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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

1H-Imidazolium, 2-[(4-aminophenyl)azo]-1,3-dimethyl-, chloride (Basic Orange 31)

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1H-Imidazolium, 2-[(4-aminophenyl)azo]-1,3-dimethyl-, chloride (Basic Orange 31)

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Ciba Specialty Chemicals Pty Ltd (ABN 97 005 061 469)
232 Settlement Rd
Thomastown VIC 3074

KPSS Australia Pty Ltd (ABN 67 003 296 366)
1A The Crescent,
Kingsgrove NSW 2208

Henkel Australia Pty Ltd (ABN 82 001 302 996)
20 Rodborough Road
Frenchs Forest NSW 2086

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication:

Spectral data
Purity and Impurities
Introduction volume

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
1H-Imidazolium, 2-[(4-aminophenyl)azo]-1,3-dimethyl-, chloride

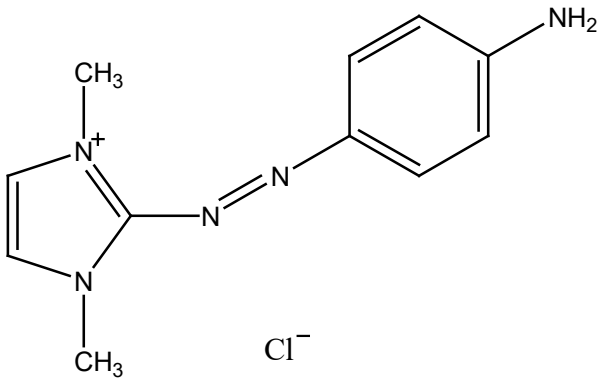
OTHER NAME(S)
Basic Orange 31 (INCI)

MARKETING NAME(S)
MIP Orange 3100
Vibracolour Flame Orange

CAS NUMBER
97404-02-9

MOLECULAR FORMULA
$C_{11}H_{14}N_5Cl$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
251.72

METHODS OF DETECTION AND DETERMINATION

METHOD	High Performance Liquid Chromatography (HPLC), Elemental Analysis
	Infrared Spectroscopy, ^{1}H Nuclear Magnetic Resonance (NMR) and UV/Vis Spectroscopy
Remarks	HPLC using UV/Vis detection allows the quantification of the notified chemical.
	Identity confirmed by elemental analysis, ^{1}H NMR and IR.
TEST FACILITY	Ciba Specialty Chemicals (1998), Ciba Specialty Chemicals (2005)

3. COMPOSITION

DEGREE OF PURITY
The current production specification for the notified chemical is > 80%.

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 component of two brands of hair dyes at up to 0.5% maximum concentration. One of the dyes may also be imported in bulk in 100 kg containers and repackaged locally.

In future the notified chemical (neat) may be imported for the formulation of hair dyes locally.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

USE
The notified chemical will be used in both oxidative and non-oxidative hair dyes. The hair dyes will be used in hair salons and in the consumer market.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Melbourne and Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
The ready to use hair dyes will be stored at the warehouses of Henkel Australia Pty Ltd and KPSS Australia Pty Ltd. Repackaging will most likely take place at a contract manufacturing site.

If in future neat notified chemical is imported, this will be stored at the Ciba Specialty Chemicals warehouse. At present, no local customers have been identified for the manufacture of hair dyes in Australia.

TRANSPORTATION AND PACKAGING
The two brands of hair dye will be imported in 80 g or 150 ml tubes and will be stored at the notifiers' warehouse prior to distribution by road to retail and hair salon outlets.

One of the brands of the hair dye may also be imported in bulk in 100 kg plastic barrels and repackaged in Australia.

In future if the hair dyes are manufactured locally, the notified chemical will be imported in sealed plastic drums (100-120 kg) or pre-weighed satchels. The notified chemical will be transported by road.

5.2. Operation description

Repackaging
The import containers containing the hair dye will be opened and the contents will be dispensed through an automated pumping system into a 1000 L stainless steel holding tanks. The equipment is cleaned with hot water and rinsed after every batch. The finished product is tested for quality assurance before being filled into retail containers using automated equipment. The filled retail containers will be packaged into carton boxes and shipped to the notifiers' warehouse for distribution to salons or retail stores.

Reformulation
At present, no local customers have been identified for the manufacture of hair dyes in Australia and hence no specific operation details are known. Typically, the notified chemical as solid material would be either introduced in pre-weighed satchels which would be cut open and emptied into the blending vessel, or the material would be weighed out from the import container and then manually emptied into the blending vessel as above. The weighing would be done in a fume hood. The blending vessel will be closed while mixing takes place. Prior to packaging, sampling and quality testing of the dye preparation is carried out in the laboratory. The dye preparation will then be automatically pumped to a multi-head filling machine for transfer into tubes or bottles.

End-use
The 150 ml tubes will be sold to the home-user market as a ready-to-use hair dye. The 80 g tubes will be sold to hair salons, where the hair dye will be mixed with other ingredients prior to use. The application instruction for the two types of the dye are as follows:

Salon use
The hair dye will be squeezed into a small mixing vessel and mixed with other ingredients before being applied to the customers' hair by brush. After a maximum of 30 minutes the hair is then rinsed into the basin and dried. The mixing and application instructions vary depending on whether the treatment is for first time highlighting or the refreshing of original highlights as follows:

First time highlights
20 mL of hair dye will be mixed approximately with 40 mL of a peroxide developer. This dye is applied to individual streaks, it is not recommended to be applied all over the head.

Refreshing service
Depending on the colour required, 5 - 15 ml of the hair dye is mixed with a non-oxidative solution (15 - 25 ml) and water (60 ml). The mixed dye is applied to whole of hair using an applicator bottle.

Home use
The hair dye can be used for individual streaks or for all over colour. For streaks the hair dye will be applied to the hair using the supplied brush applicator. For all over colour the cream is squeezed into a dish and applied to the hair by hand. No mixing will be required prior to application. Depending on the amount of hair to be dyed it is expected that up to 75ml of hair dye will be applied. After 20-30 minutes the hair will be rinsed.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Introduced as prepacked hair-dye			
Transport and warehousing	10	1-2 hours/day	20 days/year
Repackaging			
Storage and transport (from dock to repackaging site)	2-4	2-3 hours/day	2 days/year
Repackaging	6	8 hours/day	20 days/year
QC workers	1	4 hours/day	20 days/year
Formulation			
Formulation workers	9 - 12	8 hr	150 days/year
QC workers	2	4 hr	150 days/year
End-use			
Storage and transport	2 - 4	1-2 hours/day	150 days/year
Retail workers	5000	0.5 hr	100 days/year
Salon workers	1000	0.5 hr	200 days/year

Exposure Details
Storage and distribution
Initially, waterside, transport and warehouse workers will only handle the formulated hair dyes containing up to 0.5% notified chemical packed in boxes or plastic barrels. In the future, these workers may also handle drums/satchels of the notified chemical. Exposure is only likely to occur in the event of a spill from damaged containers.

Repackaging
Dermal and limited ocular exposure to up to 0.5% notified chemical may occur when opening and closing the imported containers containing the hair dye and connecting and disconnecting transfer and filling lines. Due to the automated nature of the filling process, exposure to the notified chemical is not expected, however, dermal exposure may also occur due to drips and spills and if containers are overfilled at the filling station. Skin contamination may occur when maintenance workers are cleaning equipment and during maintenance of equipment. Workers involved in the above activities will wear personal protective equipment such as, overalls, safety glasses, safety shoes, gloves hair covering and facemasks

Quality Control workers will sample and test only the final formulations containing up to 0.5% notified chemical. Dermal and possibly ocular exposure could occur during sampling and analysis. Laboratory staff will wear laboratory coats, safety glasses and impermeable gloves.

Formulation
Dermal and possible ocular and inhalation exposure to dust could occur during the weighing of the notified chemical and during the transfer from the weighing vessel/pre-weighed satchels to the blending vessel. All blending procedures are performed in sealed vessels and therefore exposure to the notified chemical is not expected. Once formulated the concentration of the notified chemical will be at up to 0.5%. Due to the automated nature of the filling process, exposure to the notified chemical is not expected except in the event of a machine malfunction. The MSDS for the notified chemical specifies

that gloves, overalls and glasses/face shield must be worn and that a disposable dust mask be worn where sufficient ventilation is not available.

Quality Control workers will sample and test only the final formulations containing up to 0.5% notified chemical. Dermal and possibly ocular exposure could occur during sampling and analysis. Laboratory staff will wear laboratory coats, safety glasses and impermeable gloves.

End-use
Retail workers will unpack the boxes and place the containers on supermarket shelves. Exposure is only likely to occur in the event of a spill from damaged containers.

Salon workers may be dermally exposed to up to 0.5% notified chemical during the mixing, application and rinsing of the hair dye. Salon workers are likely to wear protective gloves.

5.4. Release

RELEASE OF CHEMICAL AT SITE
No manufacturing of the notified chemical will take place in Australia. The finished hair dye may be imported in bulk and repackaged into retail containers locally. Reformulation of the dye into finished hair dye products may take place in Australia. However, it is envisaged that most of the notified chemical will be imported in finished products.

Repackaging
It is estimated that 2% of the dye mixture will remain in the 100 kg bulk container after emptying. Annually this equals a maximum of 6 kg of the notified chemical (calculated as 2% of annual import volume) being disposed of as residue in import container. The containers and the residual dye mixture will be disposed of by a licensed waste disposal contractor.

Process equipment, including the mixing tank and filling machines will be rinsed out and the washings sent to the on-site wastewater treatment plant. It is estimated that for each batch of final product, 2% will be sent to the on-site wastewater treatment plant (WWTP). This will equate to a maximum of 6 kg/year (2% of annual import volume) of the notified chemical being released to on-site wastewater treatment plant.

Formulation
As there are no immediate plans for importation of the raw material and local manufacture of hair dye in Australia, it is difficult to estimate the environmental releases during manufacturing. During storage of raw material at the notifiers' site the notified chemical will be in a powder form and any accidental spills which may occur will be collected by vacuuming. None of the material would be washed into the sewer from their site.

Reformulators will use typical blending and filling operations. Any solid wastes (such as residues in empty import containers) will be disposed of with the container to landfill. Approximately, 1% of import volume is expected to be sent to landfill. Rinsate from process equipment will be sent to an on-site wastewater treatment plant. The release to WWTP is expected to be 2% of the annual import volume.

RELEASE OF CHEMICAL FROM USE
The notified chemical will be released to sewer during use in salons and in the home by consumers. In the worst case, if it is assumed that 100% of the finished hair dye product being used is washed off and therefore all notified chemical used in these products could be discharged to the sewer, this equates to up to 300 kg per annum of the notified chemical being released to the sewer.

A small quantity of product (2% of container contents or 6 kg/year of the notified chemical) will remain in the container and will be disposed of to landfill via domestic garbage collection.

5.5. Disposal

The empty import bags containing up to 1.5 kg residue of notified chemical are disposed of to landfill by a licensed waste disposal operator. Spills and water used to wash process equipment will be sent to the onsite wastewater treatment plant before being released to the sewer. Hair dye packaging

containing up to 6 kg residue of the notified chemical will be disposed of to landfill as domestic waste.

