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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

2-Amino-5-ethylphenol HCl

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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 Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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2-Amino-5-ethylphenol HCl

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Cosmetic Suppliers Pty Ltd (ABN: 83 000 303 391) and Procter & Gamble Australia Pty Ltd (ABN: 91 008 396 2451)
Both at:
Nortel Building
Level 4 / 1 Innovation Road
North Ryde NSW 2113

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: purity, import volume, and use details

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
EU. Currently being evaluated by the EU Scientific committee on Consumer Products (SCCP)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
2-Amino-5-ethylphenol HCl (INCI Name)

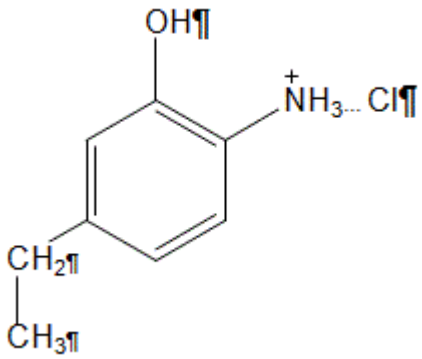
CAS NUMBER
149861-22-3

CHEMICAL NAME
Phenol, 2-amino-5-ethyl-, hydrochloride

OTHER NAME(S)
2-Amino-5-ethylphenol hydrochloride (IUPAC)

MOLECULAR FORMULA
$C_8 H_{11} NO.HCl$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
173.64 g/mol

ANALYTICAL DATA
A reference NMR spectrum was provided.

3. COMPOSITION

DEGREE OF PURITY > 96%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Beige-yellowish crystals or powder

Property	Value	Data Source/Justification
Melting Point	218°C (decomp.)	Measured
Boiling Point	225°C (decomp.) at 101.3 kPa	Measured
Density	1220 kg/m^3	MSDS
Vapour Pressure	4.1 x 10^{-9} kPa at 20°C	Measured
Water Solubility	428 g/L at 20°C (pH 1.42)	Measured
Hydrolysis as a Function of pH	Oxidatively unstable in water at pH 4 – 9. Half-lives at 30°C are 228 & 16.4 hours at pH 4 & 7.	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = 1.37 at 20°C	Measured
Adsorption/Desorption	log K_{oc} = 1.25 at 20°C	Measured
Dissociation Constant	pKa_1 = 5.42 & pKa_2 = 10.04 pKa_1 = 5.17	Calculated Measured
Surface Tension	69.94 ± 0.11 mN/m	Measured

Particle Size	Mass fraction <32 μm: 88.62% Mass fraction 32-90 μm: 11.23% Mass fraction 90-250 μm: 0.11% Mass fraction > 250 μm: 0.03%	
Measured	Mean diameter in 32-250 μm range = 45.3μm.	
Flash Point	Not determined	The notified chemical is a solid and will only be introduced as a solution in formulated products.
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400°C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Measured

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity
The notified chemical is stable under normal use conditions. It is incompatible with oxidizing agents.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in a number of formulated hair dye products at concentrations up to 1%.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	<0.2	<0.2	<0.2	<0.2	<0.2

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
The notified chemical will not be manufactured or reformulated in Australia. It will be received by the notifiers before being transported to customer sites.

TRANSPORTATION AND PACKAGING
The imported product will be packaged in 60 mL plastic tubes, with 12 tubes to a carton. These products will be imported into Australia by sea, and distributed to the notifier's warehouse, and then on to end-use sites, by road transport.

USE
The notified chemical is used as an oxidative colouring agent for hair dye formulations at concentrations up to 1%.

OPERATION DESCRIPTION
No manufacture or reformulation will occur in Australia.

End-use in hair salons
Prior to application to the hair, the oxidative colouring agent (containing the notified chemical at up to 1%) and the developer are mixed at a ratio of 1:1 to 1:3 (g dye formulation + g developer formulation) in a plastic bowl with an applicator brush. It is common practice to apply 100 g of the finished mixed product by brush. This is left in contact with the hair for the required colour time (up to 30 minutes) followed by rinse off with water and shampoo. The application may be repeated at monthly intervals.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

EXPOSURE DETAILS

Transport and Storage

As the notified chemical is introduced as a salon exclusive product, exposure is not expected during transport, storage, or distribution as long as the packaging remains intact.

Hair salons

The number of commercial salons across Australia likely to use the notified chemical is estimated in the 100s. The number of employees would range typically between two to ten per salon and these workers may use products containing the notified chemical for an average of 2 hour/day for 200 days/year.

Professional salon workers may be exposed to the notified chemical primarily by skin contact, with potential for eye contact from splattering during mixing, application or rinsing. Hairdressers may receive repeated or prolonged dermal contact to hair colour products containing the notified chemical. Good occupational hygiene practices would minimise exposure. These practices include use of personal protective equipment, consisting of impervious gloves and a plastic apron.

A recent Australian survey of 184 hairdressers and 193 trainee hairdressers found a high percentage (91.9% and 90.2% respectively) of respondents used gloves when in contact with hair dye products (Nixon et al, 2006). Gloves are most likely to be worn during the application stage of the dyeing process, while they are not usually worn when preparing the dye, shampooing/rinsing the hair or when cutting/drying the dyed hair (Lind et al, 2005 and Hueber-Becker et al, 2007). In a recent study using ^{14}C-PPD (*p*-phenylenediamine) the exposure during the various stages of the dyeing process was quantitated. The mean daily exposure (6 dye processes) during the latter stages when gloves are usually not worn (i.e. shampooing/rinsing and cutting/drying) was found to be 0.064% of daily used PPD (Hueber-Becker et al, 2007). Based on this value, as well as the same conservative estimate of the number of dye processes (i.e. 6 per day) and the amount of notified chemical used per application (1% of 60 mL = 0.6 g) an estimate for hairdresser exposure when gloves are used during application is calculated as 0.38 mg/day. In the Hueber-Becker study 0.381% of the daily used PPD was collected off the gloves used during the application phase. Therefore if gloves were not worn during the application stage (and using the same assumptions listed above) the hairdresser would be exposed to a total of 2.67 mg of notified chemical per day as a worst case. Inhalation exposure in the Hueber-Becker study could not be quantitated but was not considered to be significant.

