File No: NA/740

September 1999

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

2A 3 PYR

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

2A 3 PYR

1. APPLICANTS

Schwarzkopf Pty Ltd of 20 Rodborough Road FRENCHS FOREST NSW 2086 and Goldwell Cosmetics (Australia) Pty Ltd of 103 Yerrick Road LAKEMBA NSW 2195 have submitted a joint, limited notification statement in support of their application for an assessment certificate for 2A 3 PYR.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data, have been exempted from publication in the Full Public Report and the Summary Report.

Marketing Name: HS-P6
2A 3 PYR

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa: Beige/greyish crystals

Melting Point: 172°C

Specific Gravity: Approximately 1

Vapour Pressure: 0.133 kPa at 25°C

Water Solubility: 33.8 g/L at 25°C estimation (ACD Software)

Particle Size: Not applicable, imported in aqueous formulation

Partition Co-efficient (n-octanol/water): -0.03 log P_{ow} estimation (ACD software)

Hydrolysis as a Function of pH: Does not contain any groups which can undergo

	hydrolysis
Adsorption/Desorption:	Expected to have high soil mobility (see comments below)
Dissociation Constant:	Pyridinium ion: 3 – 6; Aryaminium ion: 1 – 5; Aromatic Hydroxyl: 8 – 10.
Flash Point:	Will be imported as an aqueous formulation and is not expected to be flammable
Flammability Limits:	Not flammable
Autoignition Temperature:	Not expected to undergo autoignition
Explosive Properties:	Not explosive
Reactivity/Stability:	Stable under ambient conditions but may undergo slow oxidation in air

Comments on Physico-Chemical Properties

The values for water solubility, partition coefficient, adsorption and dissociation constant were estimated by the notifier via the use of software package developed by Advanced Chemistry Development Inc. (ACD), which is accepted for this assessment.

No hydrolysis value was provided by the notifier on the grounds that the chemical has no groups that will hydrolyse.

The low partition coefficient and adsorption values indicate that the chemical will be highly mobile in soil and likely to remain in the water column.

Values given by the ACD software were compared for this assessment with values obtained using the USEPA ASTER estimation model. The ASTER results for the physico-chemical parameters are shown below. The ACD estimations for water solubility, partition coefficient and adsorption are within acceptable agreement with the ASTER values.

ASTER Generated Physico-Chemical Parameters

Vapour pressure	0.174 mm of Hg
Water solubility	42.9 g/L
Partition coefficient	0.975 (log P)
Adsorption	1.87 (log K_{oc})
Hydrolysis	unlikely
Dissociation (pKa)	not available

4. PURITY OF THE CHEMICAL

Degree of Purity:	99.9 %
Hazardous Impurities:	none
Non-hazardous Impurities (> 1% by weight):	none
Additives/Adjuvants:	none

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported as a component of a cream based finished hair colouring product at 0.4% w/w and used as a precursor in an oxidation hair dye.

The product, containing the notified chemical, will be imported in either finished 50 mL retail bottles, or in bulk containers, 25 kg plastic buckets or 100 kg plastic barrels lined with an aluminium foil laminate bag. Schwarzkopf Pty Ltd will import only 50 mL retail bottles. Goldwell Pty Ltd will import 50% in the bulk containers and the remainder in finished packs. The bulk component of the Goldwell import will go to a contractor for repackaging into 60 g and 80 g aluminium tubes. The tube form of the product will be available for professional (hairdresser) use only.

Import volumes of the notified chemical are: Schwarzkopf, 120 kg per annum for the next 5 years; and Goldwell, 100 kg in the first year increasing to 146 kg in the fifth year. The total import of notified chemical is 220 kg in the first year and 266 kg in the fifth year.

The notified chemical was in use in Australia from March 1996 to March 1999 under a NICNAS Low Volume Chemical permit granted under section 21U of the Act.

6. OCCUPATIONAL EXPOSURE

Transport and Storage
Imported retail packs will be transported to each notifiers warehouse prior to dispatch to retail outlets. Bulk material imported by Goldwell Cosmetics Pty Ltd is transported directly to a packaging company, prior to sale of packaged goods to hairdressing salons. Dockside, transport and warehouse workers should only be exposed in the event of a spill.