5.6. Public exposure

The public will be exposed to the notified chemical when dyeing their hair at home or attending a salon to have their hair dyed.

Salon

The public will be exposed to the notified chemical at a concentration of 0.16% (first time highlights) or 0.08% (highlight refreshing). The area of exposure is expected to be greater when highlights are being refreshed as the hair dye is applied all over. The public will be exposed for approximately 30 minutes prior to the hair dye being rinsed.

Home-use

The public will be exposed to the notified chemical at a concentration of 0.2% from dermal contact with the hair dye during application and from contact of the hair dye with the scalp during the dyeing process. Gloves supplied with the product are advised to be worn throughout the whole process including rinsing.

6. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20ºC and 101.3 kPa Violet powder

Melting Point/Freezing Point > 400ºC

METHOD	OECD TG 102 Melting Point/Melting Range EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Determined by Differential Scanning Calorimetry. No significant protocol deviations. No signals characteristic of melting were seen below 400 ºC. Decomposition was seen to occur above 192 ºC.
TEST FACILITY	Covance (2003)

Density 1310 kg/m^3 at 20.4ºC

METHOD	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Determined by gas comparison pycnometer (helium). No significant protocol deviations.
TEST FACILITY	RCC Ltd (1998a)

Vapour Pressure < 2 x 10^{-7} kPa at 20ºC

METHOD	OECD TG 104 Vapour Pressure.
Remarks	Calculated according to Schwarzenbach (1993) using the following equation as vapour pressure expected to be < 10Pa. Ln P^o = appr. [19(1 - T_b/T) + 8.5(ln T_b/T)] 101325 [Pa], where P^o = Vapour pressure in Pa at temperature T [K] T ambient temperature in [K] T_b Boiling point in [K] Boiling point assumed to be > 400 ºC which is a very conservative assumption.
TEST FACILITY	Ciba Specialty Chemicals (1999)

Water Solubility 27.5 g/L at 20ºC

METHOD OECD TG 105 Water Solubility.
EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Determine using the Shake Flask Method.
Analytical Method: HPLC
No significant protocol deviations.
TEST FACILITY RCC Ltd (1998b)

Henry's Law Constant 1.872 X 10^{-5} atm-m^3/mole (Estimated)

METHOD Estimation method – HENRYWIN v3.10

Hydrolysis as a Function of pH Hydrolytically stable.

METHOD OECD TG 111 Hydrolysis as a Function of pH.
EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH.

pH	*T (°C)*	*t½ hour*
4	50.1	6740
7	50.1	4800
9	50.1	No degradation

Remarks Kept for up to 120 hours at 50ºC. Concentrations determined by HPLC and half lives extrapolated to 25ºC.
TEST FACILITY Covance (2003)

Partition Coefficient (n-octanol/water) Could not be evaluated.

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks Analytical Method: Liquid chromatography. As the notified chemical is predicted to be in an ionised state under normal conditions, the HPLC simulation technique was not appropriate and no realistic measurement was made, despite several attempts. Log P_{OW} is expected to be low, based on high water solubility.
TEST FACILITY Covance (2003)

Adsorption/Desorption – screening test Koc = 7 to 84 L.kg^{-1} (calculated)

METHOD QSAR according to Technical Guidance Document in Support of the Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and the Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances, Part III, 2003
Remarks Determined using EPISuite. The values of 7 to 84 L/kg indicate that the notified chemical has certain tendency to adsorb to soil. Nevertheless, due to the ionic character of the substance, these values are indicative only and sensitive to pH.
TEST FACILITY Ciba Specialty Chemicals (2003)

Dissociation Constant Below a pH value of 2 at 25°C

METHOD OECD TG 112 Dissociation Constants in Water.
Remarks Determined by acid and basic titration. No significant protocol deviations.
The test substance exhibited adequate water solubility. The notified chemical will remain ionised throughout the entire environmental pH range of 4-9.
TEST FACILITY Covance (2003)

Particle Size

METHOD OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
< 5	0.00
5-8	0.23
8-15	95.2
15-32	7.77
32-40	0.35
40-63	0.22
63-90	0.30
90-125	0.27
125-160	0.14
160-200	0.16
> 200	1.37

Remarks Determined by sieving.
Inhalable fraction (<10 μm): ~32% (data taken from distribution curve)
Respirable fraction (<100 μm): ~98%

TEST FACILITY S.A.F.E Analytik (1998)

Flash Point Not determined

Remarks The flash point in the lowest temperature at which a liquid evolves vapours in such an amount that a flammable vapour/air mixture is produced. The notified chemicals melting point is > 400°C and as such the flash point is also considered to be > 400°C.

Flammability Limits Not highly flammable

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).

Remarks In the preliminary test the notified chemical burned in contact with the flame but extinguished immediately without ignition source. The test item swelled up and became porous and coloured black. There was no gleaming or burning without contact of the ignition source and therefore the substance can be considered not highly flammable.

TEST FACILITY RCC Ltd (1998c)

Autoignition Temperature 200ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.

Remarks The self-ignition temperature corresponds well with the decomposition exotherm observed in the melting point study.

TEST FACILITY Covance (2003)

Explosive Properties Potentially explosive

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.

Remarks The explosive properties were evaluated by consideration of the chemical structure and associated thermodynamic properties.

The notified chemical is considered to be potentially explosive based on the following:

1. there is a potential auxoplose/plosophore (N=N) present
2. a sharp decomposition exotherm is present
3. the enthalpy of the exotherm at 260 ºC is greater than the trigger value.

TEST FACILITY Covance (2003)

Reactivity

Remarks	The notified chemical is stable under normal conditions of use. The chemical is hydrolytically stable but decomposes at temperatures above 192 ^{o}C. The notified chemical is potentially explosive in the powder form but the chemical will be imported in aqueous mixtures.

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint	*Assessment Conclusion*
Rat, acute oral	harmful, LD50 = 1000 - 2000 mg/kg bw
Rat, acute dermal	low toxicity, LD50 > 2000 mg/kg bw
Rabbit, skin irritation (acute)	slightly irritating
Rabbit, skin irritation (repeat dose)	slightly irritating
Rabbit, eye irritation	severely irritating (100%) slightly irritating (1%)
Guinea pig, skin sensitisation – adjuvant test	no evidence of sensitisation
Skin sensitisation – LLNA	evidence of sensitisation
Phototoxicity	does not exhibit a phototoxic potential
Photoallergenicity	does not exhibit a photoallergenic and allergenic potential
Rat, repeat dose oral toxicity – 14 days.	NOEL 15.5 mg/kg bw/day
Rat, repeat dose oral toxicity – 90 days.	NOAEL 63 mg/kg bw/day, NOEL 18 mg/kg bw/day
Genotoxicity – bacterial reverse mutation	mutagenic
Genotoxicity – in vitro chromosome aberration test chinese hamster cells	clastogenic
Genotoxicity – in vitro chromosome aberration test human lymphocytes	non clastogenic
Genotoxicity – in vitro cell gene mutation test	non mutagenic
Genotoxicity – in vivo mouse micronucleus test	non genotoxic
Genotoxicity – in vivo UDS test	non genotoxic
Toxicokinetic studies	absorption 0.018±0.005 µg/cm^2
Developmental and reproductive effects	maternal and foetal NOAEL 60 mg/kg bw/day

7.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	Equivalent to OECD TG 401 Acute Oral Toxicity (Limit Test)
Species/Strain	Rat/Crl: CD(SD)IGS BR
Vehicle	Cell culture grade water.
Remarks - Method	Deviations from OECD TG401 – Limit Test Although mortality was observed in the main limit test a full acute toxicity study was not carried out at lower doses. However, in a preliminary dose finding study 3 doses were administered to 2 male and 2 female rats.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
Preliminary Test			
I (low)	2 per sex	500	0/2
II (mid)	2 per sex	1000	1/2
III (high)	2 per sex	2000	1/2
Main Test			
IV	5 per sex	2000	2/5 male; 3/5 female

LD50	1000 – 2000 mg/kg bw
Signs of Toxicity	Preliminary test One mid dose and one high dose female died on day 1. Clinical observations of salivation, slight hypoactivity, and/or lacrimation were observed prior to death. The remaining animals survived until the scheduled sacrifice and gained weight during the course of the study.

Clinical observations in survivors were salivation, red urine and/or faecal stains resolved by day 3.

Main test
Two of the males died on day 1 following observations of orange urine and faecal stains, and/or oral discharge. Three of the females died on day 0 or 1 following observations of orange urine and/or faecal stains, red discoloured faeces, soft faeces, hypoactivity, tremors and/or cold to touch. Observations in surviving animals included discoloured urine and/or faeces, urine and/or faecal stains, soft faeces, and no faeces and/or small amounts of faeces. These findings continued until study termination.

Effects in Organs

Preliminary test
Macroscopic findings in the females that died involved the stomach, ileum, duodenum and jejunum. Findings noted were red mucosal surfaces of the stomach and organs filled with red lumen fluid in both animals and distended stomach in one animal. No visible lesions were noted in the surviving animals.

Main test
Macroscopic findings in the animals that died involved the stomach, intestines, ileum, duodenum, jejunum and/or caecum with distended organs filled with orange lumen fluid in all five animals and red mucosal surface of the stomach in 2 males and 1 female. No visible lesions were noted in the surviving animals.

CONCLUSION — The notified chemical is harmful via the oral route.

TEST FACILITY — Covance (2002a)

7.2. Acute toxicity – dermal

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 402 Acute Dermal Toxicity – Limit Test.

Species/Strain	Rat/Crl: CD(SD)IGS BR
Vehicle	Test substance moistened with distilled water
Type of dressing	Occlusive
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	There were no test substance-related dermal reactions.
Signs of Toxicity - Systemic	Chromodacryorrhea and/or red nasal discharge was noted in all animals, All signs of toxicity had resolved by day 2.
Effects in Organs	No visible test substance related lesions were noted in any of the animals.

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

TEST FACILITY — Covance (2002b)

7.3. Irritation – skin (acute)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	2 males, 1 female
Vehicle	Test substance moistened with distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	0	0	n/a	0
Oedema	0	0	0	1	< 24 h	0

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

Remarks - Results

CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Covance (2002c)

7.3. Irritation – skin (repeat dose)

TEST SUBSTANCE	Notified chemical
METHOD	In house 14-day repeated dose toxicity study to assess the cumulative irritation potential when administered to the skin.
Species/Strain	Guinea pig/Himalayan Spotted
Number of Animals	Test Group: 3 male, 3 female Control Group: 1 male, 1 female
Vehicle	Bi-distilled water
Exposure Information	Concentrations applied: 5%, 3%, 1% and 0.5% Total exposure days: 14 days Dose regimen: 7 days per week Duration of exposure (dermal): 24 hours/day Post-exposure observation period: None
Type of Dressing	Test area left open.
Remarks - Method	Each test group animal had two application areas and was exposed to two different concentrations. Each concentration was applied to three different animals. The treated skin was flushed with lukewarm water prior to each new application. The animals were shaved four times during the first treatment week and three times during the second. All animals were depilated using an approved depilatory cream on day 15. After completion of the final skin assessment, the animals were sacrificed and the sites evaluated histopathologically

RESULTS

Signs of Irritation	Due to the repeated application and in spite of cleaning of the skin, a slight accumulation of the test substance was observed on the skin. Therefore, no grading scores could be recorded from day 2 to 14 as no depilation was performed during this period. On day 15 after the depilation procedure, slight erythema was observed on all sites treated with 5 and 3%, on two out of three test sites treated with the test

substance at 1% and on one out of three test sites treated with the test substance at 0.5%.

