File No: STD/1044

16 July 2003

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Trisodium Ethylene Diamine Disuccinate

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Director
Chemicals Notification and Assessment

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FULL PUBLIC REPORT

Trisodium Ethylene Diamine Disuccinate

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Procter & Gamble Manufacturing Pty Ltd (ABN 31 098 662 867)
320 Victoria Road
Rydalmere NSW 2116

A.S. Harrison and Company Pty Ltd (ABN 89 000 030 437)
75 Old Pittwater Road
Brookvale NSW 2110

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:
Acute inhalation toxicity
Bioaccumulation data

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
CEC Permit No 556, 557 March, 2003.

NOTIFICATION IN OTHER COUNTRIES
UK 1991

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
tri-sodium N,N'-1,2-ethanediylbis-L-aspartate

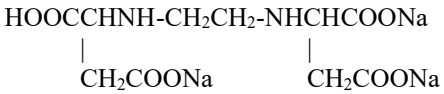
OTHER NAME(S)
[S,S] ethylenediamine disuccinic acid trisodium salt
ss-EDDS
trisodium ethylene diamine disuccinate

MARKETING NAME(S)
Octaquest E30

CAS NUMBER
178949-82-1

MOLECULAR FORMULA
$C_{10}H_{13}N_2O_8.3Na$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
358

SPECTRAL DATA

ANALYTICAL METHOD	IR, UV/VIS, and IR Spectrum
Remarks	IR A broad, ill-defined spectrum was obtained. Peaks at 3300 and 1650 cm^{-1} refer to secondary amines Peak at 1650 cm^{-1} refer to a sodium salt of carboxylic acid. UV/VIS A single peak at around 210 nm was observed. NMR Peaks at: 3.586, 3.571, 3.033, 3.018, 2.993, 2.972, 2.950, 2.935, 2.923, 2.773, 2.745, 2.672, 2.645, 2.596, 2.580, 2.567, 2.552, 2.499, 2.484, 2.471, 2.457 ppm The spectrum showed the sample was impure as it contained low levels of aspartic acid (peaks at approximately 3.8 ppm).
TEST FACILITY	BCO Analytical Services BV (1993a)

METHODS OF DETECTION AND DETERMINATION

ANALYTICAL METHOD	HPLC-UV

3. COMPOSITION

DEGREE OF PURITY
93%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Ethylene dibromide		
CAS No.	106-93-4	*Weight %*	<0.0000001%
Hazardous Properties	>0.1% conc. R45, R20/21/22 (NOHSC, 1999a)		

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)

Chemical Name	aspartic acid		
CAS No.	56-84-8	*Weight %*	3.95%

ADDITIVES/ADJUVANTS
None

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a 34% aqueous solution and reformulated in Australia to produce hair dyes. Finished hair dyes will be exported as well as used in Australia.

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

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

USE
The material will be used in the manufacture of hair dyes as a chelating agent.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, Transport and Storage

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
The notified chemical will be imported by A.S. Harrison and Company Pty Ltd, Brookvale, NSW. It will be stored at Brookvale and transported to Procter & Gamble Manufacturing Pty Ltd, Rydalmere, NSW, as required.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in 210kg plastic (HDPE) drums.

5.2. Operation Description

The notified chemical is imported in 34% aqueous solution. A pre weighed amount of the chemical is poured with fragrance into an open batch tank containing premixed water and other ingredients. The ingredients are mixed. Further ingredients are added to the batch and mixed. The product is filtered through 100-mesh stainless steel filter into the filling lines.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Waterside and Transport Workers	20	8	4
Plant Warehouse/Dispensing	3	2	100
QC Analyst	2	2	10
Manufacturing Operators	3	2	100
Packing Operators of finished goods	10	8	100
QC Analyst of finished goods	2	2	10
Distribution/Warehousing of finished goods	20-30	4	100
Retail of finished good: Supermarket, pharmacy, variety store workers	~ 10,000	1	100

Exposure Details
Categories of workers likely to be exposed to the notified chemical are those involved in the transport of the notified chemical, warehousing of the notified chemical, manufacture of final product, quality control of the finished product, warehousing and distribution of the finished product, and retail of the finished product.

Transport and Warehousing of Notified Chemical
There will be 15 deliveries per annum of the notified chemical. The notified chemical will be imported and stored at Brookvale, NSW. It will be transported to the manufacturing site in Rydalmere, NSW, as required. The notified chemical will be transported in 210kg plastic (HDPE) drums. Exposure during transport and storage is not expected, except in the event of an accident where the packaging is breached.

Hair Dye Manufacture

Blending
Exposure to the chemical may occur when it is pre weighed as an aqueous solution in the dispensary and poured into open batch tank. During the mixing process, splashing is minimised by adjusting the mixer speed. Workers involved in this process are expected to wear overalls, gloves, and eye goggles. Local exhaust ventilation will be employed over the mixing vessel to remove vapours such as ammonia and dusts such as dyes.

Quality Control testing
Samples are taken by pouring from the bottom of the tank into a container and taken to the Quality Control laboratory for testing. Worker involved in this process are expected to wear overalls, gloves and eye goggles.

Dispensing the material
Following the mixing process, product is filtered through 100-mesh stainless steel filter to filling lines. The finished product is packed in consumer packaging. The filling lines are largely automated, with some handling of the packed components.

Maintenance
There is little potential for exposure of the notified chemical for workers involved in the batch tank maintenance. Very little maintenance of the batch tank occurs and work can be done when the tank is empty.

There is some potential for exposure of the notified substance to workers involved in unscheduled maintenance (during use) of the filling lines. Workers will use recommended personal protection equipment during the maintenance work.

Quality Control of finished product
Workers involved in the quality control testing of the finished product will be exposed to the notified chemical in the finished hair dye product. Workers are expected to wear personal protective equipment including gloves, overall and safety glasses.

Transport, warehousing and retail of finished product
Exposure to the notified chemical in the finished product may occur during transport, warehousing and retail, if the packaging is breached.

Salon use of finished product
A proportion of the finished hair dye may be used by salon workers, who may apply the dyes for customers on regular basis. These workers will normally wear gloves when applying hair dyes.

5.4. Release

RELEASE OF CHEMICAL AT SITE
Release of the 'neat' raw material is not expected at the site as the filling line is largely automated. Some release is expected from residual product in the batch tank and packing line, which will be washed out during cleaning. The import drums are rinsed out and the wash water sent to on-site treatment and the drums are recycled. Plant wash water is treated on-site in a primary water treatment system (a dissolved air flotation system), which is a coagulation and flocculation system only. Since the notified chemical is a soluble salt, it is assumed that this system will not remove it from the plant wastewater, but it may chelate metals and precipitate. The concentration in the treated wastewater sent to the city sewage system is expected to be a maximum of 0.00024% w/w.

Spills are very rare but there are procedures in place to clean up should they occur. The target for bulk production losses including residues in the production system and import drums is less than 2% of the import volume.

RELEASE OF CHEMICAL FROM USE
The total quantity of the notified chemical imported annually will be incorporated into a hair colorant product and (assuming the total hair dye production is used in Australia) will almost completely be released to the environment through washing of hair. Residues in the consumer product containers,

which will be disposed of to landfill via domestic garbage collection are expected to be up to 190 kg.

5.5. Disposal

The notified chemical will ultimately be disposed of in either the sewer (major) or landfill.

5.6. Public exposure

The notified chemical will be used in hair dyes products. The maximum concentration of the product will be 2% by weight. The product is mixed with hydrogen peroxide in a 1:1 ratio prior to use. Thus the typical final consumer exposure product will contain the notified chemical at 1% by weight. The hair dye products are normally used once every 4-6 weeks.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa The chemical is white solid. In solution at 34% it is a colourless liquid with no odour.

Melting Point 311ºC (decomposition)

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.

Remarks The substance does not melt, but decomposes at temperatures about 311ºC.

The determination of the melting point was preformed by differential thermal analysis the test substance was heated at a constant temperature rate, while the heat flux of the sample was measured.

The test substance was heated up to 400 ºC. No significant heat effects were observed up to about 311 ºC. Above 311 ºC an exothermic effect was observed, it likely that this due to the reaction or decomposition of the test substance. During the preliminary and main study, the sample lost 36% and 45% of its mass, respectively. The colour of the sample changed from white to black. After the experiments, the particles were grouped in the form of a sphere.

TEST FACILITY RCC Notox (1993a)

Density 1630 kg/m^3 at 20ºC $\pm$ 1ºC

METHOD OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.

Remarks The density was determined by means of a gas comparison pycnometer. The test procedure was repeated using two independent test substance samples, with each sample measured at least in duplicate (until deviation in density <0.02 g/cm^3).

TEST FACILITY RCC Notox (1993b)

Vapour Pressure 0.0019 $\pm$ 0.0001 kPa at 25ºC

METHOD OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.

Remarks The Static Technique was used. The sample vessel was cleaned, dried and partly filled with the test substance and once attached to the measuring set-up, was evacuated for about 30 minutes at 36ºC to remove volatile impurities. A total of 98 measurements were made at 36.25ºC, 30.08ºC, and 24.55ºC. The test substance was considered to show ideal behaviour, thus the vapour pressure curve was derived according to method described by Clarke and Glew (in Trans. Faraday Soc., 62,539, 1966). The maximum deviation between the fit and data points was less than 3%, therefore, a value of 0.1 Pa (5%) was considered to be a reasonable value for the uncertainty in the final result.

The notified substance is volatile (Mensink *et al* 1995)
The Henry's Law constant (H) calculated from the molecular weight, the measured water solubility, and the estimated vapour pressure according to the following equation: H = MW (g/mol) x Vapour Pressure (Pa)/Water Solubility (mg/L) is
H = 0.68 Pa m^3/mol. Accordingly, the test substance is slightly volatile from water (Mensink e*t al*. 1995).

TEST FACILITY RCC Notox (1993c)

Water Solubility ≥1000 g/L(room temperature)

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.

Remarks The sample was stirred overnight with double distilled water in a 1:1 (w/v) ratio at room temperature by means of visual observation. The resultant phase observed visually was determined to be miscible. Based on the HPLC analysis the water solubility was determined to be ≥ 1000 g/L.
The test substance is readily soluble in water (Mensink *et al.* 1995).

