pH in three replicates was outside the range of 6-8.5 considered acceptable for this test. These factors and the very high variability among all replicates indicated the results were inconclusive.

CONCLUSION The test results were deemed inconclusive due to high variability.

TEST FACILITY BASF (1995n)

8.1.8. Ready biodegradability

TEST SUBSTANCE	Notified chemical (92.4% purity)
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
Inoculum	30 mg/L dry weight of inocula collected from 10 places in the Ludwigshafen region including natural river/lake waters and municipal and industrial waste water treatment plants.
Exposure Period	28-40 d
Auxiliary Solvent	None
Analytical Monitoring	Respirometer
Remarks - Method	Four experiments to determine the biodegradability of the notified chemical were performed using the Modified MITI test. A respirometer continuously measured the oxygen supply consumed in duplicate for each concentration of 100 and 200 mg/L (corresponding to 65.5 and 131 mg/L, respectively, theoretical oxygen demand (ThOD)). Samples were incubated at 25±1˚C.
RESULTS	
Remarks - Results	At the end of each exposure, the DOC degradation was 100% with no test substance or metabolites found. High variability in the time required for complete degradation was seen in several replicates although the general trend was the same. As the BOD was >60% generally within a 10-d window (some replicates were outside this time frame), the notified chemical was considered readily biodegradable. The reference substance aniline was also degraded indicating valid tests.
CONCLUSION	The notified chemical was readily biodegradable in these tests.
TEST FACILITY	BASF (1995o)

8.1.9. Inherent biodegradability

TEST SUBSTANCE	Notified chemical (88.5% purity)
METHOD	OECD TG 302 C Inherent Biodegradability: Modified MITI Test (II). Method for testing the biodegradability of chemical substances by microorganisms, Ministry of International Trade and Industry, Japan.
Inoculum	Sludge from 10 locations in Japan including sewage plants, rivers and bays.
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	DOC and HPLC analyses
Remarks - Method	A concentration of 30 mg/L of the notified chemical was incubated at pH 7.0-8.8, 25±1˚C and activated sludge at 100 mg/L suspended solids in triplicate.
RESULTS	
Remarks - Results	HPLC analysis found that no parent compound remained at 28 d indicating complete degradation. However, BOD analysis in the three replicates showed high variability of 6, 15 and 89% (mean of 37%) degradation at 28 d. The TOC analysis supported the high degradation at 96%. Aniline was 75% degraded by 28 d indicating valid test conditions. Therefore the notified chemical was considered to be degradable under the conditions of this test.
CONCLUSION	The notified chemical was inherently biodegradable in these tests.
TEST FACILITY	Kurume Research Laboratories (1998)

8.1.10. Ready biodegradability

TEST SUBSTANCE	Notified chemical (35.3% purity)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 evolution (modified Sturm test). Directive 92/69/EEC. International Standard ISO 9439:1988.
Inoculum	30 mg/L dry weight of activated sludge from laboratory waste water treatment plants operated with municipal and synthetic sewage.
Exposure Period	28 d
Auxiliary Solvent	None
Analytical Monitoring	Dissolved inorganic carbon
Remarks - Method	The test concentration of 162 mg/L (equivalent to 22 mg/L DOC) was incubated aerobically at room temperature for 28 d with regular sampling for DOC determination.

RESULTS

Test substance		*Diethylene glycol*	
Day	*% degradation*	*Day*	*% degradation*
5	9	8	0
8	12	13	22
13	38	16	43
16	60	23	65
19	69	27	65
28	82	28	70

Remarks - Results	The notified chemical was substantially degraded (82%) at 28 d as measured by $\%CO_2/ThCO_2$ and 98% degraded by DOC analysis. Although it was impossible to determine if the 70% degradation pass mark strictly occurred within the 10-d window of reaching 10% DOC loss, the borderline case was considered as readily biodegradable The reference substance diethylene glycol achieved 70% degradation.
CONCLUSION	The notified chemical was readily biodegradable in this test.
TEST FACILITY	BASF (1995p)

8.1.2. Bioaccumulation

No study was conducted but the log Kow is ≤4, indicating the notified chemical is unlikely to bioaccumulate in organisms.