Subcutis isolated red foci were noted at one test site treated with 1% test substance. No macroscopic findings were recorded on the other animals at scheduled necropsy. When the sites were examined histopathologically, minimal to moderate hyperplasia were observed at most application sites, in some cases with dermal inflammatory cell infiltrate. These observations were not dose-related.

Mortality: No death occurred during the course of the study.

Signs of Toxicity: No clinical signs of toxicity were noted. One male animal marginally lost body weight during the acclimatisation period but this recovered between treatment start and the end of the study. The body weight of the other animals was within the range commonly recorded for this strain and age.

Remarks - Results

CONCLUSION: The notified chemical is slightly irritating to the skin under the conditions of this study.

TEST FACILITY: RCC Ltd (2000)

7.4. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3 females per trial
- Observation Period: 21 days (trial 1); 72 hours (trial 2)
- Remarks - Method: No significant protocol deviations

In trial 1, the notified chemical was administered as supplied. In trial 2, the notified chemical was administered as a 1% solution in cell culture grade water.

Corneal injury was visually assessed using sodium fluorescein dye.

RESULTS

Trial 1: 100% Notified chemical

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	2.67	2.67	2.67	3	21 days	1
Conjunctiva: chemosis	3	3.33	2.33	4	Between days 7 and 14	0
Conjunctiva: discharge	2	1.33	1.67	3	Between days 14 and 21	0
Corneal opacity	1	1.67	0.67	2	Between days 4 and 7	0
Iridial inflammation	1.33	2	1.33	2	Between days 7 and 14	0

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

Trial 2: 1% Notified chemical

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	0.33	1	Between days 1 and 2	0
Conjunctiva: chemosis	0	0	0	0	N/A	0
Conjunctiva: discharge	0	0	0	1	< 24 h	0
Corneal opacity	0	0	0	0	N/A	0
Iridial inflammation	0	0	0	0	N/A	0

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

Remarks - Results

There was no indication of pain in any animal upon administration of the test substance or shortly thereafter. There was no evidence of corrosion noted in any animal during the course of the study.

In trial 1, test substance was present in the eye of all the animals starting at 24 hours post administration until sacrifice. Two animals in had positive sodium fluorescein tests 24, 48, 72, and 96 hours post administration of the notified chemical and on day 5. On day 5, these two animals were sacrificed due to severe ocular irritation. The remaining animal had a positive sodium fluorescein test at 24 and 72 hours and a negative sodium fluorescein test at 96 hours post administration. The findings for this animal began to resolve on day 7 through to day 21 but effects in the conjunctivae had not fully reversed by day 21

CONCLUSION

The notified chemical is severely irritating to the eye.
A 1% solution of the notified chemical is slightly irritating to the eye.

TEST FACILITY

Covance (2002d)

7.5.1 Skin sensitisation-Maximisation test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 406 Skin Sensitisation – Magnusson and Kligman method
EC Directive 96/54/EC B.6 Skin Sensitisation - Magnusson and Kligman method

Species/Strain

Guinea pig/ Himalayan spotted

PRELIMINARY STUDY

Maximum Non-irritating Concentration:
intradermal: grade 1 erythema and oedema observed at a concentration of 1%, 3% and 5%.
topical: 50% test substance in bi-distilled water.

MAIN STUDY

Number of Animals

Test Group: 20 Control Group: 10

INDUCTION PHASE

Induction Concentration:
intradermal: 5% test substance in bi-distilled water
topical: 50% test substance in bi-distilled water

Signs of Irritation

intradermal: A normal development of the local symptoms was reported in the animals of the control and test group after the intradermal injection. No details of these symptoms or individual scores were provided.

topical: The test sites were pre-treated with 10% sodium lauryl sulphate 24-hours before topical induction. Slight erythema was noted in two out of 10 animals in the control group animals. As the test substance stained the skin red, it was not possible to determine whether erythema was present in the test animals. However, no oedema was observed.

CHALLENGE PHASE

1st challenge	topical: 50% test substance in bi-distilled water
Remarks - Method	No significant protocol deviations.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50%	0/20	0/20	-	-
Control Group	50%	0/10	0/10	-	-

Remarks - Results

Approximately 21 hours after removal of the challenge application the test sites were depilated to clean them from the brown-red discolouration produced by the test substance in order to facilitate the evaluation of a possible erythema reaction.

The concentrations stated refer to the concentration of the test substance and do not take into account the purity of the notified chemical.

CONCLUSION

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

TEST FACILITY

RCC Ltd (1996a)

7.5.2. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE

Notified chemical

METHOD

Species/Strain	Mouse/CBA/J
Vehicle	Ethanol/water (50:50)
Remarks - Method	No significant protocol deviations, however, no justification was supplied as to why higher concentrations of the notified chemical were not tested. An ethanol/water mixture was chosen due to unsatisfactory solubility of the test substance in the first recommended vehicles acetone/olive oil (4/1, v/v) and dimethylformamide.

RESULTS

Concentration (%)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0	31.60	
0.25	49.01	1.55
0.5	71.46	2.26
1	102.98	3.26
2.5	79.70	2.52
5	122.73	3.88
Positive Control		
25% HCA	428.52	13.56

Remarks - Results

The study calculated an extrapolated EC3 value of 3.12%. This assumed a linear dose response and treated the value at 1% as an outlier based on the regression coefficients (R^2) calculated for the data values including

(0.61) and excluding (0.91) the 1% concentration data.

However the dose response profile (linear response to 1% (R^2=0.995) followed by a flattening out) may suggest either that saturation kinetics for absorption have been achieved or that maximal immune stimulation has been induced. (This approach would assume that the data obtained at the 2.5% concentration was the outlier). Based on this assumption the EC3 value would be estimated as 0.9%.

No mortality or clinical signs were observed during the study. No increase in ear thickness was observed in the animals of the treated groups. A red colouration of the skin which could have masked a possible discrete to moderate erythema was noted on the ears of all treated animals from day 2 up to the end of the study (day 6).

A dose-related increase in the stimulation index was noted and the threshold positive value of 3 was exceeded at concentrations of 1 and 5%.

CONCLUSION — There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY — CIT (2002)

7.6.1 Phototoxicity

TEST SUBSTANCE — Notified Chemical

METHOD — CTFA Safety Test Guidelines (1991)
OECD Draft Acute dermal photoirritation dose-response test

Species/Strain — Guinea Pig/Himalayan spotted
Number of Animals — Test Group: 10 Control Group: 5
Vehicle — Bi-distilled water
Remarks - Method — Different concentrations of the test substance was applied to the left flank test site. After 30 minutes this test site was exposed to non-erythematogenic UV-A irradiation. After irradiation the test substance was applied to the right flank but this site remained unexposed to light. Control animals were exposed to UV-A similarly, except they were treated with vehicle only. Animals were examined 24, 48 and 72 hours after application of the test substance for signs of erythema and oedema.

RESULTS

	Concentration	*Number of Animals Showing Skin Reactions after:*					
		UV-irradiated			*Non-irradiated*		
		24 h	*48 h*	*72h*	*24 h*	*48 h*	*72 h*
Test Group	50%	0/10	0/10	0/10	0/10	0/10	0/10
	25%	0/10	0/10	0/10	0/10	0/10	0/10
	15%	0/10	0/10	0/10	0/10	0/10	0/10
	10%	0/10	0/10	0/10	0/10	0/10	0/10
Control Group	0%	0/5	0/5	0/5	0/5	0/5	0/5

Remarks - Results

CONCLUSION — The notified chemical does not exhibit a phototoxic potential under the conditions of the test.

TEST FACILITY RCC Ltd (1998d)

7.6.2 Photoallergenicity

TEST SUBSTANCE Notified chemical

METHOD CTFA Safety Test Guidelines (1991)
OECD Draft Acute dermal photoirritation dose-response test method

Species/Strain Guinea pig/ Himalayan spotted

Vehicle Bi-distilled water

PRELIMINARY STUDY Maximum Non-irritating Concentration:
topical: 50% test substance in bi-distilled water (from phototoxicity study see 7.6.1)

MAIN STUDY

Number of Animals Test Group: 20 Control Group: 10

INDUCTION PHASE intradermal: 1:1 Freund's Complete Adjuvant and physiological saline
topical: 50% test substance in bi-distilled water
irradiation: 1.8 J/cm^2 UV-B and 10 J/cm^2 UV-A

Topical application followed by irradiation was repeated on day 3, 7, 9 and 11.

Signs of Irritation intradermal: A normal development of the local symptoms including erythema, oedema, necrotising dermatitis, encrustation and exfoliation were reported in the animals of the control and test group after the intradermal injection.

topical: No skin reactions were observed in the test animals.

CHALLENGE PHASE Three weeks after the induction phase concentrations of 50, 25, 15 and 10% of the test substance were applied to the left flank. The left flank of each animal was then exposed to 10 J/cm^2 UV-A irradiation. After irradiation of the left flank, the right flank was treated the test substance accordingly without irradiation. Cutaneous reactions were evaluated at 24, 48 and 72 hours after the challenge procedure.

RESULTS

	Concentration	*Number of Animals Showing Skin Reactions after challenge:*					
		UV-irradiated			*Non-irradiated*		
		24 h	*48 h*	*72h*	*24 h*	*48 h*	*72 h*
Test Group	50%	0/20	0/20	0/20	0/20	0/20	0/20
	25%	0/20	0/20	0/20	0/20	0/20	0/20
	15%	0/20	0/20	0/20	0/20	0/20	0/20
	10%	0/20	0/20	0/20	0/20	0/20	0/20
Control Group	50%	0/10	0/10	0/10	0/10	0/10	0/10
	25%	0/10	0/10	0/10	0/10	0/10	0/10
	15%	0/10	0/10	0/10	0/10	0/10	0/10
	10%	0/10	0/10	0/10	0/10	0/10	0/10

Remarks - Results A very slight discolouration produced by the test substance at the application site was observed from test day 2 to 23 (i.e. one day after the challenge application)

CONCLUSION The notified chemical does not exhibit a photoallergenic and allergenic potential under the conditions of the test. [Delete as appropriate]

TEST FACILITY RCC Ltd (1998e)

7.7.1. Repeat dose toxicity – 14 day oral toxicity study in rats

TEST SUBSTANCE	Notified chemical
METHOD	Based on OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Wistar
Route of Administration	Oral –diet
Exposure Information	Total exposure days: 14days Dose regimen: ad libitum Post-exposure observation period: 14 days (control and high dose treated animals only)
Vehicle	Feed
Remarks - Method	No significant deviations from the protocol. This study was used to select appropriate doses for a 90-day oral study and therefore only a 14-day exposure period was used. The doses were selected based on a 5-day dose range finding study.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg bw*		*Mortality*
		Nominal	*Actual*	
I (control)	5 per sex	0	0	0
II (low dose)	5 per sex	20	15.5	0
III (mid dose)	5 per sex	70	53.4	0
IV (high dose)	5 per sex	250	186.4	0
V (control recovery)	5 per sex	0	0	0
VI (high dose recovery)	5 per sex	250	186.4	0

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases.