TEST FACILITY RCC Notox BV (1993d)

Fat Solubility ≤ 0.04 mg/100 g HB 307 at 37°C

METHOD OECD TG 116 Fat Solubility of Solid and Liquid Substances and EC Directive 84/449/EEC A.7 Fat Solubility.

Remarks A preliminary study was conducted stirring 25 mL liquefied and mixed standard fat and 406 mg of the test substance overnight at 37°C and determining the test substance concentration using HPLC. In the first main study, two groups of 4 flasks each containing 25 mL liquefied and mixed standard fat and 400 mg of the test substance were placed on magnetic stirring devices in thermostatically controlled water baths at 30°C (Group I) and 50°C (Group II) and stirred for 1 hour. Thereafter, 2 flasks in each group were stirred for 3 hours and the remaining 2 for 24 hours in water baths at 37°C ± 0.5°C and the concentration of the test substance in the fat phases was determined using HPLC.

Due to the significant differences observed in the results of the main study, an additional test was conducted using smaller amounts of the test substance (100 mg in each of 4 flasks with 50 mL of liquefied and mixed standard fat). A similar procedure to that of the main study was used except the stirring period at 37°C ± 0.5°C was 48 hours. A blank test was performed in each of the main studies.
`

Significant differences between all measurements in the 2 main tests and a higher concentration in the fat phase after 3 centrifugation steps in comparison to that after 2 steps (also in the preliminary test) were observed. It was assumed that a small amount of undissolved test substance was still present in the pretreated fat phases. Therefore, the fat solubility was considered to be equal to or smaller than the largest concentration analysed.

The test substance has a low fat solubility.

TEST FACILITY RCC Notox (1993h)

Hydrolysis as a Function of pH

Remarks The submission noted that this was not determined as the data indicated that the test substance is readily biodegradable and the molecule does not contain moieties with the potential to hydrolyse.

Partition Coefficient (n-octanol/water) log Pow at 20ºC < -4.7 (estimated)

METHOD OECD TG 107 Partition Coefficient (n-octanol/water), Shake - flask method.
EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks Two preliminary tests were carried out. In the first test, 25 mL n-octanol was stirred overnight with 527 mg of the test substance at room temperature and the resulting concentration was determined using HPLC. The partition coefficient was estimated to be $\leq 2x10^{-6}$ (log $P_{ow} \leq -5.7$).

In the second preliminary test, 5 mL of a stock solution (250 mg of test substance dissolved in 50 mL double distilled water saturated with n-octanol) was shaken with 5 mL of n-octanol saturated with water for 5 minutes. Once phase separation was achieved by centrifugation for 10 minutes at 3500 g and 20ºC, the concentration of the test substance in each phase was determined using HPLC. The partition coefficient was calculated based on the test substance concentrations in the preheated water and n-octanol phases to be $<2x10^{-5}$ (log $P_{ow} < -4.7$).
As the Shake-flask Method is only applicable for substances with log $P_{ow} > -4$, a main study was not performed. However, based on the preliminary results the partition coefficient was estimated to be $< 2x10^{-5}$ (log $P_{ow} < -4.7$).

The low log P_{ow} is consistent with the high water solubility and indicates a low affinity for the organic phase and component of soils and sediments

TEST FACILITY RCC Notox (1993e)

Adsorption/Desorption – screening test log K_{oc} = 3.60 to 4.28 at room temperature.

METHOD OECD Guideline for Testing of Chemicals No.106 (Screening Version): "Adsorption – Desorption", adopted May 12 1981.

Remarks A test solution of 25.1 x 10^{-3} g/L in 0.01 M $CaCl_2$ was prepared. The soils were equilibrated with twice their weight of water before use. An amount of 5 g of each soil and an accurately weighed amount of 10 mL of double distilled water were added to 50 mL vials (in triplicate) and gently tumbled for 5 days at room temperature. The vials were then centrifuged for 10 minutes at 170 g and 20ºC and most of the supernatants were removed and weighed.

In the adsorption screening test, for each soil type, accurately weighed amounts of 25 mL test solution was added to two of the equilibrated soil samples and 25 mL of 0.01 M $CaCl_2$ was added to a third equilibrated soil sample. A blank test was performed without soil. All vials were gently tumbled for 16 hours at room temperature and then centrifuged for 10 minutes at 170 g and at 20ºC.
As the adsorption to all three soils was greater than 25%, the desorption screening test was performed with the soil samples from the adsorption test. An amount of 25 mL of fresh 0.01 M $CaCl_2$ was added to each soil sample and gently tumbled for 16 hours at room temperature. The vials were then centrifuged for 10 minutes at 170 g and at 20ºC.
Supernatants from both the adsorption and desorption test vials were weighed and the concentration of the test substance in the resultant aqueous phases were determined using HPLC.

Soil Type	*Clay Content (%)*	*Organic Matter Content (%)*	*pH*	*% Adsorption*	*% Desorption*	*Koc*
Strong silty sand	6.0	1.4	4.0	98	< 4	1.9×10^4
Strong sandy loam	13.6	1.8	7.5	83	< 3	1.6×10^3
Weak sandy loam	12.1	1.1	6.6	89	<4	4.0×10^3

Remarks The high K_{oc} values are inconsistent with the high water solubility of the test substance and indicate the mobility of the notified chemical in soil as being low. The high adsorption is likely to be due to the anionic character of the chemical and its chelating ability with metals, rather than its sorption to organic matter.

TEST FACILITY RCC Notox (1993f)

Adsorption/Desorption – main test Adsorption of the test substance to activated sludge is low.

METHOD OECD TG 106 'Adsorption – Desorption' and Proctor & Gamble Company protocol "Measurement of sorption to activated sludge"

Remarks Method Activated sludge was obtained from the control and test units of a previous test conducted on the test substance (activated sludge simulation test summarised in 8.1.2). The Mixed Liquor Suspended Solids (MLSS) levels for the blank and the test unit were 1.89 and 2.35 g/L, respectively at 105°C. The MLVSS levels at 550°C were 1.53 and 1.92 g/L and the conductivity values were 480 and 531 µS/cm for the blank and test units, respectively. Effluent from the same unit was used as overlying water for the desorption test.

The test was performed in duplicate at 2 concentrations of the non-labelled test substance (0.1 and 1.0 mg/L). The labelled material was spiked at concentrations of ± 2 x 10^6 dpm (=±0.07 mg/L).

The test containers were prepared as follows:
Unacclimated sludge
Units 1 & 2 –100 mL activated sludge + 0.05 mL labelled test material + 0.1 mL stock solution of the non-labelled test material.
Units 3 & 4 –100 mL activated sludge + 0.05 mL labelled test material + 1.0 mL stock solution of the non-labelled test material.
Acclimated sludge
Units 5 & 6 –100 mL activated sludge + 0.05 mL labelled test material + 0.1 mL stock solution of the non-labelled test material + 1 mL sodium azide (NaN_3) solution
Units 7 & 8 –100 mL activated sludge + 0.05 mL labelled test material + 1.0 mL stock solution of the non-labelled test material + 1 mL sodium azide solution

Adsorption was tested by aerating (3 hours) and centrifuging 100 mL aliquots of test solution. The test substance concentrations in the 2 mL aliquots of supernatant were measured with a Liquid Scintillation Counter (LSC). The remaining supernatant was discarded and replaced by fresh water. The solids were resuspended and the test substance concentration on the solids was measured.

Desorption was tested by aerating (3 hours) and centrifuging the suspensions. The test substance concentrations in the 2 mL aliquots of supernatant were measured with a Liquid Scintillation Counter (LSC). The remaining supernatant discarded and replaced by deionised water. The solids were resuspended and the test substance concentration on the solids was measured.

Sludge Type	*Test substance concentration (mg/L)*	*K_d Adsorption*	*K_d Desorption*

Activated	0.1	40	421
	1.0	37	334
Inhibited Sludge	0.1	71	1034
	1.0	90	1298

Remarks Recovery was between 90 and 110% for the test with unacclimated activated sludge (units 1 to 4) but was very low for the tests with acclimated sludge (units 5 to 8). Adding sodium azide to the sludge (at 200 mg/L) did not prevent biodegradation of the test substance. Further testing demonstrated the loss of test substance was due to biodegradation by the acclimated biomass despite the addition of sodium azide.
Results The results also showed that the test substance has no tendency to sorb onto the sludge and desorption was high. This is contradictory to the results of the soil adsorption/desorption study summarised above. The reason for the high adsorption observed in the soils study could be possibly due to the higher soil to water ratio and longer equilibrating time when compared to low level of suspended solids used in this study.

TEST FACILITY Lisec (1993a)

Dissociation Constant pKa1 = 2.4
pKa2 = 3.86
pKa3 = 6.83
pKa4 = 9.82

Remarks A test report was not provided. The above values were stated as the dissociation constants for the chemical with no reference to the source.

Particle Size

Remarks The notified chemical is only used in Australia in solution form.

Surface Tension 73.3 mN/m at 20°C

METHOD OECD TG 115 Surface Tension of Aqueous Solutions and EC Directive 92/69/EEC A.5 Surface Tension.

Remarks The surface tension was measured using a Krüss tensiometer type 6 based on the ring method of Lecomte de Noüy. An aqueous solution was prepared by dissolving 50.4 mg of the test substance in 50 mL double distilled water. The measurement vessel was cleaned and rinsed before being filled with the test solution and placed in a water bath at 20°C on the mobile sample table. All the instrument readings were multiplied by the calibration factor determined by calibrating the apparatus using double distilled water.
The results indicate that the test substance is not surface active.

TEST FACILITY RCC Notox (1993i)

Flash Point

Remarks Not determined as not flammable.

Flammability Limits

METHOD EEC Directive 92/69/EEC Annex V Part A.3 Methods for the determination of physico-chemical properties A-10 Flammability (solids)"

Remarks The test substance could not be ignited and coloured black with the ignition source.

TEST FACILITY RCC Notox (1993j)

Autoignition Temperature

Remarks Not determined as not flammable.

Explosive Properties

Remarks The chemical does not contain any chemically unstable or highly energetic groups which might lead to an explosion

Reactivity

Remarks Expected to be stable under normal environmental conditions.

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral LD50 >2000 mg/kg bw	low toxicity
Rat, acute dermal LD50 >2000 mg/kg bw	low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation - non-adjuvant test.	no evidence of sensitisation.
Rat, oral repeat dose toxicity - 90 days.	NOEL = 300 mg/kg bw
Genotoxicity - bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mouse lymphoma	non genotoxic
Genotoxicity – in vitro Chinese Hamster Ovary Cells	genotoxic
Genotoxicity – in vivo cytogenetic	non mutagenic
Developmental and reproductive effects	Developmental toxicity only in the presence of maternal toxicity.