While gloves are considered to be protective against exposure to the notified chemical, inappropriate glove use during the use of hair dye product, including use of the wrong type or re-using gloves, may lead to increased exposure to the notified chemical. In their occupational exposure study using the hand-rinse method in Sweden Lind et al (2005) found that dermal exposure to a number of hair dye chemicals occurred even when gloves were worn. The majority of the hairdressers in the study used the gloves more than once, and the gloves were often turned inside-out after rinsing with water and re-used. Natural rubber latex gloves were often used for 2-3 months or until they were discarded as damaged or torn. This is consistent with findings from a recent survey of Australian hairdressers, where it was found that 70% of participants re-used disposable gloves (Nixon et al, 2006). In one salon hairdressers were given one pair of disposable gloves a week and the used gloves were washed in a washing machine and dried in a dryer before re-use, which is likely to seriously affect the barrier properties of the gloves. In addition it was found to be common practice in a number of salons to wash the gloves while still on the hands, dry them and turn them inside out, then re-use the gloves with the outer contaminated surface being on the inside of the glove. In these situations the gloves are likely to be a source of contamination, rather than a protective measure.

The type of glove may also affect whether or not the gloves provide adequate protection. In the Australian survey 70% of participants used natural rubber latex (NRL) gloves (Nixon et al, 2006), and in the Swedish study the majority also used NRL gloves, with polyvinylchloride (PVC), polyethene (PE) and nitrile (NR) also being used (Lind et al, 2005). In permeability studies of hair dye chemicals through different types of gloves (NRL, PVC, NR, PE and neoprene (NP, not disposable)) it was found that all gloves gave protection for ≥ 30

min (Lind et al , 2007; and Lee and Lin, 2009). In terms of breakthrough time (elapsed time between application of chemical to the glove and its subsequent presence in the collection medium on the other side of the glove) the order was NP > NRL > NR > PVC > PE. Although suitable for the time periods expected during hair dyeing, most of the disposable gloves did show breakthrough of the chemicals and therefore are not suitable to be re-used. Due to the possible allergenicity of NRL the preferred disposable glove appears to be the nitrile (NR) glove (Lind et al, 2007; Nixon et al, 2006).

6.1.2. Public exposure

It is expected that during transport, formulation and storage, exposure of the general public to the notified chemical will be low.

The products are designed for the salon market and intended for one application per bottle. Public exposure to hair colourant products containing the notified chemical is likely to be intermittent (based on use pattern) and widespread (sold to the public and limited only by the commercial successes of the products). In the products, the notified chemical (≤ 1%) will be diluted 1:1 to 1:3 with developer, leading to maximal exposure concentrations of ≤ 0.5%. The hair dye will be used at a maximum of once per month, at up to 0.6 g of the notified chemical (1 % in 60 mL liquid product) each application. Consumers will be exposed to the hair dye product for one hour daily, 12 days per year primarily by dermal route, with the possibility of accidental ocular and oral exposure.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical and an analogue chemical, 2-amino-5-ethylphenol phosphate, were provided by the notifier. A comparison of the physical-chemical properties of these two chemicals is provided in the table below.

Property	**Notified chemical (2-amino-5-ethylphenol hydrochloride)**	**Analogue chemical (2-amino-5-ethylphenol phosphate)**
MW	173.64 g/mol	235.18 g/mol
Content free base	79.0%, w/w	58.30%, w/w
pKa	5.17	5.28 (pKa1); 7.08 (pka2)
Solubility: water/DMSO	>200/>50 g/L	>200/>50 g/L

The freebase component, i.e. 2-amino-5-ethylphenol, is expected to contribute the most to toxicity potential of the two salts. Taking into consideration the pKa and log Pow values, it is concluded that at pH values 7.0 and above both salts are available in the free base form. Therefore the phosphate salt was considered to be an acceptable analogue to determine the toxicity of the notified chemical.

The results from the toxicological investigations are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Test Substance*	*Result and Assessment Conclusion*
Acute oral toxicity in the rat	Analogue phosphate salt	LD50 > 2000 mg/kg bw low toxicity
Skin irritation/corrosion - Transcutaneous Electrical Resistance Assay	Notified chemical	Likely to be corrosive to the skin
Skin irritation – *in vitro* Reconstituted Human Epidermal Model (SkinEthic)	Notified chemical	Likely to be corrosive to the skin

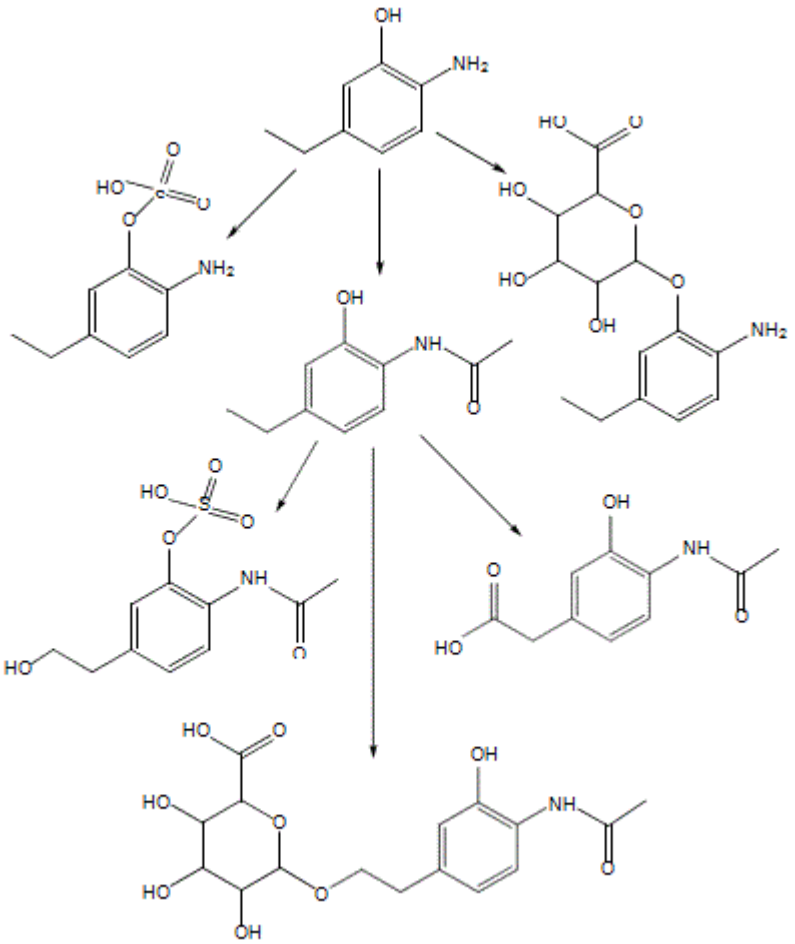
Eye irritation – *in vitro* Chicken Enucleated Eye test	Notified chemical	Severely irritating
Skin sensitisation – Murine local lymph node assay	Analogue phosphate salt	Evidence of sensitisation
Oral repeat dose toxicity – 13 week in the rat	Notified chemical	NOAEL= 16 mg/kg bw/day
Genotoxicity - bacterial reverse mutation	Analogue phosphate salt	Non mutagenic
Genotoxicity – *in vitro* cell mutation assay at the thymidine kinase locus ($TK^{+/-}$) in mouse lymphoma L5178Y cells	Analogue phosphate salt	Non mutagenic
Genotoxicity – *in vitro* induction of micronuclei in cultured human peripheral blood lymphocytes.	Analogue phosphate salt	Genotoxic
Genotoxicity – *in vivo* mouse bone marrow micronucleus test	Analogue phosphate salt	Non genotoxic
Genotoxicity – *in vivo* Comet assay in the rat	Analogue phosphate salt	Non genotoxic
Developmental and reproductive effects: Prenatal developmental toxicity study in rats	Notified chemical	NOAEL=74 mg/kg bw/day, for both dams and foetuses.
Pharmacokinetic/Toxicokinetic studies (1): Percutaneous absorption *in vitro*	Notified chemical	8.123 μg/cm^2 0.846% of applied dose
Pharmacokinetic/Toxicokinetic studies (2): Absorption, distribution, metabolism and excretion in the Wistar rat.	Notified chemical	Extensively absorbed after oral dosing, readily distributed into all organs, extensively metabolised and excreted via urine. Dermal absorption was high after 24 h exposure and low after 0.5 h exposure. Sulfation of the parent compound was the major metabolic reaction. No major qualitative differences were observed between the oral and dermal routes of administration.