Clinical Observations

Orange colouration was observed in the urine of all animals, the level discolouration increased with dose. Decreased food consumption was observed in mid (6% reduction) and high dose (11% reduction) males and high dose females (5% reduction); these observations were considered to be test substance related. During the recovery period, the mean daily food consumption improved slightly but remained less (5-8%) than that of controls. Mean body weight and mean body weight gain were lower (3-6%) in the high dose group compared to controls. No effect on the functional observation battery or grip strength were observed. The total locomotor activity in the high dose females was significantly less ($p<0.05$) than that of control females, but in the absence of a dose-response relationship or similar findings in males, this finding was considered to be unrelated to the test substance.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

A few statistically significant differences to the control values were noted in the treated males and females. A statistically significant increase (65%, $p<0.05$) in triglyceride levels was noted in the high dose males, however, the levels found were nearly identical to those noted in the control recovery males and as such the difference is considered to be incidental. A statistically significant ($p<0.05$) slight increase in albumin (3.5%) and total protein (4%) was noted in high dose females, however, these results were considered to be incidental with similar levels found in both recovery groups. All other differences were not dose dependent and considered to be unrelated to the treatment with the test substance.

Haematology

A few statistically significant differences ($p<0.05$) for certain haematological parameters were noted in high

dose animals and a prolonged activated partial thromboplastin time (20% increase) was noted in mid-dose females. These differences were generally minor, not observed in both males and females and without a dose-response relationship and were considered to be incidental.

Urinalysis
The pH of urine collected from high dose females was slightly more alkaline (pH6.6, $p<0.05$) than that of control animals. This was within the range of the historical control data and therefore considered to be incidental.

Effects in Organs
Organ Weight
No test substance-related differences to the absolute or relative organ weights were observed.

Gross Pathology
Macroscopic findings including bilateral dilation of the uterine horns and renal pelvic dilation were observed in both control and treated animals and therefore considered not to be test substance related.

Histopathology
All microscopic findings recorded were within the normal range of background findings for rats of this strain and age and occurred at similar incidences and severity in both control and treated animals.

Remarks – Results

CONCLUSION
The No Observed Effect Level (NOEL) was established as 15.5 mg/kg bw/day in this study, based on decreased food consumption and decreased body weight gain observed at higher doses.

TEST FACILITY RCC Ltd (1999a)

7.7.2 Repeat dose toxicity – 90 day oral toxicity study in rats

TEST SUBSTANCE Notified chemical

METHOD OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.
EC Directive 88/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.

Species/Strain	Rat/Wistar
Route of Administration	Oral –diet
Exposure Information	Total exposure: 13 weeks Dose regimen: Ad libitum Post-exposure observation period: None
Vehicle	Feed
Remarks - Method	Deviations from OECD TG 408 - epididymis and uterus weight not measured. No other significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg bw/day*		*Mortality*
		Nominal	*Actual*	
I (control)	10 per sex	0	0	0
II (low dose)	10 per sex	20	18 (m), 19 (f)	0
III (mid dose)	10 per sex	70	63 (m), 65.8 (f)	0

IV (high dose)	10 per sex	250	229 (m), 232 (f)	0

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

Clinical Observations
Both males and females of group IV excreted orange urine from day 3 and orange faeces from day 4 until the end of the study. One control group and high dose group male showed alopecia and/or scabbed wound on distinct parts of the body. This was considered to incidental. Mean feed consumption over treatment period was reduced in group IV males by 17% and group IV females by 8% compared with concurrent controls. Group IV males showed distinct lower body weights (24% lower at end of study) and bodyweight gain from week 5 of treatment until the end of the study. These findings, as well as a slight reduction in body weight gain of group IV females (8% lower at end of study), were considered to be treatment related and correlated with the reduction in mean food consumption.

There were no test–article related clinical signs or remarkable body weight or feed consumption changes in group II and group III animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Clinical Chemistry
The following statistically significant differences were noted in group IV animals: decreased glucose (28%, $P<0.01$) and urea (11%, $P<0.05$) levels in males, decreased creatine levels in males and females (9%, $P<0.01$), increased triglyceride (115%, $P<0.01$) and phospholipid (22%, $P<0.01$) levels in males and increased total cholesterol (29%, $P<0.01$) and triglyceride (28%, $P<0.05$) levels in females, increased albumin/globulin ratio (22%, $P<0.05$) in males and reduced absolute A1-globulin levels (18%, $P<0.01$) in males and females, decreased total protein levels (8%, $P<0.01$) in females and increased gamma glutamyltransferase (46%, $P<0.05$) in females. All findings were considered to be metabolic adaptations to the test substance.

Haematology
A significant increase in methemoglobin levels was noted in group IV males (125%, $P<0.05$) and females (100%, $P<0.05$). All other differences in haematological parameters between control and treated animals were considered to be incidental and unrelated to treatment.

Urinalysis
There were considered to be no test substance related effects.

Effects in Organs
Organ Weights
A number of differences in absolute and relative organ weight between control and group IV animals were noted. The majority of these were considered to be secondary to the reduced bodyweight observed in the group IV animals. The interpretation of these differences resulted in only two remarkable changes, a significant increase in relative kidney weight (~18%, $P<0.001$) and a decrease (but not significant) in relative heart weight (~7%).

Gross Pathology
The macroscopic findings noted in this study at the end of the treatment period did not distinguish treated from control animals.

Histopathology
The following histopathological findings were noted:
Liver: Centrilobular hepatocellular hypertrophy of minimal severity in 4 out of 10 mid-dose males. Diffuse hepatocellular hypertrophy of minimal to slight severity in all high dose males and 9 out of 10 high-dose females. This hepatocellular hypertrophy was considered to represent an adaptive metabolic response to treatment. There was no evidence of fibrosis or necrosis.

Lungs: Alveolitis of minimal to moderate degree in 1 out of 10 mid-dose males and 4 out of 10 high-dose males as well and minimally increased incidence of alveolitis in high dose females. A slightly increased incidence and minimally increased severity of alveolar histiocytosis were noted in high-dose males. A minimally to slightly increased incidence and/or severity of perivascular cuffing were noted in high-dose male

or female rats. These effects were considered to be as a result of inhalation of the test-substance during gavage.

Thymus: A slightly increased incidence of cortical atrophy was noted in mid- and high-dose males.

Mandibular lymph node: A minimally increased severity of lymphoid hyperplasia was noted in high-dose male and female animals.

The incidence, severity and morphologic appearance of all other microscopic findings noted in this study was similar in treated and control animals.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 63 mg/kg bw/day in this study, based on the treatment related effects observed at 250 mg/kg/day. The No Observed Effect Level was established as 18 mg/kg bw/day based on the histopathological findings observed in the mid-dose animals.

TEST FACILITY RCC Ltd (1999b)

7.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.

	Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver (standard) and S9 fraction from uninduced male golden Syrian hamster liver (reductive)
Concentration Range in Main Test	<u>Test 1</u> a) With standard metabolic activation: 33.3 - 3330 µg/plate (*All strains*) b) With reductive metabolic activation: 33.3 - 3330 µg/plate (*All strains*) c) Without metabolic activation: 10 – 2000 µg/plate (*S. typhimurium*) 3.33 – 500 (*E.coli*) <u>Test 2</u> a) With standard metabolic activation: 33.3 - 1000 µg/plate (*All strains*) b) With reductive metabolic activation: 33.3 - 2000 µg/plate (*All strains*) c) Without metabolic activation: 3.33 – 500 (*All Strains*)
Vehicle	Water
Remarks - Method	No significant protocol deviations. All strains were tested in the presence of both standard and reductive activation.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	333 (WP2uvrA), 33.3 (TA100)			

Test 1		10 (TA98, TA100, TA 1535), 33.3 (TA1537), 100 (WP2uvrA)	> 2000 (*S. typhimurium*), > 500 (*E.coli*)	negative
Test 2		33.3 (TA98, TA100, TA1535), 100 (TA1537, WP2uvrA)	> 500 (all strains)	negative
Present (standard)	333 (WP2uvrA, TA100)			
Test 1		333 (all strains)	> 3300 (all strains)	weak positive (TA98)
Test 2		333 (all strains)	> 1000 (all strains)	weak positive (TA98)
Present (reductive)	-			
Test 1		1000 (all strains)	> 3300 (all strains)	positive (TA98) weak positive (TA100)
Test 2		1000 (*S. typhimurium*), 2000 (E.coli)	> 2000 (all strains)	Positive (TA98)

Remarks - Results

Absence activation: In the initial assay, revertant frequencies for all doses of the test substance in all tester strains was comparable or less than those observed in the concurrent negative control cultures. Inhibited growth was observed in tester strains TA98, TA100, TA 1535 at all dose levels. This result was supported in the confirmatory assay

Standard activation: In the initial assay, a 2.3 fold increase in revertant frequency compared with concurrent controls was observed in strain TA98 when dosed at 100 µg/plate. A reduction in the background lawn and a reduction in the number of revertants per plate was observed at higher doses. Revertant frequencies for all doses of the test substance in all other tester strains was comparable or less than those observed in the concurrent negative control cultures. This weak positive response in strain TA98 was confirmed in the confirmatory assay with a 3.1 fold increase observed at dose 100 µg/plate

Reductive activation: Dose-dependent increases in revertant frequency, to approximately 7.2-fold control values (at 333 µg/plate) were observed in strain TA98. A 2-fold increase in revertant frequency was seen in the two top doses (3330 µg/plate and 5000 µg/plate) in tester strain TA100). Revertant frequencies for all doses of the test substance in all other tester strains was comparable or less than those observed in the concurrent negative control cultures. The positive response in strain TA98 was confirmed in the confirmatory assay with a dose-dependent increase to approximately 15.5-fold increase however, the weak positive response observed in TA100 in the initial assay was not repeated in the confirmatory assay.

Negative controls were within historical limits. Positive controls confirmed the sensitivity of the test system.

CONCLUSION

The notified chemical was mutagenic to bacteria under the conditions of the test.

TEST FACILITY

Covance (2002e)

7.9.1 Genotoxicity – in vitro chromosome aberration assay (Chinese Hamster cells)

TEST SUBSTANCE — Notified Chemical

METHOD — OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Cell Line	Chinese hamster V79 cells
Metabolic Activation System	S9 fraction from Phenobarbital/β-flavone induced rat liver
Vehicle	Culture medium (MEM)
Remarks - Method	No significant protocol deviations.