7.1. Acute toxicity – oral

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 401 Acute Oral Toxicity – Limit Test.
EC Directive 92/69/EEC B.1 Acute Toxicity (Oral) – Limit Test.

Species/Strain Rat/ Wistar (SPF)

Vehicle Bi-distilled water

Remarks - Method No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 /sex	2000	0/10

LD50 >2000mg/kg bw

Signs of Toxicity No clinical signs of toxicity were observed during the observation period. Body weight of the animals was not adversely affected.

Effects in Organs The macroscopic examination of the animal at study termination revealed no organ abnormalities.

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

TEST FACILITY RCC (1993a)

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 (SPF)

Vehicle	Bi-distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	Females at the start of treatment were 12 weeks old rather than the specified 10 weeks old.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5/sex	2000	0/10

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	No local skin alterations at the application site were observed
Signs of Toxicity - Systemic	No systemic clinical signs of toxicity were observed. No test substance related effects on the body weight of animals were observed during the observation period. A slight loss of body weight observed in two female animals during the first observation period was considered a consequence of the semi-occlusive dressing.
Effects in Organs	The macroscopic examination at study termination revealed no organ abnormalities.

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

TEST FACILITY RCC (1993b)

7.3. Acute toxicity – inhalation

No test reports were 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 (SPF)
Number of Animals	3
Vehicle	Not used
Observation Period	1, 24, 48 and 72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Remarks - Results	No local signs of erythema or oedema were reported.

CONCLUSION The notified chemical is non-irritating to skin.

TEST FACILITY RCC (1993c)

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	7 days
Remarks - Method	The observation period was extended to 7 days to examine the persistence of observed reactions.

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	0.33	0.33	1	1	72 hr	0
Conjunctiva: chemosis	0	0	0	0	0	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results: Slight redness of nictitating membrane and a slight discharge was observed in two male animals one hour after administration. The discharge was not observed in the males after 24 hours. The redness was not observed in the males after 48 hours. No abnormalities were observed after 48 hours in the males. Slight ventral redness of the nictitating membrane was observed in the females after 24 hours and 48 hours. Slight ventral redness of the conjunctive was observed at 72 hours. No abnormalities persisted at 7 days in the females any animals.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: RCC (1993d)

7.6. Skin sensitisation

7.6.1. Skin sensitisation (pure notified chemical)

TEST SUBSTANCE: Notified chemical.

METHOD: OECD TG 406 Skin Sensitisation -Modified Buehler.

Species/Strain: Guinea pig/Hartley derived albino

PRELIMINARY STUDY: Maximum Non-irritating Concentration: undiluted
Topical application

MAIN STUDY

Number of Animals: Test Group: 20 Control Group: 10

INDUCTION PHASE: Induction Concentration: undiluted (0.5 g)
topical application

Signs of Irritation: Not stated

CHALLENGE PHASE

1st challenge: topical application: undiluted (0.5 g)

2nd challenge: topical application: undiluted (0.5 g)

Remarks - Method: Exposure method was occlusive chamber. 1-chloro-2,4-dinitrobenzene was used as positive control in a separate group.

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	Undiluted	1/19	0/19	0/19	0/19
Control Group	Undiluted	0/10	0/10	0/10	0/10

Remarks - Results: One animal was found dead on day 20 of the study. The cause of death could not definitely be determined. Following the 1st challenge, a dermal

score of 1 was observed in one animal at 24 hour. Dermal scores in the remaining test and control animals were no reaction to slight patchy erythema (Grade ±) at similar incidence. Following rechallenge, no reaction to slight patchy erythema was observed, in both groups.

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

TEST FACILITY SLS (1995)

7.6.2. Skin sensitisation (50% aqueous solution))

TEST SUBSTANCE Notified chemical.

METHOD OECD TG 406 Skin Sensitisation - Modified Buehler.
EC Directive 96/54/EC B.6 Skin Sensitization - Buehler.

Species/Strain Guinea pig/Himalayan Spotted
PRELIMINARY STUDY Maximum Non-irritating Concentration: 50%
MAIN STUDY
Number of Animals Test Group: 20 Control Group: 10
INDUCTION PHASE Induction Concentration: 50%
Signs of Irritation None observed
CHALLENGE PHASE
1st challenge topical application: 50%
Remarks - Method Exposure method was occlusive chamber.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	50%	0/20	0/20
Control Group	50%	0/10	0/10

Remarks - Results The chemical at 50% dilution caused no positive skin reactions.

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

TEST FACILITY RCC (1993e)

7.6.3 Skin sensitisation – human volunteers

TEST SUBSTANCE Notified chemical (as a 5% aqueous solution)

METHOD
Study Design Human repeat insult patch test
Study Group 125 subjects started the study, 111 subjects completed the study. Male and female subjects were used.
Vehicle Distilled water
Induction Procedure 0.3ml of 5% solution of the test substance was applied via occlusion patch for 24 hours, three times a week for three weeks. The patch was applied to the arm or back. Grading of the test site occurred 48 hours after patch application during the induction phase or 72 hours, after patch application, after a weekend.
Rest Period 12-20 days

Challenge Procedure	A challenge patch was applied to both the original site and a similar site on the opposite side of the body and worn for 24 hours. Grading after the challenge occurred at 48 and 96 hours.
Remarks - Method	

RESULTS

Remarks - Results	None of the 111 subjects that completed the study exhibited a response during the challenge phase.

CONCLUSION	A human repeat insult patch test was conducted using the notified chemical diluted with distilled water to 5% under occlusive dressing. The notified chemical was non-sensitising under the conditions of the test.
TEST FACILITY	Hill Top Research (1993)

7.7. Repeat dose toxicity

7.7.1 Repeat dose toxicity – 28 day

TEST SUBSTANCE	Notified chemical (as a 42.3% aqueous solution)
METHOD	In house protocol: mineral balance 28 day oral toxicity study in male rats
Species/Strain	Rat/Wistar
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: none
Remarks - Method	The study also assessed the effects of the test substance on dietary minerals when administered for 28 days. During days 25-28 urine and faeces samples were collected and the mineral content of these determined. Minerals were also determined for the liver, femur, sternum, kidney and serum.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg bw/day*		*Mortality*
		Nominal	*Actual*	
I (control)	5 males	0	0	0
II (low dose)	5 males	50	59.15	0
III (low mid dose)	5 males	150	176.08	0
IV (high mid dose)	5 males	300	340.25	0
IV (high dose)	5 males	400	469.66	0

Mortality and Time to Death

There were no test substance related deaths.

Clinical Observations

No clinical signs of toxicity were observed.

At 300 mg/kg bw/day and 400 mg/kg bw/day a slight reduction in food consumption was noted during some recording periods. The reduction was not dose related. Relative food consumption was overall similar or even higher in these two groups in comparison with the control.

Water consumption was not affected by the treatment with the test substance.

No statistically significant changes in body weight or body weight gain in any of the treatment groups were observed. However, body weight and body weight gain tending to decrease with increased dose. A small decrease in body weight and body weight gain compared to control was observed at 50 and 150 mg/kg bw/day. A larger decrease in body weight and body weight gain was observed in the 300 mg/kg bw/day and 400 mg/kg bw/day treatment group.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No changes of toxicological significance were noted at the termination of treatment. All differences in haematology, clinical biochemistry, and urinalysis parameters were considered incidental and of normal variation for rats of the strain and age.

Effects in Organs

No test substance related or statistically significant changes in absolute or relative organ weights were noted.

No abnormal macroscopic findings were noted at terminal necroscopy. No test substance related abnormal microscopic findings were observed. The microscopic findings did not vary significantly in incidence or severity between the control and treated groups. All the observed abnormal findings were spontaneous and within the normal range of background morphologic alterations.

Remarks – Results

A dose related increase in urinary output of Zn was observed. Increased urinary output of Cu and Mg were observed at 300 mg/kg bw/day and 400 mg/kg bw/day, and 400 mg/kg bw/day, respectively.

The increased urinary output was compensated by a decrease faecal output of the respective minerals. For Zn, the decreased faecal output attained a statistical significance. Due to the compensatory change, the total output for these minerals remained unchanged. In addition to the effect on Zn elimination, a statistically significant reduction in Zn serum levels were observed at 300 mg/kg bw/day, and in Zn kidney levels at 50 and 150 mg/kg bw/day. These effects being test substance related cannot be ruled out, considering the changes in Zn elimination.

A statistically significant decrease in Fe output and liver content and decreased P output were noted at 300 mg/kg bw/day but not at 400 mg/kg bw/day. In the absence of dose relationship these findings were considered incidental

The P content of the femur was statistically significantly reduced at 400 mg/kg bw/day. No changes in P output, or sternum and liver P levels were observed. Hence, it is doubtful if this is treatment related.

CONCLUSION

The No Observed Effect Level (NOEL) could not be determined for this study due to increased Zn urinary excretion at all doses.

TEST FACILITY RCC (1995b)

7.7.2. Repeat dose toxicity – 90 day

TEST SUBSTANCE Notified chemical (as a 42.3% aqueous solution)

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

Species/Strain Rat/Wistar

Route of Administration Oral –diet

Exposure Information Total exposure days: 90 days;
Dose regimen: 7 days per week;
Post-exposure observation period: 4 week

Remarks - Method

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg bw/day*		*Mortality*
		Nominal	*Actual*	
I	10/sex	0	0	0
II (low dose)	10/sex	50	50.75 (male) 49.78 (female)	0

III (low mid dose)	10/sex	300	300.48 (male) 302.01 (female)	0
IV (high mid dose)	10/sex	700	722.21 (male) 703.38 (female)	0
V (high dose)	10/sex	1000	1000.45 (male) 1004.04 (female)	0
VI (control recovery)	10/sex	0	0	0
VII (high dose recovery)	10/sex	1000	1000.45 (male) 1004.04 (female)	0

Mortality and Time to Death

No test substance related deaths were noted up to and including the highest dose level of 1000 mg/kg bw/day

Clinical Observations

Food consumption:

A statistically significant reduction in food consumption was noted during the first week of treatment in females of all dose groups and males in treated at 300, 700 and 100 mg/kg bw/day. This was attributed to the palatability of the test substance in diet and not of toxicological significance. However, in the second part of the treatment period, a reduction of food consumption was noted in the males treated at 1000 mg/kg bw/day, this was test substance related. The reduction was compensated with an increase in food consumption in the recovery period.

