File No: NA/863

September 2002

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

2-[(4-Amino-2-Nitrophenyl) Amino]-Benzoic Acid

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act* 1989 (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the National Occupational Health and Safety Commission which also conducts the occupational health & safety assessment. The assessment of environmental hazard is conducted by the Department of the Environment and the assessment of public health is conducted by the Department of Health and Aged Care.

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Director

Chemicals Notification and Assessment

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1. APPLICANT

Schwarzkopf Pty Ltd of 20 Rodborough Road FRENCHS FOREST NSW 2086 (ACN 000 076 782) has submitted a limited notification statement in support of their application for an assessment certificate for "2-[(4-amino-2-nitrophenyl) amino]-benzoic acid".

2. IDENTITY OF THE CHEMICAL

The notifier has not applied for any information on the notified chemical to be exempted from publication in the Full Public Report and the Summary Report.

Chemical Name: 2-[(4-Amino-2-nitrophenyl) amino]-benzoic acid

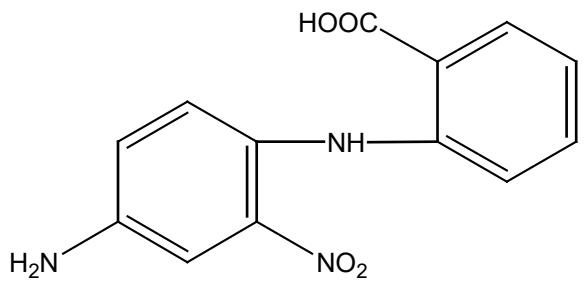
Chemical Abstracts Service (CAS) Registry No.: 117907-43-4

Other Names: 2-Nitro-4-amino-diphenylamine-2'-carboxylic acid

Marketing Name: Ro 1082

Molecular Formula: $C_{13}H_{11}N_3O_4$

Structural Formula:



Molecular Weight: 273.3

Method of Detection and

Determination: UV/Vis, IR, HPLC.

Spectral Data: Major IR absorbence peaks at 3 350, 3 050, 2 550, 1 680, 1 580, 1 510, 1 450, 1 440, 1 370, 1 350, 1 250, 1 150, 1 050, 920, 800, 750 and 650 cm^{-1}.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C & 101.3 kPa: Dark red crystal

Melting Point: >130°C (partial decomposition with thermal decomposition beginning at 200ºC).

Specific Gravity: 1.4

Vapour Pressure: <0.1 kPa at 130°C

Water Solubility: <50 mg/L at 30°C, pH 6 (Henkel KgaA, 1991a);
65 mg/L (calculated value).

Partition Co-efficient (n-octanol/water): Log P_{OW} = 0.34 at 25°C (Henkel KgaA, 1991b);
Log P_{OW} = 3.68 (calculated value).

Hydrolysis as a Function of pH: Does not containing any group which could undergo hydrolysis.

Adsorption/Desorption: K_{OC} = 2 401 (calculated value).

Dissociation Constant: The notified chemical contains the following dissociable groups with their pK_a values calculated from ACD software:

Group	pK_a
arylaminium	1.0-5.0
Diarylaminium	0-5.0
Arylcarboxylic acid	1.0-4.0

Particle Size: Not provided for dry powder.

Flash Point: > 200°C.

Flammability Limits: Not flammable.

Autoignition Temperature: Will not self-ignite.

Explosive Properties: Not expected to be explosive.

Reactivity/Stability: Will not be reactive.

3.1 Comments on Physico-Chemical Properties

Originally ACD software calculations (QSAR) were submitted for the water solubility (65 mg/L) and partition coefficient (log Pow = 3.68) on the neutral form of the molecule. However, subsequently the notifier provided test results and reports for these properties, which have been used in this section. The large variation (3 orders of magnitude) between the calculated partition coefficient (log Pow = 3.68) and experimental partition coefficient (log Pow = 0.34, determined by the EC shake flask method) suggests the notified chemical exists in the ionised form to a much greater extent than calculated from the neutral molecule. However, the water solubility results are much more closely aligned (~35 mg/L by EC test method versus an estimation of 65 mg/L) and suggest mainly the nonionised form.