Toxicokinetics

Absorption, distribution, metabolism and excretion of the notified chemical were investigated in a toxicokinetic study in rats. Rats were dosed intravenously with 75 mg/kg bw, orally with 370 mg/kg bw and topically with 20 (0.5 h) and 100 (24 h) mg/ml (equivalent to 16 and 80 mg/kg bw and to 0.2 and 1.0 mg/cm^2, respectively). The vehicle was phosphate buffered saline for the intravenous group, Milli-Q water for the oral group and DMSO for the high dose dermal group. For the low dose dermal group, a water based vehicle mimicking use conditions was used.

Notified chemical administered orally was extensively absorbed, being 101% when calculated from urine data and 60% when calculated from plasma data. The notified chemical readily distributed into all organs, was extensively metabolised and excreted via the urine. Dermal absorption of the notified chemical, including the

'potentially absorbed' fraction remaining in the skin, was high after a 24 h exposure period to a 10% solution (63% of applied dose, 680 µg/cm^2), and low after a 30 min exposure period to a 2% solution (5% of applied dose, 9.0 µg/cm^2). When absorbed, excretion took place mainly via the urine. After all routes of administration, sulfation of the parent compound 2-amino-5-ethylphenol hydrochloride was the major metabolic route. Furthermore, glucuronidation and acetylation were also relevant metabolic pathways. No major qualitative differences in the metabolite profile between the oral and dermal routes of administration were observed, although a greater proportion of acetylation was observed after dermal absorption. Based on the identified metabolites the study authors proposed the metabolic pathway shown below.



For some of the metabolites depicted, the metabolic reactions might have taken place on a different position in the molecule, e.g. the analysis could not distinguish between N- and O-acetylation.

The percutaneous absorption of ^{14}C-2-amino-5-ethylphenol hydrochloride in a representative commercial hair dye formulation (under oxidative conditions) containing 1.0% of the test item was measured by scintillation counting in a study using the pig-skin model in vitro. The study authors determined a skin penetration rate of 1.935 ± 0.762 µg/cm^2 by combining the amounts found in the receptor fluid and in the lower skin layers (lower stratum germinativum). The upper skin layers (stratum corneum and upper stratum germinativum) were excluded from this calculation. However these layers contain part of the epidermis, not just stratum corneum, and therefore may include chemical which is potentially bioavailable. In addition, deviations from the recommended protocol for in vitro dermal absorption studies for cosmetic ingredients (SCCP, 2006a) included that not enough chambers were used, and that the amount applied was significantly more than that recommended. Therefore due to several shortcomings in the study a definitive in vitro dermal absorption value cannot be determined. However, a worst case dermal absorption value of 8.123 µg/cm^2 (0.846% of applied dose) under oxidative conditions was estimated from the maximum absorption achieved in an individual cell by combining the upper skin, lower skin and receptor fluid values. This is consistent with the value derived from the in vivo ADME study under non-oxidative conditions (9.0 µg/cm^2 or 5% of the applied dose).

Although the in vivo ADME study was conducted under non-oxidative conditions it is considered to be a worst case scenario and so the dermal absorption value derived from this study (9.0 µg/cm^2, 5.0% of applied dose) will

be used in the risk assessment.

Acute toxicity

The notified chemical showed low toxicity in the acute oral toxicity study with an LD50 for the phosphate salt > 2,000 mg/kg bw in female rats. No acute toxicity studies were performed with the hydrochloride salt. Taking into account the differences in molecular weight of the hydrochloride and phosphate salts and differences in pKa (Hydrochloride salt = 5.17; Phosphate salt = 5.28), it is calculated that the hydrochloride salt will contain 148% more of the free base (assumed to be the main cause of toxicity) than the phosphate salt. This implies that a dose of 2000 mg/kg for the phosphate salt is equivalent to a dosing of 1350 mg/kg of the chloride salt (the notified chemical). Given a dose of 2000 mg/kg bw of the phosphate salt resulted in the death of 1 animal and other clinical signs of toxicity, it is expected that the LD50 of the hydrochloride salt will be less than 2000 mg/kg bw. Additionally, in the ADME study the administration of a single oral dose of 375 mg/kg bw of the hydrochloride salt to rats lead to clinical signs like lethargy, lateral recumbency and hunched posture. Accordingly, the LD50 of the notified chemical is expected to lie between the determined values of 375 mg/kg bw and 2000 mg/kg bw, and therefore the notified chemical is expected to be harmful if swallowed.

Irritation and Sensitisation

The skin corrosion and skin and eye irritation potential of 2-amino-5-ethylphenol hydrochloride was determined using *in vitro* methods.