Water consumption

A slight increase (not statistically significant) in water consumption was observed in the males treated at 700 and 1000 mg/kg bw/day. This finding was considered to be due to nature of the test substance mixed in the diet, and not of toxicological significance. During the recovery period, a reduction in water consumption (not statistically significant) was observed the males in the 1000 mg/kg bw/day treatment group compared to the respective control.

Body weight

At 1000 mg/kg bw/day a test substance related retardation of body weight gain was observed in both male and female animals. In the recovery period, the body weight gains of the control and high dose animals developed towards the same body weight curve. While males treated at 700 mg/kg bw/day had a slight retardation of the body weight gain during the last three weeks of treatment, this was considered to be a reaction of treatment with the test substance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

No toxicological significant haematological changes were observed after weeks 4 and 13 of treatment and the end of the recovery period. However, a few minor, statistically significant changes were observed in the treated rats during treatment. These were as follows:

- Slightly decreased hematocrit in male at 1000 mg/kg bw/day after 4 weeks;
- Slightly decreased mean corpuscular haemoglobin and corpuscular haemoglobin concentration indices in females at 1000 mg/kg bw/day after 4 weeks and in the males and females at 1000 mg/kg bw/day after 13 weeks;
- Slightly increased platelet count in females in 1000 mg/kg bw/day after 13 weeks;
- Slightly reduced thromboplastin time in females at 300 mg/kg bw/day and 1000 mg/kg bw/day after 4 weeks;
- Slightly prolonged thromboplastin time in males at 300 mg/kg bw/day, 700 mg/kg bw/day and 1000 mg/kg bw/day after 13 weeks.

These findings were no longer statistically significant at the end of the treatment –free recovery period.

Clinical Biochemistry
The following statistically significant effects were recorded

- Slightly increased bile acid concentration in both sexes at 1000 mg/kg bw/day after 13 weeks;
- Slightly decreased total cholesterol concentration in males at 700 mg/kg bw/day after 4 and 13 weeks;
- Slightly decreased triglyceride concentrations in males in 300, 700, 1000 mg/kg bw/day after 13 weeks;
- Slightly increased aspartate aminotransferase activity in males at 700 mg/kg bw/day after 13 weeks and, 1000 mg/kg bw/day after 4 weeks;
- Slightly increased alanine aminotransferase activity in males at 1000 mg/kg bw/day after 4 weeks;
- Slightly decrease alkaline phosphates activity in males at 700 mg/kg bw/day and males and females at 1000 mg/kg bw/day after 4 and 13 weeks;
- Slightly decreased gamma-glutamyltransferase activity in both sexes of group 700 and 1000 mg/kg bw/day after 4 weeks and increased in males at 1000 mg/kg bw/day after 13 weeks;
- Slightly increased calcium and phosphorous concentration in females at 1000 mg/kg bw/day after 13 weeks;
- Slightly increased potassium concentration in males at 1000 mg/kg bw/day after 4 and 13 weeks;
- Slightly increased chloride concentration in females of groups 700 and 1000 mg/kg bw/day after 4 weeks;
- Slightly increased albumin concentration in males at 1000 mg/kg bw/day and females at 300, 700, and 1000 mg/kg bw/day after 4 and 13 weeks;
- Slightly increased total protein concentration in females at 700 and 1000 mg/kg bw/day after 13 weeks; and
- Slightly decreased globulin concentration in males of all dose groups after 4 weeks

These changes were attributed to metabolic adaptation, and were not considered to be of any toxicological significance. These changes were no longer statistically significant and comparable to the control at the termination of the treatment recovery period. The decrease in globulin concentrations in males was considered not treatment related, but related to the higher values in the control groups.

Urinalysis
No changes of toxicological significance was observed after 4 and 13 weeks nor at the end of the treatment – free period.

Duration of oestrous cycles
There was no adverse effect on the duration of the oestrous cycles.

Sperm analysis
No effects on the sperm motility or concentration were observed in all dose groups at the end of treatment and the end of the recovery period.

At 1000 mg/kg bw/day, there was a decrease of normal sperm and an increase in sperm with head only at the end of the treatment period. While at the end of the recovery period had a higher percentage of normal sperm when compared to data obtained after the treatment period. However one male had a very low percentage of normal sperm and an increase of sperm with head only. While a slight increase in the number of sperm with abnormal hook was also recorded after the treatment free period. This indicated that treatment with a target dose of 1000 mg/kg bw/day had an adverse effect on sperm, which was largely but not completely reversed after a 4 week recovery period.

Determination of minerals elements in plasma
Plasma from allocated animals in the control and high dose group was analysed for magnesium, zinc, iron, manganese, and copper. At 1000 mg/kg a statistically significant reduction of the zinc content in plasma was noted in both male and female animals. While a statistically significant reduction in copper and magnesium, contents were observed in male animals.

Effects in Organs
No test substance related effects on the absolute or relative organ weights assessed were noted.

No test substance related macroscopic abnormalities were noted. Microscopic treatment related changes were observed in the testes and exocrine pancreas. In the pancreas, there was an increased incidence and severity of single cell death in the 700 mg/kg bw/day and 1000 mg/kg bw/day. Fatty infiltration of the exocrine pancreas was noted in the 700 mg/kg bw/day and 1000 mg/kg bw/day groups at the end of the treatment. This was still present in the 1000 mg/kg bw/day rats following the recovery period. In the testes, a minimal degree of atypical residual bodies were recorded at 1000 mg/kg bw/day males at the end of the main study.

Remarks – Results
Effects on sperm morphology and fatty infiltration of the exocrine pancreas persisted through the recovery period.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 300 mg/kg bw/day in this study, based on body weight changes, changes in sperm morphology, plasma mineral levels, and microscopic changes in the testes and pancreas, at higher doses.

TEST FACILITY RCC(1995a)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (as a 34% aqueous solution)
METHOD	in house protocol (supplied) – similar to OECD TG 471
Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, TA100 *E. coli*: WP2 uvrA (pKM101), WP2 (pKM101).
Metabolic Activation System	Arcolor 1254 induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 33 - 5000 μg/plate b) Without metabolic activation: 33 - 5000 μg/plate
Vehicle	Water
Remarks - Method	Based on a dose-range finding study 5000 μg/plate was the maximum dose used in the mutagenicity study. At this dose, neither precipitation nor appreciable toxicity was observed. The mutagenicity of test substance was evaluated using the plate incorporation method. The mutagenicity of the test substance was confirmed using the pre incubation method. All dose levels of the test substance, vehicle controls, and positive controls were plated in triplicate.

RESULTS

Remarks - Results	In the mutagenicity and confirmatory assays, no significant dose related increase in the number of revertants was seen for any strain either in the presence or absence of metabolic activation. Neither precipitation nor appreciable toxicity was observed. Appropriate positive controls were used and in all case lead to a large increase in revertant numbers, confirming the sensitivity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Microbiological Associates (1993a)

7.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (as a 34% aqueous solution)

METHOD	In house protocol (supplied) - TK+/- Mouse Lymphoma Assay
Cell Type/Cell Line	Mouse lymphoma/L5178Y TK +/-
Metabolic Activation System	2:1 Aroclor-1242 and Aroclor-1254 mixture induced rat liver S9 fraction.
Vehicle	Water
Remarks - Method	

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	2765 – 5028 μg/L	4 hours	24-48 hours	10-12 days
Test 2	2765 – 5028 μg/L	4 hours	24-48 hours	10-12 days
Present				
Test 1	2514 – 4776 μg/L	4 hours	24-48 hours	10-12 days
Test 2	2765 – 5028 μg/L	4 hours	24-48 hours	10-12 days

RESULTS

Remarks - Results

Dose range finding study: 0.5 – 5028 μg/mL
Without S9: 4% toxicity at 5028 μg/mL.
With S9: 41% toxicity at 5028 μg/mL

Experiment 1
Without S9
Suspension Growth range: 40% - 73%
Total Growth range: 35% - 65%

With S9
Suspension Growth range: 10% - 73%
Total Growth range: 10% - 96%

None of the activated or non-activated cultures cloned exhibited a mutant frequency which was at least two times the mean mutant frequency of the solvent controls. No dose dependent response was noted in the treated cultures.

Experiment 2
Without S9
Suspension Growth range: 64% - 127%
Total Growth range: 62% - 122%

With S9
Suspension Growth range: 27% - 66%
Total Growth range: 25% - 73%

None of the activated or non-activated cultures cloned exhibited a mutant frequency which was at least two times the mean mutant frequency of the solvent controls. No dose dependent response was noted in the treated cultures.

Appropriate positive controls were used and resulted in large increases in mutant frequency, confirming the sensitivity of the test system.

CONCLUSION

The notified chemical was not mutagenic to L5178Y TK +/- treated in vitro under the conditions of the test.

TEST FACILITY

Microbiological Associates (1993b)

7.10. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical (as a 34% aqueous solution)
METHOD	In house protocol (supplied) – in vitro cytogenetic assay
Cell Type/Cell Line	Chinese Hamster Ovary /(CHO-K_1)
Metabolic Activation System	Arcolor 1254 induced rat liver S9 fraction
Vehicle	Deionised water
Remarks - Method	Dose range finding study: maximum concentrations were 5000 μg/mL for the 6 and 18 hour treatment times, and 1250 μg/mL due to the severe toxicity for the 42 hour treatment time

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	79, 157, 313*, 625*, 1250*, 2500, 5000	6 hours	18 hours
Test 1	79, 157, 313, 625, 1250, 2500, 5000	18 hours	18 hours
Test 1	5*, 10*, 20*, 40, 79, 157, 313, 625, 1250	42 hours	42 hours
Test 2	79, 157*, 313*, 625*, 1250, 2500, 5000	6 hours	18 hours
Test 2	79, 157, 313*, 625*, 1250*, 2500, 5000	18 hours	18 hours
Test 2	5, 10*, 20*, 40*, 79, 157, 313, 625, 1250	42 hours	42 hours
Present			
Test 1	79, 157*, 313*, 625*, 1250, 2500, 5000	6 hours	18 hours
Test 2	79, 157*, 313*, 625*, 1250, 2500, 5000	6 hours	18 hours

*Cultures selected for metaphase analysis.