The Koc of the notified chemical was estimated by the use of ACD software and indicates a moderate adsorption to the organic component of sediments and soils. This assessment agrees this is a valid estimation, but notes that in general, all data produced using QSAR estimation should be treated with caution when conducted on ionisable compounds.

4. PURITY OF THE CHEMICAL

Degree of Purity: >98%

Hazardous Impurities: None.

Non-hazardous Impurities (> 1% by weight):

Chemical name:	Anthranilic acid
Synonyms:	Benzoic acid, 2-amino-
Weight percentage:	<2%
CAS No.:	118-92-3

Additives/Adjuvants: None.

5. USE, VOLUME AND FORMULATION

The notified chemical is an ingredient in hair dye formulations. It will be imported as a component of a water based hair colour in 50 mL bottles. It comprises up to 2% in permanent hair dyes and up to 4% in oxidation dye formulations. Since the oxidation formulations will be diluted with a developer, the final concentration of the notified chemical will be up to 2% for end use.

The notifier claimed that the products containing the notified chemical will be home-use products only, not for salons.

The notifier estimated that approximately 150 kg of the notified chemical or 7 500 kg of the hair dye formulations will be imported annually in the first 5 years.

6. OCCUPATIONAL EXPOSURE

Transport and storage
After importation, products containing 2-4% notified chemical will be transported from the dockside to the notifier's warehouse for storage, then distributed to supermarkets and retailers. The notifier estimated that there will be 1-2 waterside workers, 1-2 transport drivers and 2-4 warehouse workers who will handle the products containing the notified chemical for 1-2 hours per day and 10-15 days per year. The waterside workers, drivers and warehouse workers would only be exposed to the notified chemical if the packaging was breached.

Supermarket and retail workers
Approximately 1 000 supermarket workers and retailers will handle the hair dye products in end use packaging. They would only be exposed to the notified chemical if the packaging was breached.

7. PUBLIC EXPOSURE

The cream based final formulation containing 2% of the notified substance is mixed by the consumer before application to wet hair for approximately 30 minutes followed by washing-out. Approximately 100 mL of the dyeing mixture is applied once monthly. The absorbency of the notified chemical to hair is expected to be 10-20%. The amount of dye in contact with the scalp is very small.

The public will be exposed to a maximum concentration of 4% notified chemical in certain hair dye products before the addition of developer or 2% in other hair products, including when the developer has been added.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

The notifier has estimated that there may be a 1% loss due to accidental spills at the warehouse. Annually, this equates to 1.5 kg of notified chemical.

Once the dye has been applied to hair and allowed to develop, the dye solution is rinsed from the hair and into the sewer. The notifier has estimated that 10-20% of the notified chemical will be adsorbed onto the hair. As a worst case scenario it is assumed that 90% of the notified chemical (135 kg) will be released to the sewer with the rinse water.

The notifier has estimated that 2% of the hair dye product will remain as residue in the bottles after use, ie 3 kg/annum of the notified chemical. The containers will be disposed of in domestic garbage and end up in a landfill.

A summary of estimated maximum annual amount of waste notified chemical generated is:

spills, 1%	1.5 kg	Landfill
bottle residues, 2%	3 kg	Landfill
rinse water (users), 90%	135 kg	Sewer
TOTAL	139.5 kg	

8.2 Fate

Nearly all of the waste generated during the end use of the hair dye containing the notified chemical will be disposed to the sewer. The chemical is expected to partition from the water compartment due to its low water solubility and high estimated Koc and associate with the organic component of the sludges and sediments. However, the low experimental log Pow suggests that this will occur at a minimal rate and extent.

The residues remaining in the 'empty' import bottles and the small amount of chemical that may be accidentally spilt in storage or transport will be disposed to landfill. The small quantities involved coupled with the low water solubility and high Koc, make it unlikely that any chemical would leach from landfill.

The biodegradation and bioaccumulation potentials were not determined by the notifier but the chemical is not expected to present a significant bioaccumulation hazard to aquatic organisms due to the anticipated low concentration in water.