The *in vitro* skin corrosivity study carried out on ex vivo rat skin discs for 24 h in the Transcutaneous Electrical Resistance (TER) assay showed that 2-amino-5-ethylphenol hydrochloride has the potential to cause skin corrosion when applied as neat substance. In addition, when applied as neat substance in the *in vitro* Skin Irritation Assay on Reconstituted Human Epidermis Model (SkinEthic), the notified chemical induced significant cytotoxicity and caused tissue necrosis, which is consistent with a corrosive potential. No skin irritation was detected for aqueous dilutions of 2 and 10% (w/w).

The notified chemical was tested as neat substance and as 2% and 10% (w/w) dilutions using the Isolated Chicken Eye (ICE) Test. The 2% aqueous solution did not cause any corneal effects, whereas the 10% solution caused very slight corneal effects and the neat substance caused corneal damage. The notified chemical is therefore considered to be severely irritating to the eyes. Although the ICE test can be used for determining severe eye irritants it has not been validated for discriminating between irritants and non-irritants and therefore the results obtained on the aqueous solutions cannot be considered definitive.

The skin sensitising potential of the analogue chemical 2-amino-5-ethylphenol phosphate was investigated in a local lymph node assay in mice. The analogue chemical induced a biologically relevant immune response, resulting in an EC3 value of 8.9% when applied in DMSO as vehicle, while no sensitising potential was detected when applied in the non-standard vehicle acetone/water (1:1) mixed with olive oil (4:1). Calculation on the basis of comparison of the free base content of the phosphate and the hydrochloride salt of 2-amino-5-ethylphenol resulted in an EC3 value of 6.6% for the hydrochloride salt. Therefore, the notified chemical is also considered to have the potential to cause skin sensitisation. Based on the proposed categorisation of skin sensitising substances (SCCP, 2006b) the notified chemical would be considered a moderate sensitiser.

Repeated Dose Toxicity

A 90-day repeated dose oral toxicity study was conducted in rats with dose levels of 16, 55 and 272 mg/kg bw of the notified chemical administered via gavage in bi-distilled water. In the high dose group, the haemopoietic system, liver and kidney were identified as target organs in both sexes. The effects observed indicated that the notified chemical has the potential to cause haemolytic anaemia. At 55 mg/kg bw/day, effects on the haemopoietic system were recorded for both sexes. As no test item related adverse effects were noted at 16 mg/kg bw/day, this value was set as the no-observed-adverse-effect-level (NOAEL).

Genotoxicity

The notified chemical has not been tested for its genotoxic potential. All tests were conducted on the analogue chemical, 2-amino-5-ethylphenol phosphate, and the results are considered to be indicative of the genotoxic potential of the notified chemical.

The analogue chemical was negative in an Ames test and an in vitro mammalian cell gene mutation test in mouse lymphoma cells, but induced micronuclei in cultures of human peripheral blood lymphocytes treated both

in the presence and absence of metabolic activation in an in vitro micronucleus assay. The in vitro mouse micronucleus test is known to have high sensitivity but low specificity, i.e. yields a large proportion of 'false positives' (SCHER/SCCP/SCENIHR, 2009). In addition, the notified chemical (resulting in the same free base) has been shown to be extensively metabolised in a toxicokinetic study (see above). The S9 fraction used in the

in vitro study did not contain the relevant enzymatic cofactors required for the identified major metabolic pathway (i.e. sulfation). Therefore the relevance *in vivo* of this positive result observed *in vitro* may be questionable.

This is supported by the fact that no increase in micronuclei was noted in an in vivo micronucleus test in mice after oral administration of the analogue chemical up to the maximally tolerated dose, where discolouration of the urine and clear signs of systemic toxicity demonstrated systemic exposure. The findings in the ADME study, including rapid and extensive absorption via the oral route, with distribution of the notified chemical (or metabolites) into the red blood cells, indicate that the test substance (or metabolites) is likely to have reached the bone marrow in this in vivo micronucleus assay.

In an in vivo Comet assay conducted in rats, the liver, the duodenum and the bladder were assessed for the occurrence of DNA damage. Administration of 250, 500 or 1000 mg/kg bw of the analogue chemical to male rats did not induce a significant increase in the level of DNA migration in cells of the liver or urinary bladder. However, the analogue chemical induced a dose dependent and statistically significant increase in the level of DNA migration at 500 and 1000 mg/kg bw, which was accompanied by a concordant increase in cytotoxicity (as measured by the presence of cells with low molecular weight DNA) in cells of the duodenum. The histopathological comparison of the duodenum tissue samples from the treated animals and the vehicle controls demonstrated that the analogue chemical induced a test item related subacute inflammation in the duodenum of one animal at 1000 mg/kg bw. Based on the corresponding increase in cytotoxicity at doses with an induced increase in DNA migration, the observed clinical signs of stress in these animals after dosing, and the subacute inflammation detected by histopathological evaluation in the 1000 mg/kg bw dose group, the increase in DNA migration in the duodenum was considered to be induced by cytotoxicity and not by a specific genotoxic effect. This reasoning is strengthened by the fact that tissues which are known to be sensitive to aromatic amines (liver and bladder tissue) did not show any effect on the DNA in this assay. Therefore, it was considered that the analogue chemical is not mutagenic in the in vivo Comet assay in rats.

Based on the weight of evidence the notified chemical is unlikely to be a genotoxin in vivo. No information was available on the genotoxicity of the hair dye reaction products formed from the notified chemical during use.

Toxicity for reproduction

In a teratogenicity study, doses of 22, 74 and 369 mg/kg bw/day of 2-amino-5-ethylphenol hydrochloride in bi-distilled water were administered via gavage to rats during gestation days 6 to 20. Treatment with 2-amino-5-ethylphenol hydrochloride at 369 mg/kg bw/day resulted in marked parental toxicity, evident as severe clinical signs, associated with secondary reproductive and developmental toxicity including significantly increased pre implantation and post implantation loss, significantly increased foetal resorption and delays in ossification and development in the foetuses. Test item related effects on skeletal and cartilage development (incomplete ossification, misshapen bones) were also detected. No findings indicative of a teratogenic potential were noted. At 22 and 74 mg/kg bw/day, no treatment-related effects were observed, either in parental animals or in the offspring. The no-observed-adverse-effect-level (NOAEL) for maternal and developmental toxicity was 74 mg/kg bw/day.