RESULTS

Remarks - Results

Test 1
The test substance did not induce a significant increase in the structural aberrations in this test. In the 18-hour treatment study, there were no scoreable metaphase cells. In the 42-hour treatment, study there was a statistically significant increase in numerical aberrations at 20μg/mL ($p<0.025$, Fisher's exact test) and dose response in numerical aberrations ($p<0.05$, Cochran-Armitage test).

Test 2
The test substance did not induce a significant increase in structural or numerical aberrations in 6 hour and 18 hour treatments ($p\geq0.025$, Fisher exact test). There was a statistically significant increase in structural aberrations at 40 μg/mL in the 42 hour treatment study ($p<0.025$, Fisher exact test) and statistically significant dose response ($p<0.05$, Cochran-Armitage test). The increase in structural aberrations at this dose was within acceptable range of the historical control value and hence the increase was not considered biologically relevant. In the 42 hour treatment study there was also a statistically significant increase in numerical chromosome aberrations in 20 and 40 μg/mL ($p<0.025$, Fisher exact test) in and a statistically significant dose response ($p<0.05$, Cochrane-Armitage test)

Appropriate positive controls were used and resulted in large increases in structural aberrations, confirming the sensitivity of the test system.

CONCLUSION The notified chemical was clastogenic to Chinese Hamster Ovary (CHO-K_1) treated in vitro under the conditions of the test.

TEST FACILITY Microbiological Associates (1993c)

7.11. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical (as a 42.3% aqueous solution)
METHOD	In house protocol (supplied) - in vivo cytogenetic assay
Species/Strain	Rat/Sprague-Dawley
Route of Administration	Oral – gavage
Vehicle	Distilled water
Remarks - Method	Cell Cycle Kinetic Study The sacrifice time was based on average generation times determined in a bone marrow cell cycle kinetic study. In the cell cycle kinetic study, two groups of three male rats were dosed by oral gavage with the 2000 mg/kg.bw of the test substance or vehicle. Approximately two hours prior to this each animal was subcutaneously implanted with a 100mg 5'-bromodeoxy-uridine (BrdU) 3-day time release pellet. The animals received a 1 mg colchicine/ kg bw two hours before bone marrow collection. The animals were sacrificed at 24 hours after BrdU implantation. Bone marrow preparation were analysed microscopically and number of metaphase cells in first, second and third or greater division was determined in a total of 100 cells. The average generation time was then calculated. The average generation time was calculated to be 12.4 hours for the control and the 12.4 hours for the test substance. Based on these finding, bone marrow collection times were selected to be 10 hours for the evaluation for structural damage in M_1 cells, 16 hours for structural damage in M_1 and for numerical changes in M_2 cells and 28 and 40 hours for numerical changes in M_2 cells. Cytogenetic Assay Animals were assigned to sixteen groups of five males and five females. Two hours prior to dosing, BrdU pellets were subcutaneously implanted into the animals. The test substance, vehicle, or positive controls were administered by oral gavage at a constant volume of 10 mL/kg.bw. Where possible a minimum of 100 first division metaphase spread containing 42+1 centromeres were examined from each animal in the 10 and 16 hour sacrifice time points and were scored for chromatid-type and chromosome type aberrations. The mitotic index was recorded as the percentage of cells in mitosis based upon 500 cells counted. The percent polypoid and endoreduplicated cell were assessed by analysing 100 first division mitotic cells. Where possible the 100 second division metaphase spreads per animal in 16, 28 and 40 hour sacrifice time points were counted an recorded for total chromosome number and endoreduplication.

Group	*Number and Sex of Animals*	*Dose Mg/kg bw*	*Sacrifice Time hours*
1	5/sex	200	10, 16, 28, 40
2	5/sex	670	10, 16, 28
3	5/sex	2000	10, 16, 28
CP	5/sex	20	16
VS	5/sex	6	28

CP=cyclophosphamide. VS vinblastine sulphate.

RESULTS	
Doses Producing Toxicity	One female at the high dose died during the study period. Clinical signs of toxicity included diarrhoea in 15 of 20 males, 1 of the surviving females at 2000mg/kg bw and in one water control male animal. When compared to water control group, there was no apparent effect on the rate of body weight gain in the test substance treated groups.

Genotoxic Effects	The percentage of structurally damaged first division metaphase cells was not significantly increased in the test substance group, regardless of sex, dose or sacrifice time (p>0.025, Fisher's exact test). The percentage of numerically changed second division metaphase cell was not significantly increased in the test substance treated groups regardless of sex, dose and sacrifice time (p>0.025, Fisher's exact test)
Remarks - Results	Appropriate positive controls were used and resulted in large increases in micronuclei confirming the sensitivity of the test system.
CONCLUSION	The notified chemical was not clastogenic in this in vivo cytogenetic assay in rats under the conditions of the test.
TEST FACILITY	Microbiological Associates (1993d)

7.12 Developmental toxicity

7.12.1. Developmental toxicity – dietary administration

TEST SUBSTANCE	Notified chemical (as an aqueous solution; concentration not given)
METHOD	
Species/Strain	Charles River Cr1:CD VAF/Plus
Route of Administration	Oral – diet
Exposure Information	Exposure period: Days 6 to 15 of gestation
Remarks - Method	Complete report not provided. Results of ranging finding and definitive study provided with accompanying commentary. Review of feeding and gavage by Sullivan (1997) provided. A range finding study was performed with mated Charles River Cr1:CD VAF/Plus female rats at 0, 2000, 8000, 16000, 24000 or 40000 ppm. Five animals were used in each dose group. The pregnant animals were given treated diet from day 6 to day 15 of pregnancy. Animals were killed on day 20 and the uterus examined unopened for viable/nonviable foetuses, early/late resportions, total number of implant and corpora lutea. The highest dose selected for the definitive study was 16000 ppm. In the definitive study, 34 pregnant Charles River Cr1:CD VAF/Plus were given the test substance mixed in their diet. An additional four animal per group were dosed and killed on day 16 for plasma mineral estimation. The animals were killed on day 20 for standard teratological examination.

RESULTS

Group	*Number of Animals*	*Dose/Concentration* ppm		*Mortality*
		Nominal	*Actual*	
1	34	0	Not stated	0
2	34	2000	Not stated	0
3	34	8000	Not stated	0
4	34	16000	Not stated	0

Preliminary Study

At 2000 ppm and 8000 ppm there was essentially no adverse effects. In the 16000 ppm dose group, four rats had soft stools. A slight decrease in food intake and decrease in body weight gain during days 6 to 16. No increase in pre and post implantation loss was observed. A small decrease in gravid uterus weight was observed. At 24000 ppm one rat was emaciated and all had soft stools. There was marked reduction in food intake and severe body weight loss, with some recovery after treatment ceased. Of the four animals found to

be pregnant, three had viable foetuses. The average litter number was 5 compared to 14.5 in the controls. There was 60% post implantation loss, most were early resorption, but some foetal death was also observed. At 40000 two dams died, all animals were emaciated, had soft stool and three had body surface staining. Very marked reduction in food intake during the treatment period and severe body weight loss with some recovery after treatment were observed. In the surviving dams, there were no viable foetuses, no corpora lutea, and no implants.

Mortality and Time to Death
No mortality was observed at any of the doses.

Effects on Dams
At the highest dose, maternal toxicity was observed with statistically significant (Dunnett $p<0.01$) decreased food intake and significantly decreased body weight gain during days 6-16. There was actual body weight loss during days 6-10 and only a slight increase during days 10-16. A slight increase in body weight gain was observed when treatment ceased. The body weight gain for the 2000 and 8000 ppm groups was comparable to control animals.

There were no dams for which only resorption was observed in any group. The mean number of post implantation losses was comparable for the 0 and 2000 ppm animals. The post implantation losses for 8000 ppm and 16000 ppm was 1.8 and 2.2 foetuses per dam, respectively compared to 1.1 in the control group and 2000 ppm group.

Maternal plasma levels of zinc were significantly reduced at the mid and high dose levels and the iron and copper levels were reduced, but not significantly, at the highest dose.

Effects on Foetus
The mean foetal body weights were comparable for the control, low and mid dose groups. The mean foetal body weight for the 16 000 ppm group was reduced by approximately 40% when compared to the control group.

At 16 000 ppm, of the 28 litters evaluated, external malformations were observed in 27. A total of 395 foetuses were examined of which, 328 were malformed. The most common external malformations included limb and digital defects, tail defects, anal opening defects, cleft palate, and shortened lower jaw.

Visceral and skeletal examinations of the foetus from the 0 and 8000 ppm group were performed. In the control group, three foetuses, from three litters, were observed with developmental malformation. Four malformations were observed in four separate foetuses from three litters in the 8000 ppm group. Two malformed foetuses were found in same litter, however the malformations were not related. The skeletal and visceral malformations observed at these doses were with the historical control database for the species and strain held by test facility.

An increased incidence of three particular visceral and skeletal variation (unossified hyoid, unossified sternebrae, and bent ribs) were observed at 8000 ppm compared to the controls.

Remarks – Results
Maternal toxicity and high level of developmental malformations were both observed at the highest dose tested.

CONCLUSION
The NOAEL for the developmental effects is 8000 ppm (approximately 200 mg/kg bw/day)

TEST FACILITY International Research and Development Corporation (1994)

7.12.2. Developmental toxicity – gavage

TEST SUBSTANCE	Notified chemical (as an aqueous solution; concentration not given)
METHOD	In house protocol. Summary of method and results provided by Sullivan (1997).
Species/Strain	Charles River Cr1:CD VAF/Plus
Route of Administration	Oral – gavage
Exposure Information	Exposure period: Days 6 to 15 of gestation
Remarks - Method	Dose range finding study The test substance was given by gavage to groups of 9 pregnant Charles River Cr1:CD VAF/Plus rats at 0, 50, 200, 400, 600 or 1000 mg/kg bw/days on days 6-15 of pregnancy. Three animals from each group were killed on day 16 for plasma metal analysis. The remaining 6 animals were killed on day 20, the uterus removed, weighed, opened and the foetuses weighed, sexed, examined macroscopically and half these were dissected. Based on the dose range finding study, the highest dose in for the definitive study was 1000 mg/kg bw/day. Definitive study Thirty-six pregnant rats were dosed with the test substance once a day by gavage, on days 6 to 20. Six animals were killed on day 16 for plasma metal analysis. The remaining animals were killed on day 20. The uterus was removed, weighed, opened and the foetuses examined.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
1	30	0	0
2	30	50	0
3	30	400	0
4	30	1000	0

Preliminary Study

No adverse effects were observed at any of the dose levels. No clinical signs of toxicity, no changes in food consumption or maternal body weight gain were observed. There was no treatment related effects on the pregnancies, with no increase in resorptions, foetal deaths, foetal weights, gross or visceral or malformation or variations.