9. EVALUATION OF TOXICOLOGICAL DATA

Toxicological data is available on the notified chemical, however only full test reports of the genotoxicity studies (Section 9.3) were provided for assessment. The notifier provided a report from the European Cosmetic, Toiletry and Perfumery Association (COLIPA) (The European Cosmetic Toiletry and Perfumery Association, 1995) which contained summaries of the toxicological studies and a separate summary by Sterzel and Steiling (1996). The summary and evaluation of toxicological data, except genotoxic potential, is based on the report from the European Cosmetic, Toiletry and Perfumery Association.

9.1 Acute Toxicity

Summary of the acute toxicity of 2-[(4-Amino-2-Nitrophenyl) Amino]-Benzoic Acid.

Test	*Species*	*Outcome*
acute oral toxicity	rat	LD_{50}>2 000 mg/kg
acute dermal toxicity	rat	LD_{50}>2 000 mg/kg
skin irritation	rabbit	Not a skin irritant
skin irritation (repeat dose)	hairless mice	Not a skin irritant
eye irritation	rabbit	A severe eye irritant
skin sensitisation (Magnusson-Kligman test)	guinea pig	A skin sensitiser

skin sensitisation (Buehler test)	guinea pig	Not a skin sensitiser

9.1.1 Oral Toxicity

Species/strain:	Rat/Wistar
Number/sex of animals:	5 male and 4 female
Observation period:	14 days
Method of administration:	A single oral dose (2 000 mg/kg) by gavage. The notified chemical was suspended in an aqueous solution of 1% carboxymethylcellulose and 0.5% Cremophor.
Test method:	Not provided.
Mortality:	One male died.
Clinical observations:	Not provided.
Morphological findings:	One male had stained intestines, subcutis and muscles, and lung oedema.
Comment:	None.
LD_{50}:	> 2 000 mg/kg
Result:	The notified chemical was of very low acute oral toxicity in rats.

9.1.2 Dermal Toxicity

Species/strain:	Rat/Wistar
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	A single dermal dose (2 000 mg/kg) under an occlusive dressing for 24 hours. The notified chemical was suspended in an aqueous solution of 1% carboxymethylcellulose.
Test method:	Not provided.

Mortality:	None.
Clinical observations:	Lethargy was observed in most animals during the first 2 days. Low body weight gain was recorded up to day 7. Discolouration of black/red was seen on the exposed skin during entire study period, but no skin oedema was noted.
Morphological findings:	None.
Comment:	None.
LD_{50}:	> 2 000 mg/kg.
Result:	The notified chemical was of low dermal toxicity in rats.

9.1.3 Inhalation Toxicity

Study not included in summary.

9.1.4 Skin Irritation

Species/strain:	Rabbit/Kleinrusse (Chbb: HM)
Number/sex of animals:	5 male.
Observation period:	72 hours.
Method of administration:	An aliquot of 0.6 g of moistened notified chemical was applied to the intact shaved back skin of each animal under an occlusive dressing to 4 hours.
Test method:	OECD TG 404
Comment:	The skin irritation reactions were scored at 1, 24, 48 and 72 hours. The summary report indicated that the notified chemical was neither irritating nor corrosive to rabbit skin.
Result:	The notified chemical was non irritating to the skin of rabbits.

9.1.5 Eye Irritation

Species/strain:	Rabbit/Kleinrusse (Chbb: HM)
Number/sex of animals:	4 male
Observation period:	21 days

Method of administration: Undiluted notified chemical (0.1 g) was instilled into the conjunctival sac of the right eye, and the untreated eye served as control.

Test method: OECD TG 405

Comment: The eye irritation reactions were scored at 1, 6, 24, 48 and 72 hours and 7, 10, 14, 17 and 21 days after dosing. The summary report indicated that there were slightly increased opacity of the cornea in 2 rabbits, and mild to moderate irritation of the conjunctivae persisted during the study (21 days). Fluorescein examination revealed slight corneal epithelial damages at the end of the study. Individual Draize scores were unavailable.