Both tests were only performed with a standard metabolic activation system.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1a	5, 10, 20*, 50*, 100*, 200*	18 h	18 h
Test 1b	20, 50*, 100*, 200	28 h	28 h
Test 2a	10*, 20, 50*, 100*, 200, 300	18 h	18 h
Test 2b	50*, 100, 200, 300	28 h	28 h
Present			
Test 1a	10, 20*, 50, 100*, 200*, 500	4 h	18 h
Test 1b	50, 100*, 200*, 500	4 h	28 h
Test 2a	10, 20*, 50*, 100*, 200, 300*	4 h	18 h
Test 2b	50*, 100*, 200, 300*	4 h	28 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test**	*Precipitation*	*Genotoxic Effect*
Absent	300			
Test 1a		200	100	positive
Test 1b		100	100	positive
Test 2a		200	100	equivocal
Test 2b		100	> 50	negative
Present	300			
Test 1a		100	200	positive
Test 1b		500	200	positive
Test 2a		>300	100	positive
Test 2b		>300	100	positive

* based on <50% reduction in cell numbers

Remarks - Results

In the absence of activation in test 1 after treatment with 200 µg/mL at interval 18 h (6.5% aberrant cells exclusive gaps) and 100 µg/mL at interval 28 h (10% aberrant cells exclusive gaps) the aberration rates were significantly increased compared to the corresponding controls (0% and 0.5% respectively). These results at concentrations exhibiting precipitation, strong reduced cell numbers (24.2 % and 47.5%) and reduced mitotic indices (77.8% and 62.1%) were not reproduced in test 2. In test 2, evaluation of cultures after treatment with 200 µg/mL (18 h interval) and 100 µg/mL (28 h interval) was not feasible due to strong reduced cell numbers (38.5% and 36.1%) in combination with poor metaphase quality and the occurrence of micronuclei and fragmentation of the nuclei.

In the presence of activation in test 1 (28 h), a significant increase in the aberration frequency was observed after treatment with 200 µg/mL. In the independent test 2, cultures after treatment with 300 µg/mL revealed

significant increased aberration frequencies (18 h: 14% aberrant cells exclusive gaps; 28 h 14.5 % aberrant cells exclusive gaps). In addition, at interval 18 h, cultures after treatment with 50 µg/mL and 100 µg/mL exhibited increased aberration frequencies (5% aberrant cells exclusive gaps and 6% aberrant cells exclusive gaps) slightly beyond the historical control range (0 – 4% aberrant cells exclusive gaps).

In both experiments, no biologically relevant increase in the rate of polyploid metaphases were found after treatment with the test substance compared to the rates of the controls.

In both experiments the positive controls showed distinct increases in cells with structural chromosome aberrations.

Overall, in both experiments, in the absence and presence of activation, increased frequencies of cells carrying structural aberrations were observed. However, the induction of structural aberrations was observed only after treatment with concentrations of the test article at which precipitation was observed. An indirect mechanism for DNA damaging may be involved.

CONCLUSION — The notified chemical was clastogenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY — RCC Ltd (1997)

7.9.2 Genotoxicity – in vitro chromosome aberration assay (Human Lymphocytes)

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Cell Line	Human peripheral blood lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Cell culture grade water
Remarks - Method	No significant protocol deviations.

Both tests were only performed with a standard metabolic activation system.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	16.5, 23.6, 33.7*, 48.1*, 68.7*, 98.1*, 140, 200, 285, 408, 582, 832, 1190, 1700, 2430	3 h	22.1 h
Test 2	3.13*, 6.25*, 12.5*, 25.0*, 37.5, 50.0, 75.0, 100, 150, 200	22 h	22 h
Present			
Test 1	16.5, 23.6, 33.7, 48.1, 68.7, 98.1*, 140*, 200*, 285*, 408, 582, 832, 1190, 1700, 2430	3 h	22.1 h
Test 2	25.0*, 50.0*, 100*, 200*, 250, 349, 400	3 h	22 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test**	*Precipitation*	*Genotoxic Effect*
Absent	-			
Test 1		98.1	> 2430	weak positive
Test 2		25	> 200	negative

Present	-			
Test 1		285	> 2430	negative
Test 2		250	> 400	negative

* based on <50% reduction in mitotic index

Remarks - Results

Test 1

In the assay without metabolic activation, due to excessive toxicity only dead cells were observed on the slides treated with ≥ 285 µg/mL. No significant increase in chromosomal aberrations, polyploidy or endoreduplication was observed at the concentrations analysed, except for a weak increase in cells with chromosomal aberrations (6% aberrant cells exclusive gaps) in the cultures treated with 98.1 µg/mL, a cytotoxic dose level (54% reduction in mitotic index). Evidence of cytotoxicity was observed at 68.7 µg/mL which had no evidence of clastogenicity. This raises the strong possibility that clastogenicity observed at 98.1 µg/mL is associated with the cytotoxicity and therefore not relevant biologically, since this clastogenicity is probably induced indirectly only above a certain threshold concentration. In addition, the response was primarily in cells with simple breaks.

In the presence of metabolic activation, no significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analysed.

Test 2

In the absence and presence of activation, no significant increase in chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analysed.

CONCLUSION

The notified chemical was considered to be non-clastogenic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY

Covance (2002f)

7.9.3 Genotoxicity – in vitro mammalian cell gene mutation test

TEST SUBSTANCE

Notified Chemical

METHOD

OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

Species/Cell Line

Chinese hamster V79 cells

Metabolic Activation System

S9 fraction derived from Syrian hamsters (reductive) (test 1)
S9 fraction from Aroclor 1254 induced rat liver (test 2)

Vehicle

Cell culture grade water

Remarks - Method

HPRT test. No significant protocol deviations reported.

The assay was performed in two independent experiments with and without liver microsomal activation. Hamster S9 mix was used in the first experiment to detect possible mutagenic affects of diazonium groups as chemical moiety of the test substance. Since a negative result was obtained in test 1, rat S9 mix was used in test 2.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	3*, 10, 30*, 100*, 300*, 600*	4 h	**	**
Test 2	3*, 10, 30*, 100, 300*, 400*	4 h	**	**
Present				
Test 1	3*, 10, 30*, 100* 300*, 600	4 h	**	**

Test 2	3*, 10, 30*, 100*, 300*, 400	4 h	**	**

*Cultures selected for metaphase analysis.
** the selection and expression time were ambiguous in the report.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1		300	> 600	negative
Test 2		300	> 400	negative
Present				
Test 1		300	> 600	negative
Test 2		300	> 400	negative

Remarks - Results

The number of mutant frequencies in either the presence of standard or reductive activation or absence of activation remained well within the range of historical negative controls. Since very low numbers of spontaneous mutant colonies occurred in some of the actual negative controls, the factor of mutant colonies divided by the number of colonies in the controls exceeded the threshold of 3 in some of the test points. This effect is considered to be a result of the low negative control values rather than a mutagenic effect. Furthermore, there was no reproducible concentration dependent increase of the number of colonies.

The positive control confirmed the sensitivity of the study.

CONCLUSION

The notified chemical was not mutagenic to Chinese hamster V79 cells treated in vitro under the conditions of the test.

TEST FACILITY

RCC Ltd (1996b)

7.10.1 Genotoxicity – in vivo mammalian erythrocyte micronucleus test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/NMRI
Route of Administration: Oral – gavage
Vehicle: Deionised water
Remarks - Method: No significant protocol deviations.

Animals were treated with the test substance once. Four preliminary dose-range finding assays were conducted. According to clinical signs and toxic reactions of the mice, 300 mg/kg bw was estimated to be close to the maximum tolerated dose.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5 per sex	0	24
II (low dose)	5 per sex	30	24
III (mid dose)	5 per sex	100	24
IV (high dose 1)	5 per sex	300	24
V (high dose 2)	5 per sex	300	48
VI (positive control, CP)	5 per sex	40	24

CP=cyclophosphamide. M=mitomycin C.

RESULTS

Doses Producing Toxicity	Two group IV animals died during the study, however no deaths occurred in group V. The mean number of normochromatic erythrocytes (NCEs) was dose-dependently increased after treatment with the test substance as compared to the mean value of NCEs of the corresponding vehicle control indicating that the test substance had cytotoxic properties in the bone marrow. The effect was most pronounced at preparation interval 48 h.
Genotoxic Effects	There was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei compared to the corresponding control in any of the treated groups (II – V).
Remarks - Results	The positive control confirmed the sensitivity of the study.

CONCLUSION — The notified chemical was not clastogenic under the conditions of this in vivo mouse micronucleus test.

TEST FACILITY — RCC Ltd (1998f)

7.10.2 Genotoxicity – in vivo unscheduled DNA synthesis (UDS) test

TEST SUBSTANCE — Notified chemical

METHOD — OECD TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo.

Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Vehicle	Deionised water
Remarks - Method	No significant protocol deviations.

Animals were treated with the test substance once. Two preliminary dose-range finding assays were conducted. According to clinical signs and toxic reactions of the mice, 400 mg/kg bw was estimated to be close to the maximum tolerated dose.

The positive control for the early sampling time was chosen as N,N'-Dimethylhydrazinedihydrochloride (DMH).

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
Early sampling time			
I (vehicle control)	3 male	0	3 h
II (low dose)	3 male	100	3 h
III (high dose)	3 male	400	3 h
IV (positive control, DMH)	3 male	40	3 h
Late sampling time			
V (vehicle control)	3 male	0	16 h
VI (low dose)	3 male	100	16 h
VII (high dose)	3 male	400	16 h
IV (positive control, AAF)	3 male	100	16 h

AAF=2-acetylaminofluorene, DMH= N,N'-Dimethylhydrazinedihydrochloride.

RESULTS

Doses Producing Toxicity	No signs of systemic toxicity were noted for the main study. In the preliminary dose range finding study a reduction of spontaneous activity, eyelid closure, piloerection and apathy were noted in animals dosed with 400 mg/kg bw. In the main study, the viability of the hepatocytes was not substantially effected by the treatment.
Genotoxic Effects	No dose level of the test substance revealed UDS induction in the hepatocytes of the treated animals as compared to the current vehicle controls. Neither the nuclear grains nor the resulting net grains were distinctly enhanced due to the *in vivo* treatment. Therefore, the net grain values obtained after treatment with the test substance were consistently negative. The percentage of cells in repair did not significantly differ from the control group.
Remarks - Results	The positive controls confirmed the sensitivity of the test.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo unscheduled DNA synthesis (UDS) test.
TEST FACILITY	RCC Ltd (1999c)

7.11. Developmental toxicity

TEST SUBSTANCE	Notified chemical
METHOD	Commission of the European Communities, No. III/3387/93, according to ICH guidelines
Species/Strain	
Route of Administration	Oral – gavage/diet/drinking water
Exposure Information	Exposure days: days 6 to 17 post coitum. Post-exposure observation period: 4 days
Vehicle	Bi-distilled water
Remarks - Method	Deviation from OECD TG 414 Prenatal Developmental Toxicity Study. Treatment stopped 4 days before the females were killed and foetuses were removed. No other significant protocol deviations.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
I	22*	0	0
II	22	15	0
III	22*	60	0
IV	22	240	0

*21 females pregnant.