Mortality and Time to Death

No mortality observed

Effects on Dams

Maternal toxicity was observed at 1000 mg/kg bw/day. Clinical observations include an increase in soft stools, decreased defecation, and a significant reduction of food intake on days 6-9 and 6-16. There was dose related trend to reduction in bodyweight gain (not statistically significant) and significant decrease in carcass weight after removal of the uterus.

Effects on Foetus

No treatment related development toxicity was observed at any dose. No significant increase in resorptions, malformations, or effects on foetal weight was observed.

At 1000 mg/kg bw/day there was a small but significant increase in foetuses with variation per litter. These variations were mainly of the vertebrae and ribs. A review of historical data indicated that these variations are very common in the strain of the rats used. The variations are likely to be related to the maternal toxicity at this dose.

Eye malformation of the micropthtalmia was observed in 5 foetuses in one litter at 1000 mg/kg bw/day and one foetus in the control group.

The total number of malformation observed in the whole study are summarised below and do not indicate a dose response relationship

Dose group	Control	Low	Mid	High
Number of foetuses	5	0	1	10
Number of litters	5	0	1	3

An additional experiment investigated the mineral levels of treated pregnant rats, following gavage of the test substance at 0, 50, 400, 1000 mg/kg bw/day. The results of the study indicated a dose dependent reduction in plasma zinc levels at 2 and 4 hours after dosing. At the top dose, there was marked reduction to about 66%. There was a small and non-significant dose dependent reduction in plasma copper levels (12% at the top dose). The iron levels remained unaltered.

Remarks – Results
Original data was not provided.

CONCLUSION
The NOEL for both maternal and developmental toxicity in this study is 400 mg/kg bw

TEST FACILITY International Research and Development Corporation (Results reported in Sullivan (1997)).

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (as a 40.5% aqueous solution)
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test and EC Directive 84/449/EEC, Brussels (1984). Part C Methods for the Determination of Ecotoxicity: Biotic Degradation – Modified Sturm Test.
Inoculum	Activated sludge from a local municipal wastewater treatment facility receiving primarily domestic wastewater.
Exposure Period	34 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved Organic Carbon (DOC)
Remarks - Method	In addition to the test substance, blank samples and samples containing two reference substances (aniline and diethylene glycol) were measured. The degradation of the test material was assessed by determining the carbon dioxide produced.

RESULTS

Day	*% degradation*			
	Test substance		*Aniline*	*Diethylene glycol (DEG)*
	10 mg/L	*20 mg/L*	*20 mg/L*	*20 mg/L*
5	3.2	2.8	7.5	0.8
10	8.6	6.8	64.4	5.6
14	34.2	15.3	76.3	13.0
21	72.8	58.9	88.0	36.1
28	84.9	83.1	90.2	51.9
35	88.2	90.3	91.6	63.9

Remarks - Results	The total CO_2 evolution in the blank (9.2 mg) was less than 40 mg/L. The % DOC removal of the test substance at 10 and 20 mg/L were 94 and 93, respectively and of aniline and DEG were 98 and 67, respectively. The level of biodegradation of DEG was lower than the minimum 60% expected for a biodegradable substance. However, the test substance degraded up to 85% by 28 days thus validating the study.
CONCLUSION	The test substance is readily biodegradable.
TEST FACILITY	Lisec (1993c)

8.1.2. Ultimate biodegradability

TEST SUBSTANCE	Notified chemical (as a 40.5% aqueous solution)
METHOD	OECD TG 303 A Simulation Test – Aerobic Sewage Treatment: Coupled Units Test (1981)
Inoculum	Activated sludge from the aerators of a treatment plant dealing predominantly with domestic sewage.
Exposure Period	30 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved Organic Carbon (DOC)
Remarks - Method	Two model activated sludge 3 L plants were operated in parallel between 18°C and 25°C with a sludge residence time of 7 days and with influent passing through at a rate of 9 L/day and the test substance was added to one of the plants. The influent concentration was 20 mg C/L. The DOC concentration of each was measured. A reference substance was not

tested.

RESULTS

Time (Day)	*DR*
1	73.2
2	21.7
3	9.0
4	6.7
7	87.9
8	98.4
11	93.5
14	97.4
16	95.9
18	99.4
21	99.2
24	100.3
28	90.6
30	90.6

* DR= $T - (E - E_0)/T) \times 100$

where DR = % DOC removed with respect to the test substance

T = Concentration of the test substance in the influent (mg/L)

E = DOC concentration in the effluent of the test unit (mg/L)

E_0 = DOC concentration in the effluent of the control unit (mg/L)

Remarks - Results

The mean DOC removal was 96% with a 95% confidence interval of 94% to 97%. Seventeen data points (day 8 to 30 except day 25) were used to determine the %DR.

The results showed that the test substance is highly removed in the activated sludge unit of a wastewater treatment plant. The results of an adsorption/desorption study (summarised in 6.8b) showed that the test substance has no tendency to sorb onto sludge, which confirm that in the present study it was removed by degradation rather than by sorption onto sludge.

CONCLUSION

Based on the results of this study and the adsorption/desorption study, the test substance is removable due to biodegradation.

TEST FACILITY

Lisec (1993b)

8.1.3. Bioaccumulation

No bioaccumulation data were provided. However, if there is any release to the aquatic compartment bioaccumulation is not expected due to the high water solubility and the low log P_{ow} of the notified chemical.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical (as a 40.5% aqueous solution)

METHOD

OECD TG 203 Fish, Acute Toxicity Test –Semi-static

Species

Brachydanio rerio

Exposure Period

96 hours

Concentration Range

Nominal

100, 180, 320, 560 and 1000 mg/L

Auxiliary Solvent

None

Water Hardness	224 mg $CaCO_3$/L
Analytical Monitoring	Samples from the control and test solutions of 100, 320 and 1,000 mg/L were taken from newly prepared and spent (after 24 hours) solutions, stored in a refrigerator and sent for analysis. No details on the results of the analysis were provided.
Remarks – Method	The test solutions and control medium were slightly aerated and replaced daily. Oxygen content, pH and temperature were all satisfactorily maintained. Ten fish were tested per dose.

RESULTS

LC50	> 1000 mg/L at 96 hours (highest concentration tested).
NOEC	≥ 1000 mg/L at 96 hours (highest concentration tested).
Remarks – Results	All test solutions were completely clear (visually assessed) throughout the test. After 96 hours the condition (swimming behaviour, colour, respiratory function and other visually observable morphological or behavioural criterion) of all animals was equal to that of the control animals.

CONCLUSION	The test substance is practically non-toxic to fish.
TEST FACILITY	TNO Environmental and Energy Research (1993a)

8.2.2. Chronic toxicity to fish

The result of the chronic toxicity test conducted (test method not specified) was provided as a 30 day (ELS) NOEC of 61 mg/L. The detailed test report was not made available. Based on this NOEC value the test substance is very slightly toxic to fish (Mensink 1995).

8.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (as a 40.5% aqueous solution)
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction - Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Concentration Range	
Nominal	100, 180, 320, 560 and 1000 mg/L
Auxiliary Solvent	None
Water Hardness	224 mg $CaCO_3$/L
Analytical Monitoring	Samples from the control and test solutions of 100, 320 and 1,000 mg/L were taken from newly prepared and spent (after 48 hours) solutions (after 48 hours), stored in a refrigerator and sent for analysis. No details on the results of the analysis were provided.
Remarks - Method	Oxygen content and temperature were all satisfactorily maintained. The pH values varied between 7.1 and 7.9 but were not considered to have affected the results. Twenty daphnia were tested per dose.

RESULTS

LC50	> 1000 mg/L at 48 hours (highest concentration tested)
NOEC	
For mobility	≥ 1000 mg/L at 48 hours (highest concentration tested).
For condition	320 mg/L at 48 hours
Remarks - Results	All test solutions were completely clear (visually assessed) throughout the test. At 560 and 1000 mg/L concentrations the daphnia swam slower and with irregular movements and at 1000 mg/L they were paler than the control animals.

CONCLUSION	The test substance is practically non-toxic to daphnia.

TEST FACILITY TNO Environmental and Energy Research (1993b)

8.2.4. Chronic toxicity to aquatic invertebrates

The result of the chronic toxicity test conducted (test method not specified) was provided as a 21 day NOEC of 32 mg/L. The detailed test report was not made available. Based on this NOEC value the test substance is very slightly toxic to *Daphnia magna* (Mensink 1995).

8.2.5. Algal growth inhibition test

TEST SUBSTANCE Notified chemical (as acid form)

METHOD OECD TG 201 Alga, Growth Inhibition Test.
ISO Guideline 8692 (1989E) – Water Quality: Freshwater algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*. - for incubation conditions

Species	*Chlorella vulgaris*
Exposure Period	143 hours
Concentration Range	
Nominal	0.37, 0.75, 1.2, 2.5, 3.7, 6.1 and 12.3 mg/L
Auxiliary Solvent	None
Water Hardness	OECD standard medium and a modified medium (water hardness not specified) were used.
Analytical Monitoring	None indicated.
Remarks - Method	The toxicity of the test substance to algae in relation to the medium concentrations of trace metals Co, Cu and Zn and of the chelating agent ethylenediamine tetra acetic acid (EDTA) was studied to find an optimum medium for testing metal complexing substances. Based on a range finding test, a medium with the concentrations of Co, Cu and Zn in the test medium (2M) were ca. 100, 850 and 145 times higher, respectively than in the OECD standard medium. The trace element stock solution was prepared without the elements Co, Cu and Zn. Concentrated stock solutions of the latter elements were prepared separately. Appropriate volumes of these were added to the micropore filtrated medium containing 150 mg/L of $NaHCO_3$ (not 50 mg/L as specified in the OECD Guideline) in order to improve the buffer capacity of the medium. The OECD standard medium was used as a control. The test was extended to six days (144 h) to obtain better-defined growth curves.