Result: Insufficient data were provided to determine the degree of irritation although effects were persistent. The COLIPA report determined the notified chemical to be severely irritating to the eyes of rabbits.

9.1.6 Skin Sensitisation (Magnusson-Kligman Test)

Species/strain: Guinea pig/Pirbright White

Number of animals: Test group: 20 female;
Control group: 20 females.

Induction procedure:

test group:
day 0
- 1 injection (0.1 mL) of 0.5% notified chemical in water (pH 8);
- 2 injections (0.1 mL) of 1% notified chemical in a 1:1 mixture of Freund's Complete Adjuvant (FCA) and water.

day 7
Topical Induction:
A 48-hour occluded application of 1 g of the notified chemical (20% in vaseline).

control group: Control group was treated with FCA and vehicle only.

Challenge procedure:

day 21
Test and Control animals:
A 24 hour occluded application of 10% notified chemical in water (0.2 mL) to animal flank.

Test method: OECD TG 406

Comment:	One animal died during the study probably due to pneumonia. Animals were examined 24 and 48 hours after removal of the patches. Approximately 60% (12/19) of test animals demonstrated positive responses and no dermal effects were observed in the control animals. Individual Draize scores and the definition of a positive response were unavailable.
Result:	Individual animal data were not provided. The COLIPA report determined the notified chemical to be a skin sensitiser to guinea pigs.

9.1.7 Skin Sensitisation (Buehler Test)

Species/strain:	Guinea pig/Pirbright White
Number of animals:	Test group: 20 females; Control group: 20 females.
Induction procedure:	
test group: day 1, 8 and 15	An occlusive dermal dose of 0.2 g notified chemical (20% in vaseline) was applied to the right trunk of each animal for 6 hours.
control group:	Untreated.
Challenge procedure:	
day 29	Test and Control animals: An occluded application of 2.5% notified chemical (0.2 mL in vaseline) to both flanks for 6 hours.
Test method:	OECD TG 406
Comment:	Animals were examined 24 and 48 hours after removal of the patches. No dermal effects were observed in either the test and control animals.
Result:	The notified chemical was non sensitising to the skin of guinea pigs.

9.2 Repeated Dose Toxicity

9.2.1 Skin Irritation

Species/strain:	Hairless mouse/hr/hr

Number/sex of animals:	5 females.
Dose/Study duration:	Week 1: 2% notified chemical aqueous solution (pH 8); Week 2: 4% notified chemical aqueous solution (pH 8); Week 3: 8% notified chemical aqueous solution (pH 8).
Method of administration:	One to two drops of diluted notified chemical were applied to a defined skin area of each animal once a day for 15 consecutive working days (5 days per week).
Test method:	Unspecified.
Comment:	The skin irritation reactions were scored daily. The summary report indicated that the notified chemical did not induce any skin irritation reactions or any symptoms of systemic intoxication.
Result:	The notified chemical (up to 8%) was non irritating to the skin of mice on repeated applications.

9.2.2 Subchronic Oral Repeated Dose Toxicity

Species/strain:	Rat/Sprague Dawley
Number/sex of animals:	10/sex per dose.
Method of administration:	Oral (gavage) administration daily (5 days per week) for 13 weeks.
Dose/Study duration:	Control group: 0 mg/kg/day; Low dose group: 20 mg/kg/day; Mid dose group: 60 mg/kg/day; and High dose group: 180 mg/kg/day (vehicle: water with 1% carboxymethylcellulose and 0.5% Cremophor). Control and high dose groups had additional 5/sex as recovery groups which were terminated after a recovery period of 4 weeks.
Test method:	OECD TG 407

Clinical observations:
All animals survived the treatment.

Discolouration of urine was observed in all test animals. Fur and tail skin were coloured in a dose-related manner. Water consumption was increased in the mid and high dose females.

Clinical chemistry/Haematology
A slight increase in thrombocytes was found in males and females at high dose.

Pathology:
Yellow pigment was found in liver cells of all groups including the recovery groups. The absolute liver weight was increased in all test females with no dose-response relationship.

Comment:
The lack of detailed study results precluded the determination of a No Observed Effect Level (NOEL).