Mortality and Time to Death

There were no mortalities during the course of the study.

Effects on Dams

Signs of Toxicity

Orange discoloured cage bedding was observed from the third day of treatment and persisted to end of the study for all group III and IV animals. Food consumption was dose dependently reduced during the treatment period in groups 3 (~8.6%) and 4(~2.9%). Following the treatment period the food consumption was similar to that of the control group. Body weight gain was similarly reduced in group III and IV animals.

Necropsy findings

The uterus was reddened in one control group female. No maternal abnormalities were noted in any of the treated animals.

Reproduction data
Mean post-implantation loss and mean number of foetuses per dam were similar between treated and control dams.

Effects on Foetus
Mean foetal body weights was slightly but significantly reduced in group IV for male (3.8%, P<0.01) and female (3.6%, P<0.01) foetuses. Statistically significant differences were observed in the sex ratio of foetuses in the 60 and 240 mg/kg bw/day groups in which there was a higher proportion of female foetuses. The total litter size and total implantation loss were unaffected by treatment.

External Examination
Abdominal hernia in one low dose foetus and underweight, cleft palate and tail defects in four high dose foetuses were noted during external examination of the foetuses.

Skeletal examination (abnormal findings)]
There were a small number of abnormal skeletal findings involving abnormally shaped sternebrae or wavy ribs in all groups. The low dose animal with abdominal hernia showed bifurcation of the sternum with rib abnormalities. The four high doe foetuses with serious external abnormality showed thoracic skeletal changes including the presence of rib fragments and abnormalities consistent with the tail and limb changes.

Skeletal Examination (Stage of development)
There was an increase in findings involving absent or reduced ossification in comparison to the controls.

Remarks - Results
The difference in sex ratio is considered not to be treatment related since implantation occurred prior to dosing and there was no evidence of a sex selective effect on in utero survival.

The four high dose foetuses with serious external abnormalities were from one litter (and adjacent to each other in the same uterine horn). As no similar abnormalities were observed in any other litters, these findings are considered to be incidental. The delayed ossification is considered to be attributed to the immaturity of the skeleton and probably correlated to the lower foetus weights observed.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) for maternal and foetal effects was established as 60 mg/kg bw/day in this study, based on effects observed.

TEST FACILITY RCC Ltd (1999d)

7.12. Pharmacokinetic/toxicokinetic

TEST SUBSTANCE Hair dye formulation (0.2% notified chemical)

METHOD Dermal Absorption/Percutaneous penetration following the recommendations of the COLIPA guidelines (COLIPA, 1995) and the opinion of the Consumer Health Protection Scientific Committee (SCCNFP, 1999).

STUDY DESIGN AND OBJECTIVE

A representative hair dye formulation containing the notified chemical as 0.2% (w/w) was applied to human epidermal skin membranes mounted in Franz type diffusion cells at a target dose of 100 mg/cm^2 (200 µg/cm^2 notified chemical). After a 30 minute exposure period the skin surface was rinsed with water. Permeation of the notified chemical through the skin and into the receptor phase (pH 7.4 phosphate buffered saline/25% ethanol) was monitored over the subsequent 48-hour period. After 48 hours the diffusion cells were dismantled, the skin surface wiped, the donor chambers rinsed and the skin tape stripped and all and the remaining skin samples were analysed for notified chemical and a full mass balance calculated. Twelve replicates were conducted and the application regimen contained four replicates of three different skin donors.

RESULTS

The measured average applied dose was 202 µg/cm^2 and the average recovery of the notified chemical (from the wash, wipe, tape strips, remaining skin and receptor phase) was 99.0±0.02% of the applied dose. Permeation of the notified chemical through the skin was detected in five of the samples treated with the hair dye formulation and after 48 hours represented 0.005±0.002% of the applied dose (equivalent to 0.010±0.004 µg/cm^2). The total amount of the notified chemical recovered from the deeper layers of the skin (the remaining skin sample) amounted to 0.008±0.004 µg/cm^2 (<0.005% of the applied dose).

CONCLUSION

Considering together the amount of notified chemical in the remaining skin and receptor phase, the total amount of notified chemical absorbed from the formulation is 0.009% of the applied dose or 0.018±0.005 µg/cm^2.

TEST FACILITY An-eX Analytical Services (2000)

8. ENVIRONMENT

8.1. Environmental fate

No environmental fate data were submitted. The notified chemical is unlikely to be readily biodegradable.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - Static.
Species	*Brachydanio rerio* (Zebra Fish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Water temperature, pH and dissolved oxygen concentration.
Remarks – Method	The test medium of the only concentration of nominal 100 mg/L was prepared dissolving 400 mg of the notified chemical completely in 4 L of test water by intensive stirring for 10 minutes. The test medium was freshly prepared just before the start of the test. The water temperature, pH and dissolved oxygen concentrations in the test medium of the single test concentration and the control were measured before the start of the test and once every day during the test. The measured pH values ranged from 8.1-8.2. The dissolved oxygen concentrations were always 8.1 mg/L or higher, and thus higher than 60% oxygen saturation. The water temperature ranged from 21-22ºC. At the same time the appearance of the test medium was recorded. Throughout the test, the test medium was strongly coloured by the test item, however, the test fish were observable. The NOEC and LC0 were determined directly from the raw data. The LOEC, the LC50 and LC100 could not be quantified due to the absence of a toxic effect of the test item at the tested concentration.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3.5h*	*24h*	*48h*	*72h*	*96h*
0	0	7 per aquarium	0	0	0	0	0
100	101-109	7 per aquarium	0	0	0	0	0

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	In the control and the test medium with the test item concentration of 100 mg/L all fish survived until the end of the test and no signs of intoxication were observed.
CONCLUSION	The notified chemical is practically non-toxic to *Brachydanio rerio*.
TEST FACILITY	RCC Ltd (1998g)

8.2.2. Algal growth inhibition test

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Directive 92/69/EEC C.3 Algal Inhibition Test.
Species	*Scenedesmus subspicatus*
Exposure Period	72 hours
Concentration Range Nominal	0.46, 1.0, 22, 4.6, 10 and 22 mg/L
Concentration Range Actual	0.4mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Water temperature and pH
Remarks – Method	The test medium of the highest test concentration was prepared by dissolving 22 mg/L of the notified chemical completely in 1000 mL test water by stirring for 15 minutes. Adequate volumes of this intensively mixed test medium were added to test water to prepare the test media of the lower nominal test concentrations. The test was started by inoculation of a biomass of 10,000 algal cells per mL test medium, with cells taken from an exponentially growing pre-culture, which was set up 3 days prior to the test at the same conditions as in the test. The mean analytically determined test item concentrations in the analysed test media varied in the range from 92-98% of the nominal value. In the test media, incubated under the test conditions, the test item was sufficiently stable during the test period of 72 hours. Therefore, all biological results are related to the nominal concentrations of the test item. The E_bC50 and $E_\mu C50$ and the corresponding EC10 and EC90 and their 95% confidence limits were calculated as far as possible by Probit analysis. For the determination of the LOEC and NOEC, the calculated mean biomass and the mean growth rate at the test concentrations were tested on significant differences to the control values by a Dunnett-Test.

RESULTS

Biomass		*Growth*	
EC_b50	*NOEC*	*EC_r50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
8.5	4.6	17	4.6

Remarks – Results	The LOEC of 10 mg/L was also the lowest test concentration at which indirect effects on growth occurred by light absorption. At the start of the test, the pH value in the test media was 8.0 and at the end of the test pH values were measured between 7.9 and 9.0. The increase in pH was ascribed to CO_2 consumption via rapid growth. Growing conditions remained within the acceptable limits throughout the test.
CONCLUSION	The notified chemical was toxic to *Scenedesmus subspicatus* under the conditions of the test.
TEST FACILITY	RCC Ltd (1998h)

8.2.3. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 67/548/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Activated sludge obtained from a communal wastewater treatment plant.
Exposure Period	3 hours
Concentration Range Nominal	0.52, 1.28, 3.20, 8 and 20 mg/L
Remarks – Method	Static test at 20.1°C. Reference item: 3,5-dichlorophenol. Statistical analysis using probit model (Finney D.J., 1971).
RESULTS	
IC50	44.5 mg/L
NOEC	3.2 mg/L
Remarks – Results	The IC50 of the reference substance was 9.8 mg/L under the conditions of the test, thus validating the test.
CONCLUSION	The notified chemical was inhibitory to sludge bacteria under the conditions of the test.
TEST FACILITY	Solvias (2004)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The notified chemical will be imported as a component (0.5%) of a finished hair dye product for use in hair salons and the consumer market. Initially, no local manufacturing will occur, although reformulation in the form of repackaging may be required. Up to 95% of the notified chemical is expected to end up in the sewer during end use when excess dye is washed from the hair after treatment. A further 5% could end up in landfill as residues in used containers.

The repackaging site has a wastewater treatment system comprising a 100 000 L averaging tank, a solids separator, a grease remover, automatic pH adjustment and a dissolved air flotation (DAF) tank. It is estimated that 40 000 L/day will be treated. Based on 150 batches per year and a total of 6 kg of notified chemical, the average daily release would be 0.041 kg. It is estimated that the sewer concentration would be 1.05 mg/L, assuming that no chemical is removed by the treatment plant. Hence, the predicted environmental concentration (PEC), following treatment at the metropolitan sewage treatment plant and assuming a 1:425 dilution factor because of the relatively high flow output, is estimated to be 0.0024 mg/L. Waste water from this site feeds into the West Hornsby STP, which has an annual flow rate of 6110 ML.

Since nearly all of the notified chemical will be washed into the sewer, under a worst case scenario, with no removal of the notified chemical in the sewage treatment plant, the resultant predicted environmental concentration (PEC) in sewage effluent on a nationwide basis has been estimated as follows:

Amount entering sewer annually (Worst Case)	300 kg/y
Number of days used per year	365 d/y
Amount entering sewer per day (Worst Case)	0.822 kg/d
Population of Australia	20,100,000 persons
Daily water use per person	200 L/person/d
Daily water entering sewer	4020 ML/d
Predicted Environmental Concentration	0.204 µg/L

Based on dilution factors of 1 and 10 for inland and ocean discharges of STP-treated effluents, the PECs of the notified chemical in freshwater and marine water may approximate 0.204 and 0.020 µg/L respectively.

The notified chemical is not expected to be ready biodegradable, nor is it expected to partition significantly to sludge in an STP.

9.1.2. Environment – effects assessment

Two measured toxicity endpoints were provided for aquatic organisms. The data indicates an EC_b50 of 8.5 mg/L for algae. Using this endpoint, and assuming a safety factor of 1000 (since measured toxicity data are available for only two trophic levels), the predicted no effect concentration (PNEC) is 8.5 µg/L.