RESULTS

Effect	*Biomass - E_bC50*	*Biomass - E_bC50*	*Biomass - E_bC50*
	mg/L at 72.5 h	*mg/L at 92.5 h*	*mg/L at 143 h*
With respect to cell volumes	1.3	2.2	4.6
With respect to cell numbers	>12.3	2.4	4.4

Remarks - Results The NOEC was estimated to be 1.2 mg/L. Increased trace metal concentrations in medium 2M resulted in a lower algal growth rate compared with the standard OECD medium. A growth similar or higher than in the controls was observed when up to 1.2 mg/L of the test substance was added to the 2M medium, however, concentrations above this reduced the growth rate. The 144 h E_rC50 value was 9.5 mg/L.

	Microscopic inspection of algal cells in cultures containing different trace metal and EDTA concentrations revealed no abnormalities however, increased cell sizes and coagulation of cells were observed in the presence of the test substance. The results satisfied the validity criteria specified under the OECD Guideline (control growth rate and test medium pH) at 72.5 hours although a pH of 9.5 was reached after 143 hours (exceeding the one unit limit allowed during the 72 hour test period).
CONCLUSION	According to the E_bC50 value with respect to cell volumes, the test substance is toxic to algae. However, the report concluded that the effect pattern of the test substance on the growth of *Chlorella vulgaris* could probably be best explained by trace metal toxicity and deficiency, due to complexation of heavy metals by the test substance (decreasing the availability of the trace metals limiting the algal growth rate).
TEST FACILITY	TNO Environmental and Energy Research (1994)

8.2.6. Inhibition of microbial activity

No activated sludge respiration inhibition test was provided. In the 'adsorption-desorption' study summarised in 6.8b, addition of sodium azide did not prevent biodegradation of the test substance and it was demonstrated that loss of the test substance was due to biodegradation by the acclimated biomass. Further, the 'ready biodegradability' study summarised in 8.1.1 showed that the test substance degraded up to 85% after 28 days, demonstrating that the test substance does not inhibit the activity of activated sludge.

8.2.7. Terrestrial plant growth test

A terrestrial plant growth test was conducted base on the OECD Guideline 208 'Terrestrial Plants, Growth Test', by the TNO Nutrition and Food Research Institute. The detailed test report was not included in the submission. The species tested were *Avena sativa*, *Lactuca sativa* and *Lycopersicum esculentum*. The observed and estimated effect concentrations for these species were expressed in mg ai of the test substance per kg of dry soil were as follows:

Parameter	*Effect*	*Avena sativa (Oats)*	*Lactuca sativa (Lettuce)*	*Lycopersicum esculentum (Tomatoes)*
18 d LC50	Emergence	>1000	>1000	>1000
18 d NOEC	Emergence	≥1000	320	≥1000
18 d NOEC	Survival of seedlings	≥1000	320	≥1000
18 d EC50	Growth (wet weight)	>1000	219 (187-258)	833 (724-958)
18 d NOEC	Growth (wet weight)	1000	100	320
18 d NOEC	Condition	1000	32	100

The above indicates phytotoxic effects are unlikely at normal soil loadings.

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The notified chemical will eventually be released into the environment with the majority (up to 4800 kg) expected to be discharged into sewerage systems through washing of hair. It is expected that up to 200 kg per annum will remain in the consumer product containers and will be disposed of to landfill.

The notified chemical is expected to be highly soluble in water and as such will be mobile in both the aquatic and terrestrial compartment. It will not readily hydrolyse in natural waters at

environmental pH values, however, is readily biodegradable. Under the basic conditions generally found in the sewer (pH 8) the anionic character will lead to eventual association with soil where the notified chemical will be degraded through biological and abiotic processes to water and oxides of carbon and nitrogen plus sodium salts. Residual chemical disposed of to landfill with empty containers are also expected to slowly adsorb to soil particles and be destroyed by similar mechanisms to those operating in sediments.

As the majority of the notified chemical in the hair care products will eventually be released into the aquatic environment via the sewerage systems the predicted environmental concentration (PEC) in the aquatic environment is estimated using a worst-case scenario (Environment Australia 2003). Australia has a population of ~19.5 million people, and an average value for water consumption of 200 L/person/day has been adopted for this national-level assessment (3900 ML/day for total population).

Based on annual imports of 5000 kg per annum, and assuming the majority of this is eventually released to sewer and not removed during sewage treatment processes, the daily release on a nationwide basis to receiving waters is estimated to be 13.7 kg/day. Therefore, the concentration of notified chemical in the Australian sewerage network may approximate 3.5 μg/L (ie. 5,000 x 10^6 mg ÷ 365 days/year ÷ 3900 x 10^6 L). Based on dilution factors of 1 and 10 for inland and ocean discharges of STP-treated effluents, outfalls PECs of the notified chemical in freshwater and marine surface waters may approximate 3.5 μg/L (i.e. 3.5 x 10^{-3} mg/L) and 0.35 μg/L (3.5 x 10^{-4} mg/L), respectively.

The ready biodegradability test results showed that up to 85 % of the notified chemical was eliminated after 28 days and therefore the notified chemical was considered to be readily biodegradable. The SIMPLETREAT model (European Commission 1996) for modelling partitioning and losses in sewage treatment plants (STP) was used to estimate the proportions of the chemical partition into the different environmental compartments. The results indicate that when the chemical (5,000 kg) is released into the aqueous phase of a STP, about 9% (450 kg) partitioned to water and 91% (4,550 kg) degraded while there is no release to air through volatilisation or partitioning to biosolids. These results are consistent with the non-volatility, high solubility and low log P_{ow} values of the notified chemical and the results of the adsorption/desorption test (summarised in 6.8b) indicating that the test substance has no tendency to sorb onto the sludge.

Assuming 9% of the notified chemical (up to 450 kg) may potentially remain in solution, the following PECwater and PECsoil values were obtained (Environment Australia 2003). The worst-case scenario daily predicted environmental concentration (PEC) for the aquatic environment resulting from the nationwide release of the notified chemical into the sewage systems is reduced to 0.32 μg/L (3.2 x 10^{-4} mg/L) prior to any dilution. Based on dilution factors of 1 and 10 for inland and ocean discharges of STP-treated effluents, outfalls PECs of the notified chemical in freshwater and marine surface waters may approximate 0.32 μg/L (i.e. 3.2 x 10^{-4} mg/L) and 0.032 μg/L (3.2 x 10^{-5} mg/L), respectively.

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 0.1 m of soil (density 1000 kg/m^3). Using these assumptions, irrigation with a concentration of 3.2 x 10^{-4} mg/L may potentially result in a soil concentration of approximately 3.2 x 10^{-3} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 1.6 x 10^{-2} mg/kg and 3.2 x 10^{-2} mg/kg, respectively.

Concentration in effluent		0.32 μg/L
PECsoil (mg/kg) (assumes no degradation)		
		Recycled water
Soil concentration	1 year	3.2 x 10^{-3}
	5 years	1.6 x 10^{-2}
	10 years	3.2 x 10^{-3}

Bioaccumulation is not expected due to the high water solubility and low log P_{ow} of the notified chemical, which indicates a poor affinity to lipids. The readily biodegradable nature of the notified chemical would also limit its bioaccumulation potential.

9.1.2. Environment – effects assessment

The results of the aquatic toxicity tests are listed below. The most sensitive species was algae with a 72 hour E_bC50 of 1.3 mg/L.

Organism	*Duration*	*End Point*	*mg/L*
Fish	96-h	LC50	>1000
Daphnia	48-h	EC50	>1000
Algae	72.5-h	E_bC50 (biomass – with respect to cell volumes)	1.3

Using the lowest EC50 datum (ie. 1.3 mg/L) and a safety factor of 100 (OECD), a predicted no effect concentration (PNEC for aquatic ecosystems) of 0.013 mg/L (13 µg/L) has been derived by dividing the LC50 value by an uncertainty (safety) factor of 100.

Based on the terrestrial plant growth test results summarised in 8.2.7, the lowest NOEC is 32 mg of test substance per kg of dry soil (for lettuce).

9.1.3. Environment – risk characterisation

The risk quotient values estimated based on the scenario of discharging the entire imported notified chemical into sewage systems in Australia are less than 1. Treatment in STPs further reduces the risk as shown below. Therefore, the proposed use of the notified chemical is unlikely to pose an unacceptable risk to the aquatic life.

Location	PEC	PNEC	Risk Quotient (RQ)
Australia-wide STPs			
Aquatic			
Ocean outfall	3.5×10^{-4} mg/L (3.2×10^{-5} mg/L) #	0.013 mg/L	2.69×10^{-2} (2.46×10^{-5}) #
Inland River	3.5×10^{-3} mg/L (3.2×10^{-4} mg/L) #	0.013 mg/L	0.269 (1.04×10^{-4}) #
Terrestrial	3.2×10^{-3} mg/kg of soil	32 mg/kg dry soil	10^{-4}

PEC and RQ values calculated assuming 9% of the notified chemical partitioned into water and 91% degrades during the STP process based on SIMPLETREAT model.

Recent studies (including the study summarised in 8.2.5) have shown that the apparent toxicity of strong chelators such as the notified chemical in standard algal growth inhibition tests is related to essential trace metal bioavailability. The results of a study conducted by Schowanek et al (1996) suggested that interaction of the chelator with trace metals alters the free metal concentration and affects algal population growth, as opposed to a direct interaction between the alga and the chelator. It was illustrated that the standard algal growth inhibition test is not well suited to assess algal toxicity (sensu stricto) of strong chelators as the NOEC and the EC50 values are probably overestimated by at least one order of magnitude for the notified chemical.

Given this evidence the actual PEC/PNEC ratio for the aquatic environment can be expected to be an order of magnitude lower than the estimate above.
The risk quotient estimated based a soil concentration of approximately 3.2×10^{-3} mg/kg and the lowest NOEC reported for terrestrial plants (for lettuce) of 0.32 mg of test substance per kg of dry soil, is significantly below 1. Therefore, the proposed use of the notified chemical is unlikely to pose an unacceptable risk to the terrestrial plants.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Worker exposure to the notified chemical during hair dye manufacture will be greater than for workers handling the notified chemical in the final product. Hair dye manufacture workers will be exposed to the notified chemical as imported as a 34% aqueous solution. Those workers handling the finished product will be exposed to the notified chemical at 2% w/w in the finished hair dye product.