Toxicity of Oxidative Reaction Products

Only the in vitro dermal absorption study was conducted under oxidative conditions (i.e. in the presence of hydrogen peroxide and coupler) and therefore no information on the toxicity of the hair dye reaction products was available. These products are likely to be dimers and trimers and will therefore be of higher molecular weight than the notified chemical. Based on typical reaction kinetics for hair dye reactions it is expected that dermal exposure to the unreacted notified chemical will be greater than exposure to the reaction products during use (SCCP, 2005).

Health hazard classification
Based on the acute toxicity, skin corrosivity, eye irritancy, and skin sensitisation potential, the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

- R22: Harmful if swallowed
- R35: Causes severe burns
- R41: Risk of serious eye damage
- R43: May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
The greatest potential for exposure to the notified chemical is during the hair dyeing process in professional hairdressing salons.

The notified chemical was found to be harmful if swallowed, but the notified chemical is present at a relatively low concentration (≤ 1%) and good occupational hygiene practices are expected to be in place to avoid oral exposure. Therefore the risk of acute oral toxicity during use of the hair dye product is considered minimal.

The notified chemical was shown to be corrosive to skin and a severe eye irritant. However at the concentrations used in the imported hair dye products (≤ 1%) the risk of these effects will be greatly reduced. In addition hairdressers are expected to wear gloves during the application phase, where the greatest exposure is likely.

The notified chemical was shown to have the potential to cause skin sensitisation. The notified chemical will be present in the hair dye products at or below the cut-off for sensitisation classification (1%). Gloves are expected to be worn during the application of the hair dye, but are less likely to be worn when mixing the products, when shampooing/rinsing the dyed hair, or when cutting/drying dyed hair. In addition, inappropriate glove use, particularly the re-use of disposable gloves, may be common. Hairdressers are likely to have compromised skin barrier function due to frequent hand immersion in cleaning agents that tend to defat the skin. For some individuals, this is likely to induce increased susceptibility to sensitising agents. Therefore the risk of sensitisation cannot be ruled out. In order to reduce the risk of long-term adverse skin effects good occupational hygiene practices will be required. These should include the wearing of appropriate gloves (such as neoprene, or nitrile/natural rubber latex for disposable gloves), and not re-using disposable gloves. The product label and associated product leaflet provided by the notifier contain appropriate safety instructions regarding the potential for the product to cause skin sensitisation and the required safety measures to be taken (use of gloves, avoiding contact with skin and eyes). The inclusion of directions to not re-use disposable gloves would further reduce the risk of exposure.

Based on the weight of evidence the notified chemical is considered unlikely to be a genotoxin in vivo, and was shown to cause reproductive/developmental effects only at doses causing significant maternal toxicity. However effects on the haemopoietic system (haemolytic anaemia), the liver and the kidneys were observed after repeated oral dosing. The lowest NOAEL, from the 90 day study, was determined to be 16 mg/kg bw/day. The relevance of using data from oral toxicity studies for the risk assessment of the notified chemical is supported by good systemic bioavailability as indicated by a high oral absorption from an in vivo toxicokinetic study in rats. Comparing the oral and dermal routes of exposure in rats, the qualitative metabolite profile is similar between the dermal groups and the oral group.

Hairdressers may be exposed to the notified chemical on a daily basis. Based on studies conducted on PPD under similar use conditions the worst case dermal exposure to the notified chemical was estimated to be 0.38 mg/day when appropriate gloves are used during the application stage, and 2.67 mg/day when gloves are not used. Using the dermal absorption value determined in the in vivo ADME study (5.0% of applied dose) and a bodyweight of 60 kg this equates to a systemic exposure dosage of 0.32 μg/kg bw/day in the presence of gloves and 2.23 μg/kg bw/day in the absence of gloves. The calculation of the Margin of Exposure (MOE) for both situations is presented below:

NOAEL = 16 mg/kg bw/day
SED with gloves = 0.32 μg/kg bw/day

MOE with gloves (NOAEL/SED) = 50,000

NOAEL = 16 mg/kg bw/day
SED without gloves = 2.23 µg/kg bw/day
MOE without gloves (NOAEL/SED) = 7175

A MOE of greater than 100 is considered to be acceptable to account for intra- and inter-species differences, and therefore for both situations the risk of adverse effects after repeated exposure while hair dyeing is considered acceptable.

Overall, while the risk of sensitisation cannot be ruled out, the risk to hairdressers exposed to the notified chemical is not considered unacceptable if the appropriate good occupational hygiene practices are in place, including the use of impermeable gloves (such as neoprene), or the 'single' use of disposable, impermeable gloves (nitrile or NRL preferred).

No toxicity information was available on the reaction products formed from the notified chemical under the oxidative conditions. Based on the estimates for exposure to the notified chemical the exposure of hairdressers to these intermediates is expected to be very low. However as no information on their genotoxic potential was available the risk from exposure to these reaction products cannot be determined.

6.3.2. Public health

The public will be exposed to the notified chemical in hair dye products, at concentrations up to 1%, on an intermittent basis. The products containing the notified chemical are for salon use only and so the public will only be exposed through the skin of the scalp.

Although the notified chemical was shown to be corrosive to skin and severely irritating to eyes, the risk of severe irritancy effects will be significantly reduced due to the low concentration in use, and the safety warnings on the label to not use it for dyeing eyelashes or eyebrows.

The notified chemical was found to have the potential to cause skin sensitisation. As dermal exposure to the scalp is expected during hair dye application in the salon the risk of salon clients developing an allergy to the notified chemical cannot be ruled out. A representative product label and accompanying information leaflet have been provided by the notifier. These contain warnings regarding the possibility of allergic reaction and the need for a skin sensitivity test. In particular the product information leaflet contains the following related to public health concerns:

FOLLOW THESE INSTRUCTIONS AND ASK YOUR CLIENT TO READ THEM. HAIR COLOURANTS CAN CAUSE ALLERGIC REACTIONS, WHICH IN RARE INSTANCES CAN BE SEVERE. Thus it is important to observe certain precautions:
Do not use this product at all:

- *If your client has already experienced a reaction to any colouring products.*
- *If the scalp is sensitive, itchy or if the skin is irritated or broken; in those cases take medical advice before using any hair colour product.*
- *For dyeing eyelashes or eyebrows, as to do so may be injurious to the eye.*

SKIN SENSITIVITY TEST: This test must be performed 48 hours before each and every application of the product: Cleanse a patch of skin on the inside of the elbow or behind the ear with a mild soap and water, pat dry. Mix a small amount of [colour cream] with an equal amount of [developer] and apply to this area. Allow to dry and leave undisturbed for 48 hours.
IF DURING THE COLOURING THE FOLLOWING OCCURS:

- *Any stinging or burning and/or rash: hair should be rinsed immediately and use of the product should be discontinued as this may be an indication of a more serious reaction. FURTHER, hair SHOULD NOT be coloured again before consulting a doctor or seeking medical advice.*
- *Rapidly spreading skin rash, dizziness or faintness, shortness of breath and/or swelling to eyes/face: hair must be rinsed immediately and IMMEDIATE MEDICAL ATTENTION SHOULD BE SOUGHT.*

IF AFTER COLOURING OR ON THE FOLLOWING DAYS problems such as skin or scalp itching, skin or scalp rash, swelling to eyes/face, blistering and/or skin or scalp weeping occur, IMMEDIATE MEDICAL ATTENTION SHOULD BE SOUGHT.
Tattoos may increase the risk of allergy to hair colour products.