8.2. Ecotoxicological investigations

8.2.1.1. Acute toxicity to fish (zebra fish)

TEST SUBSTANCE	Notified chemical (83.2% purity).
METHOD	OECD TG 203 Fish, Acute Toxicity Test – static test. EEC Directive 84/449 Methods for determination of Ecotoxicity, acute toxicity for fish, 1992 – static procedure.
Species	Zebra fish (*Brachydanio rerio* HAM. and BUCH.)
Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	~250 mg $CaCO_3$/L

Analytical Monitoring	Capillary electrophoresis
Remarks – Method	Zebra fish of about 6 months old (3.2-4.2 cm body length, 0.34-0.70 g body weight) were held in mean measured treatments of 54 and 103.5 mg/L for 96 h at pH 8.1-8.3, 23˚C and dissolved oxygen 5.2-7.9 mg/L in a 16 h light photoperiod. The loading was 0.46 g bw/L with slight aeration.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
50	54	10	0	0	0	0	0
100	103.5	10	0	0	0	0	0

LC50	>103.5 mg/L at 96 h
NOEC	103.5 mg/L at 96 h
Remarks – Results	Throughout exposure, there were no adverse effects or mortalities observed in any of the treatments or the control, indicating the 96-h LC50 was >103.5 mg/L.

CONCLUSION The 96-h LC50 was >103.5 mg/L

TEST FACILITY BASF (1995q)

8.2.1.2. Acute toxicity to fish (rainbow trout)

TEST SUBSTANCE Notified chemical (82.6% purity).

METHOD OECD TG 204 Fish, prolonged toxicity test: 14-day study.
Umweltbundesamt (German Federal Environmental Protection Agency) Test guideline "Sublethal toxic effects in the zebra fish *Brachydanio rerio*" 1984.

Species	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792)
Exposure Period	28 d
Auxiliary Solvent	None
Water Hardness	2.3-2.5 mmol/L
Analytical Monitoring	Reversed phase HPLC
Remarks – Method	Juvenile rainbow trout (1.8-2.5 g body weight) were exposed to mean measured concentrations up to 98.6 mg/L under flow-through conditions. Twenty fish were used per unreplicated treatment and held in water of pH 8.0-8.4, dissolved oxygen 5.9-8.6 mg/L, 15˚C and 16 h light photoperiod.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
3.16	2.62	20	0	0	0	0	0
10.0	7.78	20	0	0	0	0	0
31.6	30.2	20	0	0	0	0	0
100.0	98.6	20	0	0	0	0	0

NOEC	98.6 mg/L at 28 d
LOEC	>98.6 mg/L at 28 d
Remarks – Results	Throughout exposure, there were no adverse effects on mean body weight, body length, general health or behaviour observed in any of the treatments or the control, indicating the 28-d NOEC and LOEC were 98.6 and >98.6 mg/L, respectively.

CONCLUSION The 28-d NOEC and LOEC were 98.6 and >98.6 mg/L, respectively.

TEST FACILITY BASF (1997c)

8.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical (75.1% purity).

METHOD EEC Directive 79/831/EEC Annex V, Part C: methods for the determination of Ecotoxicity, C2. Acute toxicity for Daphnia, updating Nov 1989.

Species	*Daphnia magna*
Exposure Period	48 h
Auxiliary Solvent	None
Water Hardness	2.46 mmol/L
Analytical Monitoring	Capillary electrophoresis
Remarks - Method	Daphnid neonates (<24 h old) were exposed to mean measured concentrations of the notified chemical up to 99.5 mg/L in four replicates of five animals each vessel. They were held for 48 h at 19.6-21.0˚C, 16 h light photoperiod, pH 8.0-8.6 and dissolved oxygen 7.6-8.1 mg/L.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
12.5	12	20	0	0
25	Not measured	20	0	0
50	Not measured	20	0	0
100	99.5	20	0	0

LC50	>99.5 mg/L at 48 h
NOEC	99.5 mg/L at 48 h
Remarks - Results	After 48 h exposure, no adverse effects, immobility or mortality was observed in any of the treatments or control, indicating the 48-h EC50 was >99.5 mg/L. No indication was given of the clarity of test solutions.

CONCLUSION The 48-h LC50 was >99.5 mg/L.

TEST FACILITY BASF (1995r)

8.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical (purity not specified).