Worker exposure may occur during the formulation of the final product. Dermal and accidental ocular exposure may occur during the pre weighing and transfer of the solution of the notified chemical to the batch mixer, mixing, and QC sampling. Exposure may also occur during the unscheduled maintenance of the filling lines. Minimal exposure will occur during the maintenance of the batch-mixing vessel.

Worker exposure will be minimised by use of the appropriate personal protection equipment. When pre-weighing, mixing and QC sampling, the operators wear uniforms, gloves and eye goggles. Local exhaust ventilation will be used over the mixing vessel to remove vapours and dust.

Worker exposure during the transport, storage, and distribution of the imported notified chemical and finished product is unlikely to occur unless there is an accidental spillage or packaging breach. Exposure for retail workers is not likely to occur, unless the packaging of the final product is breached.

Salon workers may have repeated dermal exposure to the notified chemical when apply hair dye to customers, however gloves will normally be worn to prevent exposure to other components of the dyes.

9.2.2. Public health – exposure assessment

Public exposure will be restricted to those persons using the final hair colorant product. The notified chemical will be present at 2% w/w in the final product. Before use by the consumer, the product is mixed with hydrogen peroxide, in a 1:1 ratio. Thus, the typical consumer exposure will be to a product in which the notified chemical will present at 1% w/w. The hair colorant product is used once every 4-6 weeks. Persons using the final product will be dermally exposed to the notified chemical primarily through the scalp. Dermal exposure is likely to be for 40 minutes, after which time, the product is washed off. Consumers are provided with protective gloves to be worn during the use of the hair dye product.

Accidental ocular exposure and ingestion of the notified chemical may also occur.

Direct public exposure during transport and storage or from manufacturing waste is unlikely.

9.2.3. Human health - effects assessment

The notified chemical has an estimated log Pow of <-4.7. Being highly hydrophilic, the absorption of the notified chemical through the skin is likely to be very low. Respiratory exposure is also expected to be low due to its vapour pressure and it will be in aqueous solution in the final product.

An absorption, distribution, metabolism, and excretion study of the notified chemical has been performed in rats. The notifier did not provide this study. The summary report indicates that following oral administration, the notified chemical is absorbed and distributed to a stable form in the blood to the bone marrow.

The dermal penetration of the notified chemical in rats is 0.0406 mg/cm^3/hr (Unknown, 1995).

The notified chemical has a low acute oral and dermal toxicity. The notified chemical is not a skin irritant. The notified chemical is slightly irritating to the eyes. The observed conjunctive redness is insufficient to classify the chemical as an irritant to the eye (R36). The notified

chemical is not a skin sensitiser.

The NOEL established in a 90 day repeat dose study in rats was 300 mg/kg bw/day, based on the body weight changes, changes in sperm morphology, plasma mineral level alterations, and microscopic changes in pancreas and testes at the higher doses. A 28 day study examined the effects of the notified chemical on metal concentration and found increased zinc excretion at all concentrations tested.

The mutagenicity of the chemical was examined in three *in vitro* tests and one *in vivo* test. The notified chemical was not mutagenic to bacterial cells and mouse lymphoma cells with and without metabolic activation. The notified chemical was clastogenic to Chinese Hamster Ovary cell. Zinc is essential for DNA replication, RNA polymerases and protein synthesis. All cell replication, protein synthesis, and growth processes are partially dependent on zinc (ICPS, 2001). It is possible that the positive results observed in the Chinese hamster ovary cells may be the result of the zinc deficiency caused by the chelating agent *in vitro*. The notified chemical was not mutagenic in an *in vivo* micronucleus assay. There is insufficient evidence to classify the notified chemical as R46 (mutagenic) or R40 (possible risk of irreversible effects) in accordance with the Approved Criteria for Classifying Hazardous Substances (NOHSC, 1999b).

Gavage and diet studies were undertaken to examine the developmental toxicity of the notified chemical. The NOEL for both maternal and developmental toxicity in the gavage study was 400 mg/kg bw/day. The NOAEL for developmental effects in the diet study was 8000 ppm (approximately 200 mg/kg bw/day). It has been suggested that the maternal toxicity observed in the diet study may be due to reduced mineral intake. The test substance may chelate minerals in feed. The suggested explanation for the differing results in the gavage and diet studies is that these are likely due to the increased chelation of the mineral in food and longer periods of zinc plasma level reduction in the diet study (Sullivan, 1997).

All cell replication, protein synthesis, and growth processes are partially dependent upon zinc. Zinc is essential for DNA replication, RNA polymerase, protein synthesis, and a large number of metabolic processes. In animals, zinc deficiency is characterised by reduction in growth, cell replication, adverse reproductive and developmental effects, which persist after weaning and reduced immunoresponsiveness (IPCS, 2001). Adverse reproductive effects were seen in rats on a low zinc diet. Arrest of spermatogenesis and atrophy of the germinal epithelium of testes in rats, impairment of the menstrual cycle in rats and monkeys, and ovarian follicular development retardation was observed in rats (IPCS, 2001). Zinc deficiency has been found to be injurious or lethal to the embryos and foetuses in experimental animals. Evidence indicates that adequate levels of zinc are essential for conception, blastula development and implantation, organogenesis, foetal growth, prenatal survival and parturition. Severe zinc deficiency results in high foetal resorption, with malformation of the skeleton, nervous system and viscera found in most of the surviving foetus. Impairment of the synthesis and/or metabolism of DNA is postulated to cause these abnormalities (IPCS, 2001). Numerous human health effects associated with zinc deficiency, including neurosensory changes, oligospermia, impaired neuropsychological functions, growth retardation, delayed wound healing, immune disorders and dermatitis. These are generally reversible and corrected with zinc supplementation (IPCS, 2001) These results support the conclusion that the observed developmental result are due to zinc deficiency.

In a human repeat insult patch test, the notified chemical at 5% was non-sensitising under conditions of the test.

9.2.4. Occupational health and safety – risk characterisation

Occupational exposure can occur when handling the imported 34% solution. During the formulation process, dermal and accidental ocular exposure to notified chemical may occur when the imported solution is pre weighed and poured into open the batch mixer, from splashing or vortexing during the mixing of the batch, and during QC testing. Due to its hydrophilic nature, dermal absorption may be low in humans. The notified chemical is slightly irritating to

the eyes. Operators should wear, gloves, safety glasses, and overalls.

Occupational exposure may also occur during the unscheduled maintenance of the filling lines. The concentration of the notified chemical following formulation is 2% w/w. Exposure is minimised by the use of safety glasses, gloves, and overalls.

Once the final hair dye product is packed, exposure should be low. Hence, exposure for warehousing and distribution workers and retail workers is unlikely unless the packaging is breached.

Salon workers will have regular dermal exposure to the notified chemical as well as other hair dye components. A survey of hairdressers in Melbourne found that the majority wear gloves while applying hair colours, and the potential for exposure is therefore significantly reduced. However, this indicates that a minority of hairdressers apply hair dyes without the use of gloves. The risk for these workers arising from the notified chemical is expected to be low due to its low concentration in the product low dermal absorption potential and low hazard in comparison with other hair dye components.

9.2.5. Public health – risk characterisation

The level of the notified chemical in finished hair product is low. Dermal absorption of the notified chemical is likely to be low based on its hydrophilic nature. Data from animal and human studies indicate that notified chemical is not a sensitiser, repeated dermal exposure is not of concern.

The notified chemical is present in formulated product at a level of 2% w/w. Prior to use, it mixed with hydrogen peroxide solution in a ratio of 1:1. Therefore, users of the hair colorant product are exposed to notified chemical at 1% w/w. The product remains in the hair for 40 minutes before it is washed off. The colorant product is used once every 4-6 weeks. The notifier has calculated dermal exposure using:

Systemic exposure = [dp*c*t*ssa]/bw

Where c = concentration
dp = dermal penetration
t = duration per use
bw = body weight
ssa = scalp surface area of human

The following assumptions were made:
c = 1%
dp = 0.0406 mg/cm^2/hr (based on study in rats) (Unknown, 1995)
t = 40 minutes (0.67 hr)
bw = 60 kg
ssa = 600 cm^2

Systemic exposure = 0.0027 mg/kg bw/day

Margin of Exposure calculations were undertaken, based on the NOEL and NOAEL from the subchronic study and developmental study, respectively. To determine the margin of exposure for development toxicity NOEL for the gavage study was used. The gavage study was considered more appropriate for dermal absorption due to the major effects in the dietary study being considered to relate to interaction of the notified chemical with dietary micronutrients.

Margin of Exposure
Subchronic Study
MOE = NOEL/systemic exposure
300/0.0027

111,111

Margin of Exposure
Developmental toxicity
MOE = NOAEL/Systetmic exposure
400/0.0027
= 148,148

Both Margin of Exposures exceed 100, and are hence acceptable.

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 a hazardous substance under the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

For the environment the notified chemical is classified as toxic (Acute II) to aquatic organisms (OECD 2002) according to the criteria of the Globally Harmonised System of Classification and Labelling of Chemicals (GHS).

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio the chemical is not considered to pose a risk to the aquatic 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 when used as described in the notification.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994a). 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 the notified chemical provided by the notifier was in accordance with the NOHSC *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994b). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

Labelling

- Use the following safety phrases for products/mixtures containing the notified chemical:
  - S24/25 Avoid contact with skin and eyes
  - S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection

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, as diluted for use:
  - NOSHC exposure standards for all components of the final product should be exceeded in the workplace
  - Prevent spill and splashes

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - Chemical resistant gloves, protective clothing and goggles / safety glasses

- Employers should ensure that the following personal protective equipment is used by workers to minimise exposure during occupational use of hair dyes
  - Rubber 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.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

Disposal

- The notified chemical should be disposed of to either the on-site wastewater treatment plant or to landfill in accordance with local authority requirements.

Emergency procedures

- Spills/release of the notified chemical should be handled by containing, adsorbing with inert, damp, non-combustible material and flushing the area with flooding amounts of water.
- Do not contaminate drainage or waterways.
- Avoid direct discharge into drains.

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 Section 64(1) of the Act; if

- Over 10 tonne per annum of the notified chemical is used in Australia, additional test reports on chronic toxicity to fish and daphnia and an explanation of the contradiction between the results of the two adsorption/desorption tests are required to be submitted for the notified chemical.

or

(2) Under Section 64(2) of the Act:

- If any of the circumstances listed in the subsection arise.

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