In addition, the representative product label has similar information, but also expands on the skin sensitivity test so that after describing the performance of the test as above the interpretation of this test is described as follows:

If during this period the client experiences itching or reddening of the skin recommend that the test site be rinsed off and the product not be used.
The test represents a reasonable precaution but an allergic reaction may still occur.

While the skin sensitivity test may detect some clients who have already developed an allergy to the notified chemical (or other sensitising hair dye components in the product), it is likely to also produce false negatives and will not prevent the induction of sensitisation in previously unexposed clients. This type of test is recommended for many hair dye products, but the proportion of compliance with these recommendations is unknown and may be affected by the practicalities of attending the salon 48 hours before every use of the hair dye product. In many instances the test may be performed before the first use of hair dye (before sensitisation could have occurred) but not before subsequent colourings when allergy may have been acquired. This is recognised in the company's safety information that indicates that the test should be conducted before each and every use of the product.

Concerns regarding the use of these types of 'self-tests' have been raised by the EU Scientific Committee on Consumer Products (SCCP, 2007), including: the risk of misleading and false negative results; the potential risk that these tests result in induction of sensitisation to hair dye chemicals; and the fact that very little data exists on the proportion of hair dye allergic individuals who produce a positive reaction in these type of tests.

Therefore, while the skin sensitivity test will not prevent allergic reactions to the notified chemical, and there are concerns surrounding its use, it is still considered to be a useful measure in reducing the risk of serious allergic reactions. The safety information provided on the product label and information leaflet are considered to be important means of communicating the risk of allergic reaction to the client.

As discussed above, the NOAEL from the 90 day oral study was chosen for use in the risk assessment. The dermal absorption value from the in vivo ADME study in rats (9.0 μg/cm^2) was taken as the worst case scenario, although it was not conducted under oxidative conditions. Assuming a body weight of 60 kg, the MOE was calculated as set out below:

Max absorption through rat skin *in vivo*:	A	= 9.0 μg/cm^2
Skin area surface:	SAS	= 700 cm^2
Dermal absorption per treatment:	SAS x A x 0.001	= 6.3 mg
Typical body weight of human:		= 60 kg
Systemic exposure dosage (SED):	SAS x A x 0.001/60	= 0.105 mg/kg bw
No Observed Adverse Effect Level (90-day, oral rat)	NOAEL	= 16 mg/kg bw

Margin of Exposure: NOAEL/SED = 152

A MOE of greater than 100 is considered to be acceptable to account for intra- and inter-species differences, and therefore the risk of adverse systemic effects after repeated exposure while hair dyeing is considered acceptable.

As no toxicity information was available on the reaction products formed from the notified chemical under the oxidative conditions the risk from exposure to these reaction products cannot be determined, however the exposure to these products is expected to be less than that of the notified chemical.

7. ENVIRONMENTAL IMPLICATIONS

7.1 Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured or reformulated in Australia so there will be no release of the notified chemical due to these processes.

There will be potential for environmental exposure due to accidental spills during storage, transport and distribution. However, exposure is expected to be minimal due to the packaging (plastic tubes in cartons).

RELEASE OF CHEMICAL FROM USE

The hair dye containing the notified chemical will be applied to the hair with the developer (oxidising agent) in salons and washed into the sewer. Up to 2% of the notified chemical will remain as residues in the hair dye's containers after use.

The major proportion of the notified chemical is expected to be readily oxidised by the developer while it is still in the hair. Any small quantities of the notified chemical that are not oxidised at this stage, will be released to the sewer from the washings at the hair salon.

RELEASE OF CHEMICAL FROM DISPOSAL

The residues in the empty tubes (up to 2%) will most likely be disposed to general garbage which will go to landfill.

7.1.2 Environmental fate

The notified chemical is oxidatively and photochemically unstable in aqueous solution. As a result of the use pattern, most of the notified chemical is expected to be degraded to oxidative dimers in the salon. The dimers will be washed to the sewer or trapped on hair. The degradants on hair will gradually be washed to the sewer or, those on hair clippings, will be disposed to landfill. The structure of one of the dimers was characterised by LC/MS in a laboratory hydrolysis study. The fate of this dimer will be taken to be representative of the other similarly structured oxidised dimers. The identified dimer's fate was based on physico-chemical values calculated by the EPIWIN (v 3.20) model (US EPA, 2007). Although the dimer is moderately water soluble (predicted range 157 – 595 mg/L), it is expected that a significant proportion will partition to the soil, in landfill, or sludge, in the sewage treatment plant (STP), due to the high predicted K_{oc} value of 5012. On the basis of the predicted low water/octanol partition coefficient (log K_{ow} of 2.9) and moderate water solubility, this dimer, and related dimers, are not expected to bioaccumulate.

The minor proportion of the notified chemical that does not oxidise in the salon will be washed to the sewer, while the residues in the empty tubes will go to landfill. As the notified chemical is oxidatively unstable in water, most of the small quantity that is discharged to sewer is expected to react with organic matter and be recovered in sewage treatment plants with sludge. The residues of the notified chemical that are disposed to landfill may be mobile, as indicated by an adsorption/desorption study (K_{oc} value of 18) performed under neutral pH conditions. However, in an aerobic soil environment, oxidation will occur to produce less mobile dimers which will partition to the soil. On the basis of the low water/octanol partition coefficient and high water solubility, the notified chemical is not expected to bioaccumulate. For the details of the physico-chemical studies and the environmental fate studies of the notified chemical please refer to Appendices A and C, respectively.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC in water can be estimated as tabulated below based on the hypothetical worst case assumption that all of the notified chemical will be discharged to sewer, and subsequently released to receiving waters.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	200	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	200	kg/year
Days per year where release occurs	313	days/year
Daily chemical release:	0.64	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.2	million
Removal within STP	0%	
Daily effluent production:	4 232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.15	µg/L
PEC - Ocean:	0.02	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1300 kg/m^3). Using these assumptions, irrigation with a hypothetical worst case concentration of 0.151 µg/L may potentially result in a soil concentration of approximately 1.16 x 10^{-3} mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 5.81 x 10^{-3} mg/kg and 1.16 x 10^{-2} mg/kg, respectively.