METHOD EEC Guideline XI/681/86, Draft 4: “Prolonged toxicity study with *Daphnia magna*: effects on reproduction”.

Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	None
Water Hardness	2.46 mmol/L
Analytical Monitoring	Reverse phase HPLC
Remarks - Method	Daphnid neonates (<24 h old) were exposed to mean measured concentrations of the notified chemical up to 99.3 mg/L in 10 replicates of one animals each vessel. They were held for 21 d at 19.9-21.7˚C, 16 h light photoperiod, pH 7.8-8.6, hardness 2.2-3.2 mmol/L and dissolved oxygen 7.4-9.6 mg/L. The static solutions were changed three times per week with daily feeding of daphnids with live green algae. No indication was given of the clarity of test solutions.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual on Day 0*		*24 h*	*48 h*
1.56	1.6	10	0	0
3.13	Not measured			
6.25	Not measured			
12.5	11.6	10	0	0
25	Not measured			
50	Not measured	10	0	0
100	99.3	10	0	0

NOEC	99.3 mg/L at 21 d
LOEC	>99.3 mg/L at 21 d
Remarks - Results	After 21 d exposure, no adverse effects on fecundity was observed in any of the treatments or control, indicating the 21-d NOEC and LOEC were 99.3 and >99.3 mg/L, respectively. Although two daphnids died in the lowest concentration of 1.6 mg/L on Day 11, this was unlikely to be treatment related as all higher concentrations showed no mortality. Indeed there was a significant dose response stimulation of fecundity with increasing concentration.
CONCLUSION	The 21-d NOEC and LOEC were 99.3 and >99.3 mg/L, respectively
TEST FACILITY	BASF (1997d)

8.2.4. Algal growth inhibition test 1

TEST SUBSTANCE	Notified chemical (purity not specified).
METHOD	EEC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus* CHODAT
Exposure Period	72 h
Concentration Range	
Nominal	6.25-100 mg/L
Actual	Not specified for individual treatments but within 78-96% of nominal.
Auxiliary Solvent	None
Water Hardness	Not specified.
Analytical Monitoring	Methodology not specified.
Remarks - Method	Algae were incubated (three replicates per treatment) at 23±2˚C and pH 6.8-9.6 for 72 h under constant light with mean fluorescence measured every 24 h. A strong yellowish colour was observed in treatments ≥25 mg/L while lower treatments had a light yellow colour.

RESULTS

Biomass		*Growth Rate*	
72-h E_bC50 > 100 mg/L	72-h E_bC10 = 16.7 mg/L	72-h E_rC50 > 100 mg/L	72-h E_rC10 > 100 mg/L

Remarks - Results	A linear dose-response relationship was observed with the highest nominal concentration of 100 mg/L causing a 33% reduction in biomass after 72 h compared to the control. This translated into a 9.3% inhibition of growth rate giving 72-h E_bC50 and E_rC50 values of >100 mg/L.
CONCLUSION	The 72-h E_bC50 and E_rC50 values were >100 mg/L.
TEST FACILITY	BASF (1998b)

8.2.5. Algal growth inhibition test 2

TEST SUBSTANCE	Notified chemical (purity not specified).

METHOD	EEC Directive 92/69/EEC C.3 Algal Inhibition Test. OECD Guideline for Testing of Chemicals, No. 201, Algal growth inhibition test. US EPA Ecological Effects Test Guidelines, OPPTS 850.5400, Algal toxicity, tiers I and II.
Species	*Scenedesmus subspicatus* CHODAT
Exposure Period	96 h
Concentration Range	
Nominal	0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 100 mg/L
Actual	within 102% of nominal for those concentrations measured.
Auxiliary Solvent	None
Water Hardness	150 mg/L $CaCO_3$
Analytical Monitoring	Reverse phase HPLC with UV detection.
Remarks - Method	Algae were incubated (three replicates per treatment) at 21-25˚C and pH 7.9-8.4 for 96 h under constant light with mean fluorescence measured every 24 h.