Repackaging – Tube Filling (150 to 200 days/year)
Two operators are involved in repackaging. One transfers the bulk material and the other operates the tube filler. The bulk hair colour is described as of cream consistency and flowable. The hair colour is pumped from the original import containers into the hopper of a metal tube filler. The product is then automatically filled into metal tubes and sealed.

Tube filling is carried out in a single shift. Upon completion of a particular shade, the hopper and filling machine are washed out with hot water. The amount of hair colour in the wash out is approximately 10 to 50 g bulk product. The submission indicates on average 540 shade changes per annum. Approximately 30 g of notified chemical per annum would be present in the wash out.

Skin contact with the bulk hair colour from drips and spills is possible during loading of the bulk material to the hopper. During wash out there may be potential for whole body contact from splashes and spray. The notifier states that operators are required to wear long vinyl gloves, safety glasses and overalls.

Hairdressing Salon
This number of commercial salons across Australia is estimated in the 1 000s. The number of employees would typically range between two to ten per salon.

The professional use hair colour products containing the notified chemical will be available in either 60 g or 80 g tubes. Prior to application to the hair, the hair colour is prepared in a plastic bowl and mixed with an applicator brush. The colour is applied by brush, left in contact with the hair for the required colour time (up to 30 minutes), then rinsed away with water. It is claimed that at least 50% of the dye will be adsorbed to hair. The amount prepared for hair colouring would depend on the desired shade and the amount of hair coverage required. Exposure is primarily by skin contact, with potential for eye contact from splattering during mixing, application or rinsing. Personal protective equipment, where worn, would likely be cuff length disposable plastic or latex gloves and plastic apron.

Retail Outlet
Retail hair colour products containing the notified chemical are packaged in 50 mL bottles and sold to the public through retail outlets. Retail workers should only be exposed in the event of a spill.

7. PUBLIC EXPOSURE

Public exposure is likely to be intermittent but widespread, with the extent of the population exposed limited only by the commercial success of the product. Hair colour is usually applied approximately every 4 to 6 weeks as new growth becomes visible at the base of the hair, thus the frequency of exposure is low. The duration of exposure will be approximately 20 to 30 minutes, following which the colour preparation is washed from the hair.

In the event of transport accident involving the imported finished product the extent of a spill will be limited by the small pack size of the product (50 mL bottle). Given the nature of the formulation, cream based, dispersion would be minimal and significant public exposure from this source is unlikely. Any spillage would be readily recoverable on adsorbent material with the residue being washed to drain. Bulk formulated product imported in 25 or 100 kg buckets has slightly more potential for dispersion but as transport will be limited to movement from the docks to the contract repackager, the overall risk of exposure from this source is likely to be low.

8. ENVIRONMENTAL EXPOSURE

Release

At warehouse facilities, spills may account for up to 1% loss of notified chemical, that is up to 2.2 kg/year in year 1 and 2.7 kg/year in year 5.

In the repackaging process the filling machinery is washed between every hair dye colour change.

Amount of Notified Chemical in Washwater per Year

Number of colour changes per month:		45
Amount of waste product:		
per colour change (estimated):		10 to 50 g;
per year in washwater (estimated worst case):		45 x 12 x 50
	=	27 000 g
Amount of notified chemical in washwater per year, if present at 0.4% w/w concentration:		108 g

The amount 108 g is approximately 0.05% and 0.04% of the annual amount of notified chemical imported in year 1 and 5, respectively.

The resultant washwater, containing 108 g of notified chemical, is combined with other waste effluent streams and is sent to the on-site treatment plant. In the treatment plant the effluent

initially has the solids removed then undergoes chemical/biological treatment. The treated water is recycled in the plant for other process activities or used for garden maintenance. The resultant sludge is disposed of by a waste contractor and assumed to go to landfill or an incinerator.

The notifier has estimated that 10 to 20 g of product will be left in the bags lining the bulk imported containers. This equates to 0.01% to 0.08% depending on container size (25 kg or 100 kg). It is estimated that the bulk container residue will retain less than 0.1% notified chemical. Thus of the 50 kg of notified chemical that undergoes repackaging in the first year, less than 50 g is wasted. The internal liner prevents the outside plastic bulk container from being contaminated with product. These containers will be disposed of to landfill.