Result:
A NOEL is not established based on effects seen at the lowest dose tested.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay

Strains:	TA98, TA100, TA1535, TA1537 and TA1538.
Metabolic activation:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254.
Concentration range:	First experiment: 4 to 2 500 μg/plate; Second experiment: 8 to 5 000 μg/plate. Positive controls (without metabolic activation): Sodium azide for TA100 and TA1535; 4-nitro-o-phenylenediamine for TA98 and TA1538; 9-aminoacridine for TA1537. Positive control (with metabolic activation): 2-aminoanthracene for all strains.
Test method:	OECD TG 471
Comment:	Precipitation and toxic effects were observed at 5 000 μg/plate. The notified chemical induced reverse mutation in all tested strains in the absence and presence of S9-mix. Results of the experiments were unavailable.
Result:	The notified chemical was mutagenic under the conditions of the test.

9.3.2 Gene Mutation Assay at the HGPRT *Locus* in Chinese Hamster Lung Cells (Heidemann, 1988)

Cells: V79 Chinese hamster lung cells.

Metabolic activation system: Liver fraction (S9 mix) from rats pretreated with Aroclor 1254.

Dosing schedule:

Metabolic Activation	Experiment Number	Test concentration (μg/mL)	Controls
-S9	1 and 2	treatment time = 4 hours 10, 30, 60 and 100 μg/mL	Positive: EMS Negative: unspecified negative control and a solvent control
+S9	1 and 2	treatment time = 4 hours 10, 30, 60 and 100 μg/mL	Positive: DMBA Negative: unspecified negative control and a solvent control

EMS - ethyl methanesulphonate
DMBA – 7, 12-dimethylbenz(a)anthracene

Test method: OECD TG 476

Comment: In experiment 1, mutant frequencies were increased by a factor of 3-5 compared to solvent control, but the results were within historical control ranges and non-reproducible.

No enhanced genetic alteration of the HGPRT locus in V79 Chinese hamster lung cell line could be detected, both with and without S9-mix.

Result: The notified chemical did not show mutagenic activity (point mutation) under the conditions of the test.

9.3.3 Chromosomal Aberration Assay in Chinese Hamster Lung Cells (Heidemann, 1989)

Cells: V79 Chinese hamster lung cells.

Metabolic activation system: Liver fraction (S9 mix) from rats pretreated with Aroclor 1254.

Dosing schedule: The test was performed in duplicate and 100 metaphases per culture were scored for structural chromosomal aberrations.

Metabolic Activation	Test concentration (μg/mL)	Controls
-S9	treatment time = 4 hours 7 hr preparation interval: 100; 18 hr preparation interval: 10, 60, and 100; 28 hr preparation interval: 100.	Positive: EMS Negative: unspecified negative control and solvent control
+S9	treatment time = 4 hours 7 hr preparation interval: 100; 18 hr preparation interval s: 10, 60, and 100; 28 hr preparation interval s: 100.	Positive: CPA Negative: unspecified negative control and solvent control

EMS - ethyl methanesulphonate
CPA - cyclophosphamide

Test method: OECD TG 473

Comment: At 100 μg/mL, the plating efficiency of V79 cells and the mitotic index at 7 hours with S9-mix, and at 18 and 28 hours without S9-mix were decreased.

No enhanced structural aberrations in the cell line could be detected at each fixation interval, both with and without S9-mix.

Result: The notified chemical was non clastogenic under the conditions of the test.

9.4 Other Studies

9.4.1 Cutaneous Absorption Study *in vitro* (excised rat skin)

Species/strain: Female rat/Wistar

Formulation details: 1.36% notified chemical aqueous solution containing basic emulsion *Bth 66* B (mix of fatty alcohols and fatty alcohol polyglycol sulfate) and ammonia (pH 9.8).

Method of administration: Excised rat skin was exposed to the formulation (0.253 mg notified chemical per cm^2) for 20 hours in a non-occlusive manner at 32°C.

Test method: Unspecified.

Comment: The *in vitro* dermal penetration rate was measured as 3.18% (n=5, 20 hours) which corresponds to 0.506 mg/cm^2.