RESULTS

Biomass		*Growth Rate*	
96-h E_bC50 = 2.64 mg/L	96-h E_bC10 = 0.70 mg/L	96-h E_rC50 > 100 mg/L	96-h E_rC10 = 2.68 mg/L

Remarks - Results	The highest nominal concentration of 100 mg/L caused a 92% reduction in biomass after 96 h compared to the control. However, the growth rate in this treatment was 49.3% of the control at the same time giving a 96-h E_bC50 of 2.64 mg/L and a 96-h E_rC50 of >100 mg/L. This is considered moderately toxic (Mensink et al. 1995).
CONCLUSION	The 96-h E_bC50 and E_rC50 values were 2.64 and >100 mg/L, respectively.
TEST FACILITY	BASF (1999)

8.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical (37% purity)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test. International Standard ISO 8192.
Inoculum	Activated sludge from laboratory waste water treatment plants fed with municipal and synthetic waste water.
Exposure Period	3 h
Concentration Range Nominal	1,000 mg/L
Remarks – Method	A single concentration of 1,000 mg/L of the notified chemical was incubated with 1 g/L inoculum at 20±2˚C for 0.5 and 3 h.
RESULTS	
	10% respiration inhibition after 0.5 h 3% respiration inhibition after 3 h
Remarks – Results	The 3-h EC50 of the notified chemical was >1,000 mg/L. The 3-h EC50 of the reference substance 3,5-dichlorophenol was 24 mg/L (no confidence limits reported) which was within the validity limits of this method.
CONCLUSION	The 3-h EC50 of the notified chemical was >1,000 mg/L.

TEST FACILITY	BASF (1995s)

8.2.7. Inhibition of bacterial growth

TEST SUBSTANCE	Notified chemical (~75% purity)
METHOD	DIN 38412 part 8.
Inoculum	*Pseudomonas putida* bacteria
Exposure Period	16 h
Concentration Range	
Nominal	11 concentrations from 0.98 to 1,000 mg/L
Remarks – Method	Cultures of bacteria in agar growth media (replicated four times per treatment) were incubated at 21±1˚C for 16 h. The optical density of the suspension was measured at a wavelength of 436 nm to determine the extent of cell multiplication.
RESULTS	
Remarks – Results	The 16-h EC50 was given as 5.47 mg/L (no 95% confidence limits were reported).
CONCLUSION	The 16-h EC50 to bacteria cell growth was 5.47 mg/L (no confidence limits).
TEST FACILITY	BASF (1995t)

8.5E. Earthworm toxicity

TEST SUBSTANCE	Notified chemical (80% purity)
METHOD	OECD TG 207 Earthworm, acute toxicity test. DIN/ISO 10 390 Bodenbeschaffenheit, Bestimmung des pH-Wertes, 1993.
Remarks - Method	Earthworms (*Eisenia foetida*) of 9 months age (about 300 mg) were incubated in artificial soil (comprising sand, clay and sphagnum peat) for 14 d under constant light, 19-25˚C, 35% water content and pH 6.5. Four replicates containing 10 worms each were run for each nominal test concentration of 62.5, 125, 250, 500 and 1,000 mg/kg soil. Mortality and body weight was assessed after 7 and 14 d.
RESULTS	
Remarks - Results	The 14-d LC50 was 142 (122, 166) mg/kg soil with no clear effect on body weight. As the control mortality was <10% and the chloracetamide reference substance 14-d LC50 was 25.3 mg/kg soil (95% confidence limits not reported), the test was considered valid.
CONCLUSION	The 14-d LC50 was 142 (122, 166) mg/kg soil, which is considered slightly toxic to earthworms (Mensink et al. 1995).
TEST FACILITY	BASF (2001c)

8.5E. Terrestrial plant emergence and growth

TEST SUBSTANCE	Notified chemical (80% purity)
METHOD	OECD TG 208 Terrestrial plants, growth test. International Standard ISO 11269-2:1995 (E) soil quality – determination of the effects of pollutants on soil flora – part 2: effects of chemicals on the emergence and growth of higher plants.