It is estimated that 2% of the contents of the end use container will remain after emptying. These containers will generally be disposed of via the domestic garbage system. Thus, approximately 2% of the imported volume, i.e. 4.4 kg/year in the first year increasing to 5.3 kg in year 5, will end up in landfill or be incinerated.

At each user site, whether at home or in a salon, the notified chemical has the potential to end up in the sewerage system. However, since the notified chemical is a precursor, it should be totally consumed when mixed in the appropriate proportions in the dye preparation and applied. Thus, no pure notified chemical, and only a proportion of the final dye will be washed into the sewer.

A worst case scenario would be if a number of the bulk containers simultaneously lost their contents into the sewer. In the PEC calculation below it is assumed that the contents of three of the 100 kg bulk containers of product, that is, 1.2 kg of the notified chemical, is lost to sewer. The PEC for this situation is calculated below.

Metropolitan Sewage Treatment Plant (STP):

Unreacted notified chemical released:	1.2 kg
Water handled by the STP:	250 ML/day
Dilution in receiving water:	1:10
PEC in receiving water:	0.0048 ppm

Fate

The notified chemical is consumed when mixed with the other dye components. Once applied and allowed to cure for the 20 to 30 minutes, the majority of the dye will become bound to the hair. As indicated above the worst case scenario of the loss of three, 100 kg bulk product containers to sewer gives a daily PEC of <0.0048 ppm.

Summary of the Estimated Annual Amount of Notified Chemical Wastes in Year 5

Spills	1%	2.7 kg
Repackaging washwater	<0.05%	108 g
Bulk container residue	<0.1%	0.27 kg
User container residue	2%	5.3 kg

Thus, approximately 3% of the imported chemical will end up in landfill or incinerated. Due to the low partition coefficient and adsorption value, the chemical is likely to be highly mobile in soil and readily become suspended in water. Thus, the unreacted chemical is likely to leach out of a landfill but in very low concentrations and in a diffuse manner.

The estimated environmental partitioning calculated by the ASTER model, given below, indicates that the chemical will not adhere to soil or sediments but will remain dissolved in water.

ASTER Estimated Environmental Partitioning, Mackay Level 1 Partitioning

2.01% into air

0.07% into soil

97.85% into water

0.07% into sediment

The bioconcentration factor estimated by ASTER is 2, indicating the chemical is unlikely to bioconcentrate.

9. EVALUATION OF TOXICOLOGICAL DATA

The notifier stated that only summary reports could be accessed for the notified chemical.

Summary of the acute toxicity of 2A 3 PYR

Test	*Species*	*Outcome*	*Reference*
Acute oral toxicity	Rat	LD_{50} 156 mg/kg	(IBR Neviki Assoc 1998)
Acute dermal toxicity	Summaries or full studies not available		
Acute inhalation toxicity	Summaries or full studies not available		
Skin irritation	Rabbit	Non-Irritating	(IBR Neviki Assoc 1998)
Eye irritation	Rabbit	Irritating	(IBR Neviki Assoc 1998)
Skin sensitisation	Guineapig	Mild Sensitiser	(IBR Neviki Assoc 1998)

9.1 Toxicokinetics – Absorption, Distribution and Excretion (OFS 1989)

Species/strain:	Rat/Sprague-Dawley (Him:OFA, SPF)
Number/sex of animals:	5/sex/group
Test Material:	For cutaneous application: 3.4 mL of ^{14}C-labelled test substance dissolved in 95% ethanol, concentrated to 0.5 mL, then mixed with non-labelled test substance, p-phenylenediamine-dihydrochloride; before application, the mixture was mixed (1:1) with a developer containing hydrogen peroxide (to mimic the hair product as applied); For oral administration: 0.2 mL of ^{14}C-labelled test substance dissolved in 95% ethanol, mixed with non-labelled test substance to 25 mL of water.
Study Design:	
Cutaneous Application	Group I and II animals: the mixture was applied to an area (9 cm^2) of clipped dorsal skin; after 30 minutes, remaining test substance was rinsed away with water. Group I animals were sacrificed after 72 hours and samples were drawn from the rinse water, treated skin, urine, faeces and carcass. Group II animals were sacrificed after 24 hours and radioactivity measured in 13 organs (thyroid, adrenal, fat, gonads, liver, lung, brain, muscle, heart, spleen, femur, skin and kidney), blood and carcass.
Oral Administration	Group III and IV animals: 0.5% of test substance mixture administered by gavage. Group III animals were sacrificed after 72 hours and samples drawn from urine, faeces, 13 organs, and carcass *sans* GI tract. Group IV animals were sacrificed after 24 hours and radioactivity measured in 13 organs, blood and carcass *sans* GI tract.
Findings:	
Cutaneous Application	Rinsewater contained 94.6% of the radiolabel, ^{14}C; Absorbed ^{14}C was excreted mainly via the urine (87%) and the remainder in the faeces (13%); The mean percutaneous absorption was 0.074%; Excretion was rapid; 86% was eliminated in the first 24 hours; Accumulation was low, the mean concentration of blood and 13 organs were below or near the detection limit; The application site contained 2.91% (Group I) and 2.32% (Group II) of the applied ^{14}C.