9.1.3. Environment – risk characterisation

Location	*PEC (µg/L)*	*PNEC (µg/L)*	*Risk Quotient (RQ)*
From Reformulation	2.400	8.5	2.82×10^{-1}
Australia-wide STPs (worst case)			
Inland river	0.204	8.5	2.41×10^{-2}
Ocean outfall	0.020	8.5	2.41×10^{-3}

On the basis of the RQ values provided in the table above, the low volumes used, and nationwide and diffuse use of the notified chemical, it is not considered to pose an unacceptable

risk to the health of aquatic life based on its reported use pattern.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

Storage and distribution

Waterside, transport and warehouse workers exposure to the notified chemical is expected to be negligible except in the event of an accident.

Repackaging

Exposure to the notified chemical during repackaging processes is expected to be low, due to the low concentration of the notified chemical (0.5%), the use of engineering controls (transfer lines, automated filling process) and the use of PPE. Quality control workers exposure to the notified chemical is also expected to be low due to the low concentration of the notified chemical (0.5%), the small samples involved and the limited exposure time and would be limited by the use of PPE.

Formulation

Dermal and possibly ocular and inhalation exposure to the notified polymer may occur during weighing of the notified chemical and during the transfer from the weighing vessel/pre-weighed satchels to the blending vessel. The estimated typical case dermal exposure is 3000 mg and 900 mg respectively using measured data for the exposure scenario 'dumping of powders in a formulation facility' (European Commission, 2003). Therefore, for a 70 kg worker and a 100% dermal absorption factor, reasonable worst-case and typical case dermal exposure is estimated to be 43 mg/kg bw/day and 13 mg/kg bw/day respectively.

As the percutaneous absorption of a 0.2% solution of the notified chemical was estimated to be 0.009%, this is expected to be an overestimate of the actual exposure. Exposure would be further limited by the use of personal protective equipment (PPE).

The estimated atmospheric concentration of notified polymer due to dust is 5 - 50 mg/m^3, based on EASE model (EASE) using reasonable worst-case defaults (European Commission, 2003). Therefore for a 70 kg worker, assuming an inhalation rate of 1.3 m^3/hour, 8 hour exposure time and 100% bioavailability, inhalation exposure is estimated to be 0.7 – 7.4 mg/kg bw/day.

Following formulation of the hair dye exposure to the notified chemical is expected to be very low due to the low concentration of the notified chemical (<0.5%), the automated nature of the filling process and the use of PPE.

Retail workers

Exposure to the notified chemical is expected to be negligible except in the case of an accidental spill. However, even in the event of an accident, exposure to the notified chemical is expected to be low due to the low concentration (<0.5%) and the small pack size.

Salon workers

Currently only one of the hair dyes is supplied for professional use, although, there is the potential for future professional use products containing the notified chemical. Salon workers could be exposed to the notified chemical at a concentration of up to 0.5% premixing and up to 0.16% (first time highlights) and 0.08% (refreshing service). When mixed with peroxide (first time highlights), the notified chemical will react with the other components of the formulation, however, residual chemical may still be present. Percutaneous absorption of the notified chemical has been estimated to be 0.009% although this was without reaction with a developer. Overall, salon worker exposure to the notified chemical is expected to be low due the low concentrations involved and the expected low percutaneous absorption. Exposure would be limited by the use of gloves. In addition, it is expected that exposure will either be avoided or the hands rinsed following exposure due to staining.

9.2.2. Public health – exposure assessment

The public will be exposed to the notified chemical at a maximum concentration of 0.2% when

dyeing their hair at home or attending a salon to have their hair dyed.

Salon
Consumers will be exposed to up to 20g (15g refreshing service) of the hair dye (containing 0.5% notified chemical but diluted in use) per application. The period between the first application of the hair dye to the refreshing service in a minimum of 6 weeks. The refreshing service can be used up to 2 times (each 6 weeks apart) before the initial treatment needs to be reapplied. Therefore worst case acute exposure is estimated to be as follows:

Product	*Application Quantity (g/application)*[a]	*Application Frequency*[b]	*Retention Factor (%)*[b]	*% Notified Chemical in Product*	*Exposure to notified Chemical per application (mg/kg bw)*[c]	*Exposure to Notified Chemical (mg/kg bw/day)*[c]
Professional use (1st time highlighting)	20	1/month	10	0.5	0.17	0.005
Professional use (refreshing)	15	1/month	10	0.5	0.13	0.004
Use pattern described above						0.005[d]

a) amount of product
b) data from EU SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (SNCNFP, 2003a)
c) assuming 60 kg bodyweight
d) (0.17 + 2*0.13)/90

Alternatively the percutaneous absorption of the notified chemical applied to the skin for 30 minutes in a hair dye formulation at 0.2% without mixing with peroxide i.e. the refreshing treatment was calculated to be 0.018 µg/cm^2. Based on this exposure could be calculated to be (0.018x580)/60 = 0.17 µg/kg bw/application which indicates that the exposure based on the amount applied is an over estimate of actual exposure

The notified chemical is a potential sensitiser. The relevant dose metric for skin sensitisation potential is the amount of chemical per unit area of the allergen on the skin.

Predicted maximum dermal exposure (for hair dye)

Product	*Application Quantity (g/application)*[a]	*% Notified Chemical in Product*	*Partition coefficient*[b]	*Area of exposure (cm^2)*[c]	*Dermal Exposure (µg/cm^2)*
Professional use (1st time highlighting)	20	0.5	0.1	580	17.2
Professional use (refreshing)	15	0.5	0.1	580	12.6

a) amount of product
b) amount applied to scalp (SNCNFP, 1999)
c) data from EU SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (SNCNFP, 2003a)

Home-use
Consumers will be exposed to up to 75g of the hair dye containing 0.2% notified chemical per application. The colour is reported to last up to 10 washes. Therefore worst case acute exposure is estimated to be as follows:

Product	*Application Quantity (g/application)*[a]	*Application Frequency* [b]	*Retention Factor (%)*[b]	*% Notified Chemical in Product*	*Exposure to notified Chemical per application (mg/kg bw)*[c]	*Exposure to Notified Chemical (mg/kg bw/day)*[c]
Semi-permanent hair dye	75	1/week	10	0.2	0.25	0.036

a) amount of product
b) data from EU SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (SNCNFP, 2003a)
c) assuming 60 kg bodyweight

Alternatively the percutaneous absorption of the notified chemical applied to the skin for 30 minutes in a hair dye formulation at 0.2% was calculated to be 0.018 µg/cm^2. Based on this exposure could be calculated to be (0.018x580)/60 = 0.17 µg/kg bw/application which indicates that the exposure based on the amount applied is an over estimate of actual exposure.

The notified chemical is a potential sensitiser. The relevant dose metric for skin sensitisation potential is the amount of chemical per unit area of the allergen on the skin.

Predicted maximum dermal exposure (for hair dye)

Product	*Application Quantity (g/application)*[a]	*% Notified Chemical in Product*	*Partition coefficient*[b]	*Area of exposure (cm^2)*[c]	*Dermal Exposure (µg/cm^2)*
Semi-permanent hair dye	75	0.2	0.1	580	26

a) amount of product
b) amount applied to scalp (SNCNFP, 1999)
c) data from EU SCCNFP (Scientific Committee on Cosmetic Products and Non-food products intended for Consumers) (SNCNFP, 2003a)

9.2.3. Human health – effects assessment

Toxicokinetics, metabolism and distribution.
In an *in vitro* percutaneous absorption study the total absorption was estimated to be 0.018 µg/cm^2 when a dose of 202 µg/cm^2 was applied as a component of a hair dye formulation. The toxicological studies involving dermal application did not provide evidence of percutaneous absorption as no colouration of the urine or faeces or urine was recorded. The colouration observed in the urine and faeces in the oral repeat dose studies indicate that the notified chemical and/or its coloured metabolites are absorbed from the gastrointestinal tract and excreted via urine and faeces.

The notified chemical was not tested in the presence of an oxidising agent and hence the potential absorption of the notified chemical (or reaction products) when used in an oxidative hair dye is not known.

Acute toxicity.
The notified chemical is considered to be harmful if swallowed but of low dermal toxicity. Although inhalation toxicity has not been established inhalation exposure is considered to be an unlikely route of exposure.

Irritation and Sensitisation.
Based on the results of the two skin irritancy studies (single application and prolonged repeat exposure), the notified chemical is considered to be slightly irritating to skin. Although based on the ocular lesions observed in an eye irritation study in rabbits the notified chemical would only be classified as irritating, as irreversible colouration of the eyes occurred, the notified chemical is considered to be severely irritating to the eye. The eye irritancy potential was also tested at a

concentration of 1%, at this concentration the notified chemical was only slightly irritating to the eye.

The notified chemical was negative in a skin sensitisation adjuvant test in guinea pigs, although in a mouse local lymph node assay (LLNA), the notified chemical induced delayed contact hypersensitivity and as such the notified chemical is considered to be a potential skin sensitiser. As the LLNA can be interpreted in a number of different ways the worst-case EC_3 value for the notified polymer is 0.9%. The notified chemical did not exhibit a phototoxic or photoallergenic potential.

Repeated Dose Toxicity.
In a 14-day oral toxicity study in rats the No Observed Effect Level (NOEL) was established as 15.5 mg/kg bw/day, based on decreased food consumption and decreased body weight gain observed at higher doses. In a 90-day oral toxicity in rats the No Observed Adverse Effect Level (NOAEL) was established as 63 mg/kg bw/day based on a number of treatment related effects (reduced bodyweight, clinical chemistry, organ weights and histopathological observations) observed in the high dose animals. A NOEL of 18 mg/kg bw/day was also established in this study.

Mutagenicity.
The notified chemical has been tested in bacteria and mammalian cells for gene mutation, in mammalian cells for chromosomal aberrations *in vitro* and in two *in vivo* tests (bone marrow micronucleus and UDS tests).

The notified chemical showed evidence of mutagenic activity in the bacterial tester strain TA98 in the presence of reductive activation in two independent tests. A weak positive result in this tester strain (2.3 – 3.1 fold increase in revertant frequency) was also observed in the presence of standard activation. A weak positive response in the tester strain TA100 was observed in the presence of reductive activation but this was not observed in a confirmatory assay.

The chromosome aberration test in chinese hamster cells increased frequencies of cells carrying structural aberrations were observed in the absence and presence of activation. However, the induction of structural aberrations was observed only after treatment with concentrations of the test article at which precipitation was observed. An indirect mechanism for DNA damaging may be involved. An *in vitro* chromosome aberration test in human lymphocytes was negative in the presence of a normal metabolic system. The notified chemical was not tested in the presence of a reductive metabolic activation system in either of the chromosome aberration test.

An *in vitro* test for gene mutation in mammalian cells showed that the test agent is non mutagenic in the absence of activation system and under normal or reduced activation systems. The *in vivo* micronucleus test in mice and UDS on rat hepatocytes gave negative results.

Based on the weight of evidence approach, the notified chemical is classified as mutagenic.

Toxicity for reproduction.
In a prenatal developmental toxicity study the No Observed Adverse Effect Level (NOAEL) for maternal and foetal effects was established as 60 mg/kg bw/day.

Carcinogenicity
The principal risk posed by azo dyes is the potential carcinogenicity of aromatic amines produced by cleavage of the azo bond *in vivo*.