Remarks - Method	Fresh seed of oats (*Avena sativa*), oilseed rape (*Brassica napus*) and vetch (*Vicia sativa*) were sown in a field soil (pH 7.7, 1.7% OC) in PVC pots adjusted to 40% of the maximum water capacity. The test substance was dissolved in demineralised water and added in aliquots to the pots to achieve treatment concentrations of 62.5, 125, 250, 500 and 1000 mg/kg soil. After 19 d at 19-22˚C, 60-70% relative humidity, the four replicates of 10 seeds/replicate for each treatment were assessed for germination, fresh/dry weight and shoot length. Seedlings were thinned to five per pot.
RESULTS	
Remarks - Results	There was no adverse effect on emergence of seedlings at the highest concentration. The dry weight of oilseed rape and vetch were similarly unaffected, but the NOEC and LOEC values for oats were 500 and 1,000 mg/kg soil, respectively. The sensitivity of all three species was identical with shoot length and fresh weight as the most sensitive indicators. The NOEC and LOEC values were 250 and 500 mg/kg soil, respectively.
CONCLUSION	The NOEC and LOEC values were 250 and 500 mg/kg soil, respectively.
TEST FACILITY	BASF (2001d)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

Based on the intended use pattern of the notified chemical in detergents, degreasers and cleaning agents, nearly all of the imported amount will eventually be released to the aquatic environment via the sewerage systems through formulation and use (washing off surfaces and cleaning activities). Only wastes from spills during normal operating procedures are expected to be disposed of to landfill or incinerated. The chemical is expected to partition to water due to its high solubility and biodegrade to oxides of carbon and nitrogen as it is readily biodegradable.

Based on maximum annual imports of 20 tonnes/year, and assuming a worst-case scenario that all is eventually released to sewers on a nationwide basis, the daily release to sewers is estimated to be 54.79 kg/d. Assuming a national population of 20 million with each person contributing an average 200 L/d to overall sewage flows and that the chemical is not removed during sewage treatment processes, the worst-case predicted environmental concentrations (PECs) in sewage effluent, ocean and inland river on a nationwide basis are estimated as follows:

Amount of notified chemical entering sewer annually	20,000 kg
Population of Australia	20 million
Amount of water used per person per day	200 L
Number of days of release in a year	365
Amount partitioned to water (based on solubility)	100%
$PEC_{aquatic}$ (sewage effluent)	13.7 µg/L
$PEC_{aquatic}$ (river)	13.7 µg/L
$PEC_{aquatic}$ (ocean)	1.37 µg/L

The worst-case PEC in sewage effluent on a nationwide basis is estimated as 13.7 µg/L (EA 2003). Based on the respective dilution factors of 0 and 10 for inland and ocean discharges of effluents, the PECs of the notified chemical in freshwater and marine water may approximate 13.7 and 1.37 µg/L, respectively.

Another worst-case scenario is considered assuming that products containing the notified chemical would only be used in a major metropolitan centre such as Sydney (with a population of 4.1 million) and the PECs for release to inland river and ocean are estimated to be 67 and 6.7 µg/L, respectively.

The notified chemical was considered readily biodegradable in nine tests and was inconclusive in another. Given that the Henry's Law Constant was not provided (nor could it be calculated as the vapour pressure was not provided) and the log Pow was < -4, the amount of chemical partitioning to air, water, sludge or biodegrading could not be estimated accurately. However, as a worst case, the Simpletreat model (European Commission 2003) for estimating partitioning and losses in sewage treatment plants (STP) indicates the maximum partitioning to water for a readily biodegradable chemical is 13% with the remainder degraded in the STP.

Based on the partitioning for a readily biodegradable chemical, the revised worst-case PECs for freshwater and marine water from the nationwide release of the notified chemical into the sewage systems are 1.8 and 0.18 µg/L, respectively. On the metropolitan scale of Sydney, the PECs are 8.7 and 0.87 ng/L, respectively. The revised PEC for the concentration in STP effluent is 1.8 µg/L.

The notified chemical is not expected to partition to biosolids in STPs based on the Simpletreat model, therefore the PEC of the notified chemical is 0 mg/kg in receiving soil.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation rate is assumed to be 1,000 $L/m^2/y$ (10 ML/ha/y). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 0.1 m of soil (density 1,000 kg/m^3). Using these

assumptions, irrigation with a concentration of 1.8 µg/L is expected to give a soil PEC of 0.018 mg/kg soil after a year assuming no biodegradation. As the notified chemical is readily biodegradable and expected to percolate deeper than just the top 0.1 m of soil, the soil PEC is expected to be much lower.