Oral Administration	Excretion ^{14}C of was mainly via the urine (96%) with the remainder excreted in the faeces (4%); The mean excretion was rapid: 99% was eliminated within the first 24 hours, indicating oral absorption is almost complete; Peak levels of ^{14}C in blood were reached prior to sampling at 24 hours; At 24 and 72 hours, accumulation of ^{14}C was highest in the thyroid, liver and adrenals and to a lesser extent in muscle, heart, and fat tissue; The study authors reported excretion after dosing was slightly slower in females than in males, however, the recovery of radioactive label was not reported.

9.2 Acute Toxicity

9.2.1 Oral Toxicity (IBR Neviki Assoc 1998)

Species/strain:	Rat/Hannover-Wistar
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	10% suspension of test substance in 1% methyl cellulose at doses of 100, 123, 151, 185 or 280 mg/kg
Test method:	OECD TG 401
Clinical observations:	Piloerection, slight dyspnoea, tremor and strong convulsions, was noted soon after treatment, followed by mortality in animals with convulsions; Survivors showed a tendency to recover from the 2nd hour after dosing and appeared normal after 2 to 3 days
Mortality:	A dose response relationship of toxicity was found in males; in females 4/5 died at 151 mg/kg and 3/5 died at 185 mg/kg
Morphological findings:	At necropsy, day 1 or day 14, no substance related changes were observed
LD_{50}:	156 mg/kg
Result:	2A 3 PYR was of moderate acute oral toxicity in rats

9.2.2 Skin Irritation (IBR Neviki Assoc 1998)

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	6 males
Observation period:	7 days
Method of administration:	0.5 g of test substance on gauze applied to a dorsal skin site; after 4 hours, the remaining test substance was removed with water
Test method:	OECD TG 404
Comment:	No individual Draize scores were given; The test substance was reported non irritating.
Result:	2A 3 PYR was non irritating to the skin of rabbits

9.2.3 Eye Irritation (IBR Neviki Assoc 1998)

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	6 males
Observation period:	21 days
Method of administration:	0.1 g of test substance instilled into the conjunctival sac of the left eye of each animal
Test method:	OECD TG 405
Comment:	No individual Draize scores given; Irritation index at 24 hours was 33; at 48 hours it was 27; Irritation was reversible after 7 days; The test substance was reported irritating;
Result:	2A 3 PYR was irritating to the eyes of rabbits

9.2.4 Skin Sensitisation (IBR Neviki Assoc 1998)

Species/strain:	Guineapig/DHP
Number of animals:	20 test animals; 10 positive control animals; 20 negative control animals.
Induction procedure:	*Test animals* Day 1: three pairs of intradermal injections as follows:

- Freund's Complete Adjuvant (FCA) with distilled water (1:1);
- test substance, 1% in distilled water;
- test substance 1% in distilled water in FCA emulsion (1:1).

Day 7: test substance (1% in distilled water) applied and held under occlusive dressing for 48 hours;

Control animals:
Negative control animals were treated similarly, but omitting the test substance and using distilled water;
Positive control animals were treated similarly, but omitting the test substance and using 0.1% dinitrochlorobenzene (DNCB).

Challenge procedure: Day 21: 1% of test substance in distilled water held under occlusion for 24 hours;
Positive control animals were treated similarly, but omitting the test substance and using 0.02% DNCB.