Result: The *in vitro* dermal penetration rate of the notified chemical is low in excised rat skin.

9.4.2 Cutaneous Absorption Study *in vitro* (excised rat and pig skin)

Species/strain:	Female rat/Wistar; Male pig/Schweizer Edelschwein.
Formulation details:	Cream (without developer): 2% notified chemical Complete formulation: 2.28% notified chemical and 6% H_2O_2. No further details of the formulation were provided.
Method of administration:	Cream (without developer) was introduced to excised pig skin (91 mg, 16.9 mg/cm^2) and excised rat skin (99 mg, 18.2 mg/cm^2) for 30 minutes, and rinsed off. Complete formulation was introduced to excised pig skin (122 mg, 22.5 mg/cm^2) and excised rat skin (100 mg, 18.5 mg/cm^2) for 30 minutes, and rinsed off. Each penetration rate was derived from 6 measurements. A dynamic chamber model (2 hour intervals) was chosen to follow the absorption kinetics over a period of 22 hours after exposure.
Test method:	Unspecified.
Comment:	Cream (without developer) had penetration rates of 1.86% and 0.56% in excised rat and pig skin, respectively. Complete formulation had penetration rates of 1.17% and 0.09% in excised rat and pig skin, respectively.
Result:	The *in vitro* dermal penetration rates of the notified chemical were low in excised rat and pig skin.

9.4.3 Cutaneous Absorption Study in Rats *in vivo* (complete formulation)

Species/strain:	6 Female rat/Sprague Dawley.
Formulation details:	[^{14}C] labelled and unlabelled notified chemical, p-toluylene diamine sulfate, resorcinol, sodium sulfite and ammonium sulfate, basic emulsion *BHT 66* (mix of fatty alcohols and fatty alcohol polyglycol sulfate), water and ammonia. The concentration of the notified chemical in above formulation was 4% before 1:1 dilution with a developer

dispersion containing 6% H_2O_2.

Method of administration: The diluted (1:1) formulation containing 4.02 mg notified chemical (0.45 mg/cm^2) was applied to intact clipped rat skin for 30 minutes under a semi-occlusive dressing.

Observation period: 72 hours.

Test method: Unspecified.

Comment: The percutaneous absorption rate of the notified chemical was 0.19% (0.45 mg/cm^2).

Most notified chemical (92.2%) was removed by washing, 0.04% was found in dermis and 0.22% was found in the *stratum corneum*. Excretion through urine and faeces was 59% and 41%, respectively, mainly in the first 24 hours (73%).

The labelled material was near or below the detection limit in all analysed organs.

Result: The *in vivo* dermal penetration of the notified chemical is low in rats.

9.4.4 Cutaneous Absorption Study in Rats *in vivo*

Species/strain: 6 Female rat/Sprague Dawley.

Formulation details: [^{14}C] labelled and unlabelled notified chemical, ammonium sulfate, basic emulsion *BHT 66* (mix of fatty alcohols and fatty alcohol polyglycol sulfate), water and ammonia.

The concentration of the notified chemical in above formulation was 4% (pH 9.5).

Method of administration: The formulation containing 7.89 mg notified chemical (0.89 mg/cm^2) was applied to intact clipped rat skin for 30 minutes under a semi-occlusive dressing.

Observation period: 72 hours.

Test method: Unspecified.

Comment: The percutaneous absorption rate of the notified chemical was 0.12% (0.89 mg/cm^2).

Most notified chemical (94%) was removed by washing, 0.02% was found in dermis and 0.16% was found in the

	stratum corneum. Excretion through urine and faeces was 52% and 48%, respectively, mainly in the first 24 hours (70%). The labelled material was near or below the detection limit in all analysed organs.
Result:	The *in vivo* dermal penetration of the notified chemical is low in rats.