Based on the ready biodegradability of the notified chemical, the high water solubility and diffuse release to the sewer Australia wide, there is little expected potential for bioaccumulation.

9.1.2. Environment – effects assessment

The toxicity of the notified chemical to various organisms is summarised in the following table.

Organism	*Duration*	*Endpoint*	*Value*
Zebra fish (*Brachydanio rerio*)	96 h	LC50	>103.5 mg/L
Rainbow trout (*Oncorhynchus mykiss*)	28 d	NOEC	98.6 mg/L
		LOEC	>98.6 mg/L
Waterflea (*Daphnia magna*)	48 h	LC50	>99.5 mg/L
	21 d	NOEC	99.3 mg/L
		LOEC	>99.3 mg/L
Alga (*Scenedes-mus subspicatus*)	72 h	E_bC50	>100 mg/L
		E_rC50	>100 mg/L
	96 h	E_bC50	2.64 mg/L (no confidence limits)
		E_rC50	>100 mg/L
Microorganisms in activated sludge	3 h	EC50	>1,000 mg/L
Pseudomonas putida bacteria	16 h	EC50	5.47 mg/L (no confidence limits).
Earthworm (*Eisenia foetida*)	14 d	LC50	142 (122, 166) mg/kg soil
Oat (*Avena sativa*)	19 d	NOEC	250 mg/kg soil
Oilseed rape (*Brassica napus*)		LOEC	500 mg/kg soil
Vetch (*Vicia sativa*)			

The ecotoxicological data indicate the notified chemical has low toxicity to fish, aquatic invertebrates and microorganisms in activated sludge, and moderate toxicity to algae. It had some toxicity to one bacteria and was slightly toxic to earthworms.

An aquatic predicted no effect concentration (PNEC) of 0.264 mg/L was calculated by dividing the most sensitive endpoint of 2.64 mg/L (96-h E_bC50 for algae) by an assessment (safety) factor of 10 as chronic toxicity data were available for three trophic levels – fish, invertebrates and algae (OECD 2003). For the terrestrial environment, the most sensitive endpoint of 142 (122, 166) mg/kg soil for earthworms divided by an uncertainty factor of 100 (acute data available for two trophic levels) gave a terrestrial PNEC of 1.42 mg/kg soil. A factor of 100 was used rather than 1,000 because the notified chemical is not expected to persist in the environment (OECD 2003).

9.1.3. Environment – risk characterisation

Location	PEC (µg/L)	PNEC (µg/L)	Risk Quotient (Q)
Australia-wide STPs – no mitigation			
Ocean outfall	1.37	264	0.0052
Inland River	13.7	264	0.052
Major metropolitan centre (eg. Sydney) – no mitigation			
Ocean outfall	6.7	264	0.025
Inland River	67	264	0.25
Australia-wide STPs – with mitigation*			
Ocean outfall	0.18	264	0.00068
Inland River	1.8	264	0.0068
Major metropolitan centre (eg. Sydney) – with mitigation*			
Ocean outfall	0.87	264	0.0033
Inland River	8.7	264	0.033

*PEC and Q values calculated assuming 13% of the notified chemical remains in the effluent water after the STP process based on the SIMPLETREAT model.

The worst-case risk quotients (Q = PEC/PNEC) with no mitigation for the freshwater environment if the notified chemical is used nationwide and in a major metropolitan centre such as Sydney are 0.052 and 0.25, respectively. The respective Q values for ocean are 0.0052 and 0.052. As these values are all less than 1, there is low concern to the aquatic compartment.

The ready biodegradability of the notified chemical will reduce the PEC in sewage effluent and the Q values. In the Sydney metropolitan area, these mitigation factors reduce the Q values for river and ocean to 0.033 and 0.0033, respectively.

Given that the use pattern of the chemical indicates widespread and diffuse release, rather than within a single metropolitan base, the PECs and Q values will be lowered further. The Q values for river and ocean with nationwide use are 0.0068 and 0.00068, respectively, indicating an acceptable risk.

For the terrestrial environment, the worst case PEC of 18 µg/kg soil (from effluent re-use for irrigation to agricultural soils nationwide) and the PNEC of 1.42 mg/kg soil would give a Q value of 0.013, which is indicative of a low risk.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Transport and Storage

Exposure to the notified chemical is expected to be negligible except in the case of an accidental spill.