Test method: OECD TG 406, Magnusson and Kligman Maximisation Test

Challenge outcome: No individual dermal reaction scores given;
The test substance was reported mildly sensitising

Result: 2A 3 PYR was mildly sensitising to the skin of guineapigs

9.3 Repeated Dose Toxicity (Centre International de Toxicologie 1989)

Species/strain: Rats/Sprague-Dawley Crl(SD)BR

Number/sex of animals: 6/sex/group

Method of administration: Oral, (gavage)

Dose/Study duration: 0, 10, 22, 47 or 100 mg/kg/day (dose volume 5mL/kg)

Test method: OECD TG 407

Remarks:

No deaths occurred during the study. Dose related hypersalivation was observed at 47 and 100 mg/kg/day. This effect lasted for approximately 5 minutes and was mostly observed, immediately after treatment.
Opacity of the right eye was noted in one male of the 47 mg/kg/group, possibly due to trauma; chromorhinorrhea was noted observed in one male of the 100 mg/kg/group from Days 7 to 19.

No other treatment related alterations or pathological symptoms were observed.
Based on hypersalivation at 47 and 100 mg/kg/day the No Observed Effect Level (NOEL) established for this study was 22 mg/kg/day.

Result: The NOEL established for this study was 22 mg/kg/day.

9.4 Developmental Toxicity (OFS 1994)

Species/strain:	Rat/Wistar Crl:W1 BR
Number/sex of animals:	Female
Method of administration:	Oral (gavage)
Dose/Study duration:	0, 15, 45 or 135 mg/kg bw from days 6 to 15 of gestation; The high dose was reduced to 90 mg/kg bw after 5 days due to excess mortality at 135 mg/kg/ bw; Dams were sacrificed on day 20 of gestation
Maternal in-life findings:	At 135 mg/kg bw, two animals died, within the first two days of dosing, suffering from severe convulsions and hypersalivation; hypersalivation occurred in some animals immediately after dosing; Between Day 6 and 11 of gestation, significantly lower bodyweight gains and food consumption were recorded at 135/90 mg/kg bw.
Foetal findings:	Significantly increased incidence of foetuses with incomplete ossified caudal vertebrae (15 and 45 mg/kg bw/day) and of the pelvis (45 mg/kg bw/day); Increased incidence of slightly enlarged brain ventricles at 15 and 45 mg/kg bw/day; At 135/90 mg/kg bw/day, significantly more foetuses with rudimentary lumbar ribs and 'variations in general' (not defined) were observed and considered to be due to maternal toxicity.
Test method:	Not stated, but similar to OECD TG 414
Result:	Based on clinical signs in dams at 135/90 mg/kg bw/day, the NOEL for maternal toxicity was 45 mg/kg bw/day; Based on developmental effects at all doses and the brief description given in the summary, the Lowest Observed Adverse Effect Level (LOAEL) for developmental toxicity is 15 mg/kg bw/day.

9.5 Genotoxicity

9.5.1 *Salmonella typhimurium* Reverse Mutation Assay (Toxicol. Laboratories Limited 1986)

Strains:	*Salmonella typhimurium* TA 1535, TA 1537, TA98, TA 100
Concentration range:	0, 8, 40, 200, 1 000, 5 000 μg/plate
Metabolic Activaton System:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Test method:	Ames BN et al (1975) Mutation Research, **31**:347-364; plate incorporation method
Comment:	Toxicity in all strains at 5 000 μg/plate as evidenced by reduced background lawns; No significant increases in the number of revertants in any strain tested in the presence or absence of metabolic activation;
Result:	2A 3 PYR is not considered mutagenic in the bacterial strains tested.

9.5.2 DNA Damage and Repair Test in *Escherichia coli* (Labor L + S GmbH 1988)

Strain:	*Escherichia coli* WP2 (repair component); *Escherichia coli* CM871 (repair deficient).
Metabolic activation system:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Dosing schedule:	Two independent experiments were conducted, with and without metabolic activation (S9), as follows: Test substance: 0, 4.88 to 5 000 μg/mL: Positive Controls: 4-nitro-quinoline-1-oxide 0.00097 to 1 μg/mL (-S9), 2-aminoanthracene 0.0486 to 6 400 μg/mL (+S9); Negative Control: ampicilline, 0.0048 to 5 μg/mL
Test method:	Not stated
Comment:	The test substance caused a similar induction of cell growth in both strains with and without addition of metabolic activation indicating that no DNA-damaging activity under these test conditions