7.2 Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 0.255 mg/L	Very toxic
Daphnia Toxicity	EC50 5.3 mg/L	Toxic
Algal Toxicity	E_rC50 2.01 mg/L	Toxic
Inhibition of Bacterial Respiration	IC50 186 mg/L	Not harmful

The results of the ecotoxicity tests indicated the notified chemical is very toxic to fish, and toxic to aquatic invertebrates and algae. The notified chemical is not harmful to micro-organisms in the sewage sludge at feasible influent concentrations.

The major oxidised degradant dimer identified in laboratory studies is not expected to be as toxic to fish as the notified chemical, based on the predicted endpoints (96 h LC50 = 9.8 – 74.8 mg/L) from the EPIWIN (v 3.20) model (US EPA, 2007). The modelled endpoints for daphnia and algae are comparable to those for the notified chemical.

7.2.1 Predicted No-Effect Concentration

Based on the endpoint for the most sensitive trophic level tested (fish) and applying an assessment factor of 100, the Predicted No-Effect Concentration (PNEC) has been calculated as follows:

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Fish Toxicity (LC50)	0.255	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	2.55	µg/L

7.3 Environmental risk assessment

Using the PEC and PNEC values calculated above, the risk quotient Q has been calculated as follows:

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.15	2.55	**0.06**
Q - Ocean	0.02	2.55	**< 0.01**

The calculated risk quotients for river and ocean discharge of sewage treatment plant effluent indicate that the notified chemical is not expected to pose a risk to the environment when it is used as proposed in hair salons, as a component of hair dyes.

A risk assessment was not performed for the major degradant since it is expected to be less toxic than the notified chemical, based on values determined by the EPIWIN (v 3.20) model (US EPA, 2007).

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)], with the following risk phrases:

- R22: Harmful if swallowed
- R35: Causes severe burns
- R41: Risk of serious eye damage
- R43: May cause sensitisation by skin contact

and

As a comparison only, the classification of the 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.
Skin Corrosion/irritation	1	Causes severe skin burns and eye damage
Serious eye damage/eye irritation	1	Causes serious eye damage
Skin Sensitiser	1	May cause an allergic skin reaction
Acute aquatic toxicity	1	Very toxic to aquatic life

Human health risk assessment

Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk of adverse systemic effects. However the risk for skin sensitisation to hairdressers cannot be ruled out, and therefore appropriate work practices, such as the 'single' use of impermeable gloves (neoprene, nitrile or natural rubber latex preferred), are required.

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk of adverse systemic effects. However the risk for skin sensitisation to hair dye users cannot be ruled out. Therefore appropriate communication of this risk and the recommendation for skin sensitivity testing before every use is required.

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following risk phrases for health hazard classification and safety phrases for the notified chemical:
  - R22 Harmful if swallowed
  - R35: Causes severe burns
  - 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 ≥ 25%: R22; R35; R41; R43
  - 10% ≤ Conc < 25%: R35; R41; R43
  - 5% ≤ Conc < 10%: R34; R41; R43
  - 1% ≤ Conc < 5%: R36; R38; R43

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

Health Surveillance

- As the notified chemical is a sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

Material Safety Data Sheet

- An Australian MSDS should be made available for all imported hair dye products being used in hairdressing salons.

CONTROL MEASURES
Occupational Health and Safety

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

- Avoid contact with eyes
- Do not re-use disposable gloves worn during handling of the hair dye product

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical in the imported hair dye products:
  - Impermeable gloves (neoprene, nitrile or natural rubber latex preferred)

  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 *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] 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 to minimise the risk of serious allergic reactions to the notified chemical:
  - Product labels and associated information leaflets should include warnings regarding the risk of allergic reaction;
  - Hairdressers should advise clients of the risk of allergic reaction and provide the product information leaflet for clients to read;
  - Skin sensitivity tests should be conducted prior to each and every use of the hair dye products containing the notified chemical.

- The notified chemical should not be used for dyeing eyelashes or eyebrows.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS 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;
  - the concentration in hair dye products has increased from 1%;
  - the notified chemical is used in hair dye products intended for sale to the public;
  - the notified chemical has begun to be formulated into products in Australia;

- additional information becomes available to the person as to the adverse effects of the oxidative reaction products created during hair dye use.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from hair dye ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased from 1 tonne, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet
The MSDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Water Solubility 428 g/L at 20°C

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

Remarks The water solubility at 20°C was evaluated using the flask method with quantitation by a validated HPLC-UV method. The pH of the solution was 1.42 reflecting the high concentration of the weakly acidic compound in distilled water.

Test Facility Dr. U. Noack-Laboratorien (2006a)

Hydrolysis as a Function of pH Oxidatively unstable in water at pH 4 – 9

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)*	*$t_{½}$ <hours>*
4	30°C	228
7	30°C	16.4
9	50°C	< 2.4

Remarks Photolytic effects were avoided by excluding light from the tests. The notified chemical was unstable under all test conditions. The stability decreased at higher pH and higher temperature. The notified chemical undergoes oxidative dimerisation in aqueous solution as confirmed by LC/MS. The degradation process does not conform well to a pseudo first order kinetic model, especially at pH 7.0. Nevertheless, degradation half-lives were extrapolated from a best fit linear regression analysis based on a first-order decay model.

Test Facility Dr. U. Noack-Laboratorien (2006b)

Partition Coefficient (n-octanol/water) log P_{ow} = 1.37 at 20°C

Method OECD TG 117 Partition Coefficient (n-octanol/water) - HPLC
EC Directive 92/69/EEC A.8 Partition Coefficient

Remarks The study deviated from the guidelines by using a small inorganic salt, sodium nitrate, for the determination of the dead time (t_0) instead of the usual organic compounds since it has less interaction on a reversed phase column. The mobile phase was 70% methanol/30% phosphate buffer at pH 7.0.