Reformulation

Exposure to the notified chemical from drips and splashes could occur during transfer of Trilon M liquid and the final formulated product. The notified chemical will be present at a concentration of 40% and 1-10% respectively. Exposure to the notified chemical during transfer of the final formulated product is more likely during the manual filling of larger packages rather than the automatic filling of smaller packages where exposure is expected to be negligible.

For manual operations, where intermittent exposure may occur, the estimated dermal exposure during reformulation is 0.04-0.4 mg/cm^2/day, based on EASE model (EASE) and assuming the notified chemical is present at concentration of 40%. Therefore, for a 70 kg worker with surface area for hands at 820 cm^2 and forearms at 1140 cm^2 and a 10% dermal absorption factor, systemic exposure is estimated to be 0.11-1.12 mg/kg bw/day. A 10% absorption factor was chosen due to the very low log Pow and the lack of effects observed in the acute dermal toxicity

study. Dermal exposure during automatic formulation processes will be much lower.

Exposure to the notified chemical would be reduced by the use of PPE.

It is considered that the physico-chemical properties of the notified chemical and the nature of its use constitute a low risk of inhalation exposure.

Laboratory Staff & Maintenance Workers
Minimal exposure will occur during the laboratory testing since it will only take a few minutes per batch.

Incidental skin contact is also identified for workers drum recycling. However, personal protective equipment will be worn, thus minimising any dermal exposure.

End Use
Incidental dermal exposure to the notified chemical (concentration of 1-10%) may occur during dilution of the final cleaning product before application. The estimated dermal exposure during dilution is 0.01-0.1 mg/cm^2/day, based on EASE model (EASE) and assuming the notified chemical is present at concentration of 10%. Therefore, for a 70 kg worker with surface area for hands at 820 cm^2 and forearms at 1140 cm^2 and a 10% dermal absorption factor, systemic exposure is estimated to be 0.028-0.28 mg/kg bw/day

During end use application, dermal contact is the most likely route of exposure with wipe on cleaning products. There is an increased risk of inhalation exposure with spray on products. The concentration of the notified chemical in diluted form will be 0.025 –0.25%.

9.2.2. Public health – exposure assessment

The formulated products containing the notified chemical are to be supplied for industrial use only. There is potential for contact with residues of the notified chemical at a concentration of up to 0.25% on cleaned surfaces. Overall, public exposure is expected to be negligible.

9.2.3. Human health - effects assessment

Toxicokinetics, metabolism and distribution.
An absorption and excretion study consisting of three experiments (single low dose, single high dose, repeated (7 day) high dose) for notified chemical was provided. It was assumed that the notified chemical is exclusively excreted via the urine and that no metabolism occurs. In all cases the notified chemical was rapidly absorbed from the gastrointestinal tract. Absorption, however, was incomplete amounting to 17-33% of the dose applied. The excretion of the test substance was rapid with urinary excretion half life of about 3-6 hours. There was no indication that induction or saturation of urinary excretion of the notified chemical occurs after repeated oral administration.

An accumulation of the notified chemical is not expected, owing to its rapid excretion.

Acute toxicity.
The notified chemical was of low oral and dermal toxicity in acute rat studies.

Irritation and Sensitisation.
In the skin irritation study, mechanical skin lesions were observed in two of the three rabbits. In one animal this skin reaction was non-reversible after 15 days. The notified chemical is considered to be a slight skin irritant. Minimal to moderate conjunctival irritation was noted in all treated eyes and persisted up to 72 hours. Treated eyes appeared normal after 8 days. The notified chemical is considered to be a slight eye irritant. The notified chemical was negative in a skin sensitisation adjuvant test in guinea-pigs.

Repeated Dose Toxicity.
In a 90 day oral repeat study in rats, dose dependent signs of toxicity were noted, characterised mainly by impairment of food consumption and body weight gain, increased water consumption,

increased blood in the urine, decreased fat content in the liver and the presence of focal vacuolisation of tubular epithelia in the kidneys and focal hyperplasia of the urothelium in the renal pelvis. The kidney was the target organ. Besides an increase in urinary zinc (thought to be due to the chelating ability of the notified chemical), no substance related findings were observed at 2400ppm. The No Observed (Adverse) Effect Level (NO(A)EL) was established in this study as 2400 ppm (170 mg/kg bw/day in males, 204 mg/kg bw/day in females).