Result:	2A 3 PYR was negative for DNA damage in the bacterial strains tested

9.5.3 DNA Damage and Repair/Unscheduled DNA Synthesis (UDS) in Mammalian Cells *in vitro* (AATU 1991)

Cells:	Primary hepatocytes from Wistar rats
Dosing schedule:	Two independent experiments were conducted as follows: Test substance: 0, 1.95 or 125 µg/mL of cell culture: Positive Control: 7,12-dimethylbenz[a]anthracene 10µg/mL
Test method:	Butterworth et al (1987) Mutation Research, **189**:113-121.
Comment:	The test substance was toxic for the indicator cells at 125 µg/mL. The test substance did not induce DNA repair in liver cells under the conditions of the test.
Result:	2A 3 PYR was negative for unscheduled DNA synthesis

9.5.4 *In vitro* Mammalian Chromosome Aberration Test (Labor L + S GmbH 1988)

Cells:	Chinese Hamster Ovary (CHO)
Metabolic activation system:	liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Dosing schedule:	Test substance in dimethylsulfoxide (DMSO), with and without metabolic activation (S9), as follows: -S9 0, 10, 20, 40, 60, 80, 100 µg/mL for 20 to 25 hours; +S9 0, 312.5, 625, 1250, 2 500 µg/mL for 18 to 23 hours; Positive controls: Methylmethanesulphonate, 30µg/mL (-S9); Cyclophosphamide, 12.5 µg/mL (+S9).
Test method:	Not stated
Comment:	The test substance induced significant increases in induced chromosomal aberrations in CHO cells, both in the presence and absence of metabolic activation
Result:	2A 3 PYR induced chromosomal aberrations in CHO cells

9.5.5 Sister Chromatid Exchange (SCE) Assay in Mammalian Cells, *in vitro* (Fraunhofer Institut 1991)

Cells:	Chinese hamster lung cells (V79)
Metabolic activation system:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Concentration range:	Test substance in Ham's F10 medium, in duplicate, with and without metabolic activation (S9) as follows: -S9, 3 hour incubation: 0, 250, 500, 1 000, 1 500 µg/mL 24 hour incubation: 0, 5, 10, 15, 20, 25, 50, 75 µg/mL. +S9, 0, 250, 500, 1 000, 1 500 µg/mL. Positive Controls: ethylmethansulfonate 200 µg/mL (-S9); cyclophosphamide 2.0 µg (+S9).
Test method:	OECD TG 479
Metaphase Analysis:	25 metaphases per group were analysed
Comment:	The test substance induced a biologically significant and dose-dependent increase in SCE in the presence and absence of metabolic activation; The results were interpreted as indication of an interaction between the test substance and DNA.
Result:	The notified chemical induced SCE in Chinese hamster lung cells *in vitro.*

9.5.6 Malignant transformation of C3H-mouse M2 Fibroblasts *in vitro* (Marquardt 1994)

Cells:	Mouse, C3H M2 - fibroblasts
Dosing schedule:	Test substance in DMSO, tested with and without metabolic activation, as follows: -S9 0, 50, 100, 200, 300 µg/mL; +S9 0, 100, 500, 750, 1 000 µg/mL;

	Positive Controls: N-methyl-N'-nitro-N-nitrosoguanidine 0.5 μg/mL; 2-acetylaminofluorene 10 μg/mL; 3-methylcholanthrene 10 μg/mL.
Test method:	Not stated
Comment:	The test substance was tested up to concentrations inducing significant cytotoxicity; the compound was reported as 'detoxified' by microsomal metabolism; No induction of malignant transformation *in vitro*; The positive controls yielded the expected results indicating the proper functioning of the indicator cells and the external metabolising system.
Result:	2A 3 PYR was negative for malignant transformation *in vitro.*

9.5.7 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Safepharm Laboratories Limited 1992)

Species/strain:	Mouse/CD1
Number and sex of animals:	35/sex
Doses:	Test substance: 200 mg/kg (dose volume of 10mL/kg in arachis oil); Positive control: 50 mg/kg cyclophosphamide; Negative control: arachis oil.
Method of administration:	Single oral dose. Test and negative control animals were sacrificed at 24, 48 or 72 hours post treatment; Positive control animals were sacrificed at 24 hours.
Test method:	OECD TG 474
Comment:	Bone marrow toxicity (a significant change in the ratio of normochromatic to polychromatic erythrocytes) was observed in the 24 hour dose group; No significant increase in the frequency of micronuclei in polychromatic erythrocytes
Result:	2A 3 PYR was non genotoxic

9.6 Overall Assessment of Toxicological Data

The submission contained only summary reports on the notified chemical, 2 A 3 HP, which had been submitted to the European Cosmetic, Toiletry and Perfumery Association (COLIPA).