The notified chemical is not to expected to be reductively cleaved to release one or more of the aromatic amines listed in either the Appendix to EC Directive 76/769/EEC: (http://europa.eu.int/comm/enterprise/chemicals/legislation/markrestr/consolid_1976L0769_en.pdf) or the annexes of the European Union SCCNFP/0495/01, Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers concerning ``The Safety Review of the Use of Certain Azo-Dyes in Cosmetic Products," 2/27/02. http://europa.eu.int/comm/food/fs/sc/sccp/out155_en.pdf (prepared in the context of Directive

76/768/EEC).

Overseas Opinion
The SCCNFP has reviewed this toxicological data (except for the chromosome aberration assay in Chinese Hamster Cells) in the 'Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP) concerning Basic Orange 31' (SCCNFP, 2003b). The SCCNFP is of the opinion that the notified chemical might be regarded as safe in general. However the data were insufficient for a final evaluation. Further information requested:
Identification of all impurities
Data on stability of test material in the experimental investigations and in hair dye formulations
Percutaneous absorption study in accordance with guidance
The safety dossier should fulfil the demands of the SCCNFP strategy paper as to mutagenicity of possible reactions products.

Use information supplied in the report was as follows:
Non-oxidative hair dye formulations: maximum of 0.2%
Oxidative hair dye formulations: 0.1% after mixing with the oxidative agent.

The safety dossiers for all permanent and non-permanent hair dyes are required to be submitted to the SCCNFP by July 2005 at the latest with information relating to the safety of the combination of ingredients to be submitted by December 2007.

Restrictions
Phenylenediamines are listed in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). Although the notified chemical is not covered by this entry in the SUSDP the following relevant listing information is provided for information purposes.

Phenylenediamines are currently listed in the SUSDP in **Schedule 6** with the entry:

> "in hair dye preparations **except** when the immediate container and primary pack are labelled with the following statements:
>
> KEEP OUT OF THE REACH OF CHILDREN, and
>
> WARNING – This product contains ingredients which may cause skin irritation to certain individuals. A preliminary test according to the accompanying directions should be made before use. This product must not be used for dyeing eyelashes or eyebrows; to do so may be injurious to the eye.
>
> Written in letters not less than 1.5 mm in height."

Please note that this would only impact products sold for consumer use.

The notified chemical is not currently listed as itself in either Annex II or Annex III of the EU cosmetic directive although this may change pending the full safety evaluation by the SCCNFP. p-Phenylenediamines, and their N-substituted derivatives (which are structurally related to but do not include the notified chemical) are listed in Annex III Part 1 of the Cosmetics Directive (List of substances which cosmetics products must not contain except subject to restrictions and conditions laid down). The maximum authorised concentration in the finished product is 6% (calculated as free base). For **general use**, the label must contain the following warning statements; "can cause allergic reaction" "Contains phenylenediamines" and "Do not use to dye eyelashes or eyebrows". For **professional use** the label must contain the following warning statements; "For professional use only" "Contains phenylenediamines" "Can cause an allergic reaction" and "Wear suitable gloves".

Observations on Human Exposure.
No observations on human exposure have been provided for the notified chemical. The chemical, p-phenylenediamine (PPD), is a widely used permanent hair dye. PPD is known to have skin and eye irritation and skin sensitisation potential (EC3 0.06 –0.2%). PPD has been

linked to incidences of severe allergic and other skin reactions, with incidence figures quoted for Europe at about 3 in every million users.

Hazard classification for health effects.
Based on the available data, the notified chemical is classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004). The classification and labelling details are:

R22 Harmful if swallowed
R41 Risk of serious damage to eyes
R43 May cause sensitisation by skin contact

9.2.4. Occupational health and safety – risk characterisation

Except for in the event of an accident only repackaging workers, formulation workers, quality control workers and salon workers will be exposed to the notified chemical. The risk to these workers has been assessed below.

Repackaging, quality control and salon workers
All of these workers will come into contact with the notified chemical at a maximum concentration of 0.5%. At this concentration the notified chemical is unlikely to be a skin irritant, is expected to only be a slight eye irritant and is below the EC3 value for sensitisation. Therefore the risk of local adverse effects is considered to be low, however, the risk of sensitisation cannot be ruled out. Due to the limited exposure expected for these workers, the risk of adverse systemic is also considered to be low. As a precaution workers should avoid contact with skin and eyes and wear gloves when handling products containing the notified chemical.

Formulation
Due to the eye irritation and sensitisation potential of the notified chemical workers involved in the handling of the neat notified chemical should avoid contact with skin and eyes and are recommended to wear coveralls, gloves and eye protection. A disposable dust mask should be worn where sufficient ventilation is not available. Reasonable worst-case exposure to the notified polymer was estimated to be 50.4 mg/kg bw/day. Based on a NOAEL of 63 mg/kg bw/day, derived from a 90-day rat oral study the margin of exposure (MOE) is calculated as 1.25. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Whilst this margin of exposure is lower than the acceptable value, actual exposure is expected to be a lot lower than that estimated due to the conservative nature of the EASE model, the expected low dermal absorption of the notified chemical and the recommended use of PPE due to sensitisation potential. As such the risk of adverse systemic effects is considered to be low.

9.2.5. Public health – risk characterisation

Irritation and Sensitisation
The public will be exposed to the chemical at a maximum concentration of 0.2%. At this concentration the notified chemical is unlikely to be a skin irritant, is expected to only be a slight eye irritant and is below the EC3 value for sensitisation. Therefore the risk of local adverse effects is considered to be low, however, the risk of sensitisation cannot be ruled out.

The highest level of amount of chemical per unit area of the allergen on the skin was calculated as 26 μg/cm^2 for the home-use product. Based on a worst-case EC-3 value of 0.9% from interpretation of the LLNA study data, a skin potency value for the notified chemical was calculated at 225 μg/cm^2. This calculation was done using the fact that the LLNA was performed by applying 25 μL of test solution to < 1cm^2 of the rabbit's ear, therefore, the concentration of test solution applied to the mouse ear was estimated to be 25 000 μg/cm^2 (assumed that 1mL = 1 g). As the calculated EC3 concentration was 0.9%, the quantity of chemical causing sensitisation is estimated to be 0.9% x 25 000 μg/cm^2 = 225 μg/cm^2.

This gives a margin of safety of 8.6 for the estimated maximum dermal exposure of 26 μg/cm^2. Although this is lower than the desired margin of safety of 100, the notified chemical is considered to be a less potent sensitiser than PPD which has been reported to effect only about 3 in every million users. The product labelling (for the two currently proposed hair dyes) advise that the product may cause an allergic reaction or cause skin irritation. A preliminary skin test is also advised which should identify individuals susceptible to sensitisation.

Systemic effects
The highest public exposure to the notified chemical was estimated as 0.036 mg/kg bw/day (although due to expected low percutaneous absorption this is expected to be an overestimate). Based on the lowest NOEL of 15.5 mg/kg bw/day, derived from the 14-day rat oral study the lowest MOE is calculated as 435. MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Therefore the risk of systemic effects from use of hair dyes containing the notified chemical is considered to be low.

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

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*. The classification and labelling details are:

R22 Harmful if swallowed
R41 Risk of serious damage to eyes
R43 May cause sensitisation by skin contact

and

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

	Hazard category	***Hazard statement***
Acute toxicity	4	Harmful if swallowed.
Serious eye damage/eye irritation	1	Causes serious eye damage
Skin Sensitiser	1	May cause allergic skin reaction
Chronic hazards to the aquatic environment	2	Toxic to aquatic life with long lasting effects

10.2. Environmental risk assessment

On the basis of the PEC/PNEC ratio and based on its reported use pattern, the notified chemical is not considered to pose an unacceptable risk to the environment.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Moderate Concern to occupational health and safety when handling the neat notified chemical due to the risk of sensitisation.

10.3.2. Public health

There is No Significant Concern to public health when used in the proposed manner and provided the hair dye formulations are adequately labelled to indicate sensitisation potential.

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 1994). 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 1994). The accuracy of the information on the label remains the responsibility of the applicant.

The label for the products containing the notified chemical contained warning statements consistent with those required for p-phenylenediamine.

12. RECOMMENDATIONS

REGULATORY CONTROLS

Hazard Classification and Labelling

- The ASCC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical:
  - R22 Harmful if swallowed
  - R41 Risk of serious damage to eyes
  - R43 May cause sensitisation by skin contact
  - S24/25 Avoid contact with skin and eyes
  - S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
  - S37 Wear suitable gloves
  - S39 Wear eye/face protection

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc $\geq$ 25%: R22; R41; R43
  - 10% $\leq$ Conc < 25%: R41; R43
  - 5% $\leq$ Conc < 10%: R36; R43
  - 1% $\leq$ Conc < 5%: R43

- The National Drugs and Poisons Standing Committee (NDPSC) should consider the notified chemical for listing on the SUSDP.

- Products containing the notified chemical and available to the public must carry the safety directions consistent with the following on the label:
  - WARNING: This product contains ingredients which may cause skin irritation to certain individuals. A preliminary test according to the accompanying directions should be made before use. This product must not be used for dyeing eyelashes or eyebrows; to do so may be injurious to the eye.
  - If in eyes wash out immediately with water.
  - Keep out of reach of children.

Health Surveillance

- As the potential for skin sensitisation exists, the notifier's MSDS should be provided to the authorised medical practitioner responsible for health surveillance in the hair dye manufacture facility. Employers should carry out health surveillance for any worker

who has been identified in the workplace risk assessment as having a significant risk of adverse health effects. Sensitised persons should be transferred to another workplace.

CONTROL MEASURES

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical and during hair dye manufacture:
  - Local Exhaust ventilation should be implemented where there is a likelihood of exposure to dust.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified during hair dye manufacture:
  - Minimise dust generation
  - Do not breathe dust
  - Avoid contact with skin and eyes

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical in the hair dye formulation:
  - Avoid contact with skin and eyes

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during hair dye manufacture:
  - Protective eyewear, chemical resistant industrial clothing, impermeable gloves and respiratory protection (if required).

- Employers should ensure that the following personal protective equipment is used by workers (including salon workers) to minimise occupational exposure to the notified chemical in the hair dye formulations:
  - impermeable gloves

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

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

- 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.

Public Health

- The following measures should be taken by the notifiers to minimise public exposure to the notified chemical:
  - Gloves should be supplied with hair dyes containing the notified chemical intended for sale to the public

Environment

- Avoid release of concentrated notified chemical to the aquatic environment.

Disposal

- The notified chemical should be disposed of by thermal decomposition in incinerators or to secure landfill.

Emergency procedures

- Spills/release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

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
- the importation volume exceeds one tonne per annum notified chemical; or
- any additional information for the notified chemical generated as a result of the SCCNFP data requirements becomes available; or
- An updated SCCNFP opinion concerning the notified chemical becomes available or there is any change in status of the notified chemical in the EU Cosmetic Directive; or
- the notified chemical is included in hair dye preparations at a concentration of > 0.5% or is intended to be applied at a concentration of > 0.2% (as mixed);

or

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

The Director will then decide whether secondary notification is required.

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