In a 28 day oral repeat dose study in rats, dose-dependent signs of toxicity were seen at 12000 and 3000ppm. These included impairment of food consumption and body weight gain, increased sera urea, increased kidney weight and the presence of focal or multifocal vacuolisation of the tubular epithelia in the renal cortex. The kidney was the target organ. All findings were reversible after withdrawal of the test substance. The No Observed (Adverse) Effect Level (NO(A)EL) was established as 750 ppm (82 mg/kg bw/day) in this study.

Mutagenicity.
In an in vitro chromosomal aberration study in Chinese Hamster V79 cells, a clear increase in the number of aberrant metaphases was observed at 2700 μg/mL in the absence of metabolic activation and a slight increase in chromosomally damaged cells at 2700 μg/mL in the presence of metabolic activation. No increase in aberrant metaphases was observed at lower doses. The notified chemical was negative in an Ames test and an in vitro cell gene mutation assay in Chinese Hamster Ovary (CHO) cells. In an in vivo mouse micronucleus test, the notified chemical was found to be non genotoxic.

Hazard classification for health effects.
Based on the available toxicological data, the notified chemical is not classified as a hazardous substance under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999b).

9.2.4. Occupational health and safety – risk characterisation

The notified chemical is a slight skin and eye irritant. Adverse effects on the kidney were observed in repeated dose studies.

Reformulation

Exposure and hence the risk of irritation is most likely during the initial transfer of Trilon M liquid to the mixing vessel and during the filling of larger packages of the final formulated products.

For manual operations, exposure to the notified chemical was estimated to be 0.11 – 1.12 mg/kg bw/day. The margin of exposure (MOE) was based on the lowest NOAEL of 82 mg/kg bw/day, derived from a 28-day rat oral study. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Based on the above, the MOE is calculated as 73 -910. Taking into account the worst-case exposure scenario and the severity of systemic effects, the risk of adverse systemic effects using modelled worker data is low for reformulation workers involved in manual operations. The risk is much lower for workers employed in automated formulation processes.

Due to the possible risk of adverse effects (irritancy and systemic) the following personal protective equipment should be worn during reformulation: Protective eyewear, chemical resistant industrial clothing (coveralls) and impermeable gloves.

End Use

Exposure to the notified chemical during dilution was estimated to be 0.028 – 0.28 mg/kg bw/day. The MOE based on the lowest NOAEL of 82 mg/kg bw/day, derived from a 28-day rat oral study is calculated as 292 –2928. Therefore, the risk of systemic effects due using modelled worker data is low for end use workers

Although exposure to the notified chemical can occur during application of the diluted product,

the risk of an adverse reaction is expected to be low due to the low concentration of the notified chemical.

9.2.5. Public health – risk characterisation

Exposure to the notified chemical is expected to be negligible. Therefore the risk to public health is also deemed to be negligible.

10. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

10.1. Hazard classification

Based on the available data the notified chemical is not classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

and

As a comparison only, the classification of notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations, 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Corrosive to metals	1	May be corrosive to metals
Skin corrosion/irritation	3	Causes mild skin irritation
Serious eye damage/eye irritation	2B	Causes eye irritation

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio:

The chemical is not considered to pose a risk to the environment based on its reported use pattern.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

There is Negligible Concern to public health based on its reported use pattern.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of Trilon M Liquid provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd Edition* (NOHSC, 2003). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

The label for Trilon M Liquid provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994). The

accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

CONTROL MEASURES
Occupational Health and Safety

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

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Protective eyewear, chemical resistant industrial clothing (coveralls) and impermeable gloves;

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

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

Environment

Disposal

- The notified chemical should be disposed of to approved licensed facilities. Treatment options include incineration or secure landfill.

12.1. Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Subsection 64(1) of the Act; if
  - the notified chemical is imported as Trilon ES Powder
  - the notified chemical is intended for other uses, e.g. those indicated in the technical bulletin.

or

(2) Under Subsection 64(2) of the Act:
  - if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

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