Toxicokinetic studies with a hair-dye formulation of 2 A 3 HP revealed a very low percutaneous absorption (0.074%) after a 30 minute exposure. Extent of recovery of oral administered dose was not reported. However, 96% of recovered label was excreted in the urine. This indicates absorption via the GIT. Excretion was rapid; with the majority of the absorbed dose by both routes excreted in urine within the first 24 hours. Organ accumulation was very low.

The notified chemical was of moderate oral toxicity in rats (LD_{50} 156 mg/kg), producing dyspnoea and convulsions prior to death. The notified chemical was reported as non irritating to rabbit skin. Based on the reported primary irritation indices, the notified chemical is a moderate to severe eye irritant in rabbits. The notified chemical was mildly sensitising to guineapig skin in an adjuvant type study.

In a 28 day repeat oral dose study, dose related hypersalivation, immediately after dosing was the only clinical sign observed in rats. The NOEL was 22 mg/kg bw/day. In a developmental study in rats, the highest dose tested (150 mg/kg bw/day) was reduced to 90 mg/kg bw/day after 5 days because of excess mortality. No evidence of teratogenicity or foetal toxicity were observed but incomplete ossification of the caudal vertebrae (15 and 45 mg/kg bw/day) and of the pelvis (45 mg/kg bw/day) were seen. At the high dose 2 dams died and the remainder had significantly lower body weight gains and food consumption. The foetuses at this dose had significantly more variations in general (not defined). The NOEL for maternal toxicity was 45 mg/kg bw/day and the LOAEL for developmental toxicity was 15 mg/kg bw/day.

The notified chemical was not mutagenic in an Ames test (*S.typhimurium*), and did not produce DNA damage in *E.coli*. In mammalian cell *in vitro* test systems, it produced a significant increase in chromosome aberrations in CHO cells, did induce SCE in Chinese Hamster lung cells and was negative in the UDS assay using rat liver cells. *In vivo*, the notified chemical induced bone marrow toxicity but not genotoxicity in the mouse micronucleus test.

In consideration of the submitted summary reports, the notified chemical is classified as a hazardous substance under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999), on the basis of acute oral toxicity, skin sensitisation and eye irritation. The overall hazard classification is Toxic (T) with risk phrases R25 – Toxic if Swallowed; R43 – May Cause Sensitisation by Skin Contact; and R36 – Irritating to Eyes, assigned.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

No ecotoxicological data were provided. However, the following table is a summary of the ecotoxicological data estimated by the ASTER model.

ASTER Generated Ecotoxicological Data

Test	*Species*	*Results*
Acute Effect LC50	Water flea *(Daphnia magna)*	338 mg/L
	Fathead minnow	750 mg/L
	Bluegill	548 mg/L
	Rainbow trout	366 mg/L
Chronic Effect	Fathead minnow	96 mg/L

This data indicates that the chemical is practically non-toxic to aquatic organisms.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The hazard posed by the chemical is very low. A worst case PEC calculated in Section 8 was less than 0.0048 ppm which is orders of magnitude below the LC_{50} values. The worst case concentration is unlikely to be reached, because the actual estimated amount of notified chemical reaching the environment is around 3%, that is, 8 kg per annum. This amount will most probably be disposed of by landfill or incineration. Unreacted chemical is likely to leach out of a landfill. However, this will be in low concentrations and in a diffuse manner.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Toxicokinetic studies with the hair dye formulation of 2 A 3 HP revealed a very low percutaneous absorption (0.074%) after a 30 minute exposure and rapid excretion. The notified chemical is acutely toxic, irritating to eyes, non-irritating to skin and has skin sensitisation potential. Dose related hypersalivation immediately after treatment was the only significant clinical sign in rats treated by gavage at up to 100 mg/kg bw/day for 28 days. The NOEL was 22 mg/kg bw/day. In a reproductive toxicity study, the NOEL for maternal toxicity was 45 mg/kg/day and the LOAEL for developmental effects was 15 mg/kg/day.