9.4.5 Excretion After Intestinal Absorption in Rat

Species/strain:	Rat/Sprague Dawley.
Number/sex of animals:	6 females.
Method of administration:	A single oral dose of 20 mg/kg [^{14}C] notified chemical in DMSO/water (7/3) was administrated by gavage.
Observation period:	3 days
Test method:	Unspecified.
Comment:	The minimum peroral absorption rate of the notified chemical via intestine was 37.7% (found in urine during the first 24 hours). Excretion through urine and faeces was 38% and 62%, respectively, mainly in the first 24 hours (93.2%). After 72 hours, the highest levels of labelled material were found in kidneys, liver and blood.
Result:	Most of the chemical was excreted in the first 24 hours with some evidence of accumulation in liver and kidney.

9.4.6 Embryotoxicity and Teratogenicity in Rats

Species/strain:	Rat/Wistar
Number/sex of animals:	25 dams/group.
Method of administration:	Oral (gavage) administration daily from day 6 to 15 of pregnancy.
Dose/Study duration:	Control group: 0 mg/kg/day; Low dose group: 50 mg/kg/day; Mid dose group: 150 mg/kg/day; and

High dose group: 450 mg/kg/day
(vehicle: water with 4% carboxymethylcellulose).

All the dams were sacrificed on day 21 of gestation for examination.

Test method: Unspecified.

Maternal response to the treatment:
Discolouration of red urine was observed. Temporary decrease in bodyweight gain due to decreased food consumption in high dose animals during the first half of the treatment period.

Examination of foetuses:
One foetus at mid dose had malposition of the right hind paw.

Comment:
The notified chemical was neither embryolethal, embryotoxic nor teratogenic.

Result:
Insufficient data were provided to determine the NOEL. The NOAEL for embryotoxicity and teratogenicity was 450 mg/kg/day.

9.5 Overall Assessment of Toxicological Data

The COLIPA report consisted of study summaries only and detailed results were unavailable for assessment. Based on the COLIPA report, the notified chemical was of very low acute oral toxicity and low acute dermal toxicity in rats. It was not a skin irritant but a severe eye irritant in rabbits. In guinea pigs, the notified chemical was found to be a sensitiser in a Magnusson and Kligman test but not a sensitiser in a Buehler test. Taking account the proposed use and the difference between the 2 test methods, the notified chemical is not considered to be a skin sensitiser under the use conditions.

In a 3-week dermal repeat dose study, the hairless mice tolerated the notified chemical up to 8% without showing any skin irritation. In a 13-week oral repeat dose study in rats, coloured urine was seen in all test animals and the discolouration of fur and tail skin was presented in a dose related way. No NOEL is established based on effects seen at the lowest dose tested and the lack of a complete study report. When the notified chemical was administered in pregnant rats, no embryotoxicity and teratogenicity were observed up to 450 mg/kg/day.

The notified chemical was mutagenic in an Ames test. However, it did not show any evidence of mutagenicity at the HGPRT *locus* or clastogenicity in a chromosomal aberration study in the Chinese hamster lung cells. Full test reports were provided for both end points. The notifier did not provide a study report on micronucleus assay in the bone marrow cells *in vivo*.

Cutaneous absorption of the notified chemical was investigated with excised rat and pig skin models *in vitro* and *in vivo*, in the presence or absence of hydrogen peroxide. The

percutaneous absorption rates were found to be higher in rat skin than in pig skin *in vitro*, and in rats were higher in *in vitro* studies than in *in vivo* studies.

More than 70% of the notified chemical was excreted in the first 24 hours after dermal absorption in rats, distributed equally in urine and faeces. When a gavage dose was administered in rats, the minimum peroral absorption rate was 37.7%. Excretion through urine and faeces was 38% and 62%, respectively, mainly in the first 24 hours (93.2%).

Based on the severe eye irritant and skin sensitisation effects, the notified chemical is classified as a hazardous substance with R41 (Risk of serious damage to eyes) and R43 (May cause sensitisation by skin contact) according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

No ecotoxicological data has been supplied by the notifier which, according to the Act, is acceptable for chemicals with import volumes < 1 tonne per year.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notified chemical is expected pose a low environmental hazard if used as specified by the notifier.