The notified chemical was non mutagenic in an Ames test and did not induce DNA damage *in vitro*. However, it was clastogenic *in vitro*. The notified chemical did not cause malignant transformation of mouse fibroblasts *in vitro.* In vivo, in the mouse micronucleus assay, the

notified chemical induced bone marrow toxicity, but no increased frequency of micronuclei.

No adverse effects during its use in Australia under a Low Volume Chemical permit have been noted.

Based on the summary data provided the notified chemical is classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999). The hazard classification is Toxic (T) and risk phrases: R25 – Toxic if Swallowed; R36 – Irritating to Eyes; and R43 – May Cause Sensitisation by Skin Contact, assigned.

The notified chemical is not hazardous at the imported concentration of 0.4%. However, the imported product is described as hazardous.

Occupational Health and Safety
The notified chemical will be imported as an ingredient (0.4% w/w) of a hair colour product in both bulk material for subsequent repackaging into professional use hair colour products, and retail hair colour products.

The risk of adverse health effects to dockside, transport or warehouse workers is expected to be negligible except in the event of a spill. Exposure after a spill would be controlled by use of the recommended practices for spillage clean up given in the Material Safety Data Sheet (MSDS) supplied by the notifier.

During repackaging of the imported bulk material into metal tubes, potential for dermal and eye exposure may occur as hoppers are loaded or during wash out of the tube filler. Dermal absorption is not expected to be significant given the low percutaneous absorption and low concentration of notified chemical in the hair product. Therefore, the risk of adverse health effects from repeated or prolonged exposure to the notified chemical, is considered to be negligible. Skin sensitisation is a possibility, particularly in atopic individuals. To protect workers from the hazardous nature of the hair colour product, the notifier states that operators are required to wear long vinyl gloves, safety glasses and overalls during these procedures. These measures will protect against any potential health effects of the chemical present at 0.4%.

Hairdressers are likely to receive frequent, repeated or prolonged dermal contact to hair colours containing the notified chemical. As above, there is negligible risk of systemic toxicity following repeated or prolonged exposure. However, the risk of skin sensitisation cannot be excluded. Particularly so as hairdressers by occupation are likely to have a compromised skin barrier function and for some individuals an increased susceptibility to sensitising agents. The notifiers product labels list the dye ingredients, and warn of the potential for skin irritation or sensitisation. Good hygiene practices, such as prompt removal of contaminants from the skin and the wearing of plastic or cotton lined gloves will be required to reduce the risk of adverse skin effects.

The risk of adverse health effects to retail outlet workers is expected to be negligible except in the event of a spill.

Public Health Effects
Based on the brief summaries provided for the range of toxicological studies performed on the notified chemical, the chemical presents little toxicological hazard at the levels of use,

and for the purpose, proposed by the applicant. Exposure will be potentially widespread, but will be intermittent, limited to the dermal route, and of short duration, so the potential for adverse effects is negligible. Based on the toxicology, physicochemical characteristics and the proposed use pattern, the notified chemical is not considered to pose a significant hazard to public health.

13. RECOMMENDATIONS

To minimise occupational exposure to hair products containing the notified chemical the following guidelines and precautions should be observed:

Packaging workers:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (Standards Australia 1994) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand 1992);
- Industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia 1987) and AS 3765.1 (Standards Australia 1990);
- Impermeable gloves should conform to AS/NZS 2161.2 (Standards Australia 1998);
- All occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand 1994);
- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly with absorbents which should be put into containers for disposal; and

Hairdressers:

Hairdressers are encouraged to consult guidance documents for identifying and managing health risks in hairdressing that have been published by some state occupational health and safety authorities (Division of Workplace Health and Safety 1994; WorkCover NSW 1997; WorkCover Corporation 1996). The notifier should advise the hairdressing industry of the availability of state government publications in addition to any current industry codes.

All workers:

- Good occupational hygiene should be practised to minimise the potential for skin and eye contact and ingestion. In addition, there should be prompt removal of skin and eye contaminants; and
- A copy of relevant MSDS should be easily accessible to employees.

If the conditions of use are varied, greater exposure of the public to the notified chemical may occur. Under such circumstances, further information will be required in order to assess the risks to public health.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 1994).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

16. REFERENCES

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