The residues remaining in the 'empty' import bottles (up to 3 kg/annum) and the small amount of chemical that may be accidentally spilt in storage or transport (up to 1.5 kg/annum) will be disposed to landfill. The low quantity placed in landfill is unlikely to leach.

The notifier estimated a 10-20% uptake of dye by the hair which means that up to 90% will be rinsed from the hair and end up in the sewer. The use of the hair dye would be dispersed over Australia, so a PEC for the notified chemical could be calculated as follows:

Amount of notified chemical entering sewer	135 kg
Population of Australia	18 million
Amount of water used per person per day	150 L
Number of days in a year	365
Estimated PEC	0.000013 mg/L (0.013 ppb)

No aquatic toxicity data are available, however the estimated PEC is likely to be well below toxic levels. A low hazard may be expected from the proposed use pattern.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Based on the COLIPA report, the notified chemical was of very low acute oral and low acute dermal toxicity. It was not a skin irritant but a severe eye irritant. Under the proposed use conditions, the notified chemical is considered to be non-sensitising. The notified chemical

had a low percutaneous absorption rate. A No Observed Effect Level (NOEL) could not be established in a 13 week oral study in rats. The notified chemical was found mutagenic to the bacterial strains tested but non-mutagenic at the HGPRT *locus* and non-clastogenic in cell cultures. The notified chemical is classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) based on the effects of severe eye irritation and skin sensitisation. The overall classification is Irritant (Xi) and the risk phrases R41 (Risk of serious damage to eyes) and R43 (May cause sensitisation by skin contact) are assigned.

Occupational Health and Safety

As the notified chemical is introduced as an end use consumer product ready for sale to the public, exposure is not expected during transport, storage, distribution or retail as long as the packaging remains intact. The risk of adverse health effects for transport, storage and retail workers is considered negligible.

Should the notified chemical be manufactured or formulated in Australia, the occupational exposure scenario will change and industrial controls and personal protective equipment particularly eye and skin protection will be needed to minimise the health risk. Workers who become sensitised should not continue to handle the notified chemical.

Public Health

The public exposure to the hair dye products containing up to 4% notified chemical will be widespread and repeated. However, given its low concentration, the short exposure time, its low absorption rate and low toxicity, the risk to the public health induced by the notified chemical is considered to be low.

13. RECOMMENDATIONS

To minimise occupational exposure to "2-[(4-Amino-2-Nitrophenyl) Amino]-Benzoic Acid" the following guidelines and precautions should be observed:

- The label for "2-[(4-Amino-2-Nitrophenyl) Amino]-Benzoic Acid" should include risk phrases R41 (Risk of serious damage to eyes) and R43 (May cause sensitisation by skin contact).

- Goggles should conform to the specifications detailed in Australian Standard (AS) 1336 (Standards Australia, 1994) and Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand, 1992); industrial clothing should conform to AS 2919 (Standards Australia, 1987) and AS 3765.2 (Standards Australia, 1990); impermeable gloves should conform to AS/NZS 2161.2 (Standards Australia/Standards New Zealand, 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 swept up and put into containers for disposal.

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

- Workers who become sensitised should not continue to handle the notified chemical.

If products containing the notified chemical are hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999), workplace practices and control procedures consistent with State and Territory hazardous substances regulations must be in operation.

To minimise exposure to end use consumer products containing 2-[(4-Amino-2-Nitrophenyl) Amino]-Benzoic Acid the following guidelines and precautions should be observed:

- Label for the product containing the notified chemical should carry the Warning Statement 21, Appendix F of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP);

- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly with absorbents and put into containers for disposal;

- The concentration of the notified chemical in hair dye formulations should not exceed 2%.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the product containing 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 may 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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Sterzel, W and Steiling W (1996) 2-Nitro-4-amino-diphenylamine-2'-carboxylic acid, COLIPA No. B 087, Hankel KgaA, Dusseldorf.

The European Cosmetic Toiletry and Perfumery Association (1995) COLIPA Number: B 087, Bruxelles.

Attachment 1

The Draize Scale (Draize, 1959) for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale (Draize *et al*., 1944) for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
		Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
Diffuse beefy red	3 severe	Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe

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MSDS