Test Facility Dr. U. Noack-Laboratorien (2006c)

Adsorption/Desorption – screening test log K_{oc} = 1.25 at 20°C

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using HPLC

Remarks Only one test was performed (at pH 7) for the test substance. A K_{oc} value of 18 was determined and hence the notified chemical is likely to be very highly mobile in soil (McCall et al., 1980). However, the notified chemical may have an affinity towards silicates and organic matter since a significant proportion is in the form of an ammonium salt at low environmental pH values (4 – 5).

Test Facility Dr. U. Noack-Laboratorien (2006d)

Dissociation Constant

1. pKa_1 = 5.42 and pKa_2 = 10.04 (Sparc calculator)
2. pKa_1 = 5.17 (measured by titration)

Method
1. Sparc Calculator
2. Potentiometric titration according to Hasselbach-Henderson equation.

Remarks
1. pKa_1 is the prediction for the dissociation of a proton from the aryl ammonio group and pKa_2 is the prediction for the dissociation of a proton from the phenolic group. The prediction for pKa_1 is within 0.3 pH units of the measured value.
2. pKa_1 for the notified chemical was determined using potentiometric titration but pKa_2 wasn't determined using this method.

The values obtained for pKa_1 and pKa_2 are close to those expected for an aryl ammonio group and a phenolic group, respectively (Ripin & Evans, 2009). The notified chemical will be dissociated in water.

Test Facility Wella Analytical (2007)

Particle Size

Method In-house method using sieve analysis

Range (μm)	*Mass (%)*
<32	88.59
32-45	7.43
45-63	2.56
63-75	0.53
75-90	0.21
90-125	0.09
125-250	0.02
>250	0.03

Remarks The fine dust (particle size < 32 μm) was removed by preliminary sieving. The mean particle diameter in the range 32-250 μm was calculated to be 45.3 μm.

Test Facility Dr.U.Noack-Laboratorien (2006e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	2-Amino-5-ethylphenol phosphate (WR 802433) Batch No: GST 079-03/52-09 Purity: 99.9%
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Directive 2004/73EC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar SPF
Vehicle	Purified water
Remarks – Method	Females only Observation 1, 2, 3 and 5 hours, then 2/day on Days 2-15

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3F	2000	1
2	3F	2000	0

LD50	> 2000 mg/kg bw
Signs of Toxicity	Slight to marked sedation in all animals up to 5 hrs. Slight lacrimation in all animals up to 3 hrs. Hunched posture observed in all animals, persisting up to Day 5 in some animals. Ruffled hair in all animals up to Day 5. Pale skin was observed in 3 animals up to Day 8, with muscle twitching and hypothermia up to 5 hrs. Tachypnoea in one animal up to 2 hrs post-administration. All animals had orange urine from 3 hrs and up to Day 5. Cyanosis was observed in all animals up to 2 hrs, persisting in 1 animal up to 3 hrs and 3 animals up to Day 2. In killed animal, rales were also observed at 2-hrs. No clinical signs observed in any of the surviving animals after Day 9. Body weights were normal.
Effects in Organs	No macroscopic findings.
Remarks – Results	One animal was killed in extremis for ethical reasons 2.5 hrs after administration.
CONCLUSION	The test substance is of low toxicity to female rats via the oral route.
TEST FACILITY	RCC Ltd (2006a)

B.2. Corrosion-skin: Transcutaneous Electrical Resistance Assay

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 430 *In vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)
Remarks – Method	Positive control, hydrochloric acid (approx. 36%) and negative control, sterile distilled water, were used. No significant protocol deviations.

RESULTS

Notified chemical Contact Period	Mean Electrical Resistance ± SD
24 hrs	3.7 ± 1.0 kΩ

Remarks – Results	The TER measurement was below the threshold value of 5 kΩ and the skin discs were pale by end of test. The positive and negative controls produced results as expected.
CONCLUSION	The notified chemical is considered to be likely to produce skin corrosion.
TEST FACILITY	Safepharm Laboratories Ltd (2006a)

B.3. Irritation-skin: *In Vitro* Reconstituted Human Epidermal Model (SkinEthic)

TEST SUBSTANCE	Notified chemical
METHOD	OECD Guidelines not available but recently validated by ECVAM (ESAC, 2008)
Remarks – Method	The procedure used was as per Kandarova et al (2006), except that less test substance was used (16 µg or mL versus 20 µg or mL) and the Formazan extraction was conducted overnight (rather than for 2 hrs). This protocol differs from that evaluated by ECVAM in that the exposure period is shorter, only 15 minutes, compared to the 42 minute exposure period in the ECVAM validated protocol. The notified chemical was applied neat and at two concentrations, 2% and 10% (w/v) in distilled water to one sample of freeze killed tissue and triplicate samples of viable tissue, per concentration. The treatment period was 15 minutes followed by a rinsing step and a 42 ± 2 hr post-treatment incubation treatment. The positive control used was SDS (Sodium Dodecyl Sulphate). The two endpoints, cytotoxicity in the colourimetric MTT reduction assay, expressed as percentage viability of treated cultures in comparison to negative controls, as well as morphological changes identified by histological examination, were evaluated. - Notified chemical is considered to be non-irritant to the skin if the tissue viability is > 50%. - Notified chemical is considered to be irritant to the skin if the tissue viability is ≤ 50%. Evidence of cellular damage or disruption of the tissue morphology was used as an additional parameter to confirm the occurrence of reduced tissue viability, as determined using the MTT assay, or to detect changes in the tissue that have not resulted in a reduction in tissue viability. In a pre-test the notified chemical was shown to have the ability to directly reduce MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). Therefore a modified procedure was used which incorporated parallel testing on viable and freeze-killed tissues. In addition an 'MTT Correction plate' was used to correct for any interference from the colour of the test material.
RESULTS	Relative viabilities of notified chemical treated tissues compared to the negative control tissues: 2% w/v in distilled water: 100.9% 10% w/v in distilled water: 106.2% Neat: 0%

Remarks – Results

The 2% and 10% concentrations of the notified chemical did not induce significant cytotoxicity in the MTT assay. The neat notified chemical induced significant cytotoxicity. The colour of the notified chemical did not interfere with the MTT test. The notified chemical was considered to have the ability to directly reduce MTT but not to have caused interference with the MTT test.

Histological evaluation revealed that the notified chemical induced no significant epidermal effects at 2% and 10% when compared to the negative controls. When applied neat, tissue necrosis was observed.

The positive control produced the expected result. The negative control viabilities (0.758) were slightly below the predicted assay acceptance criterion of 0.8. However the test laboratory considered this to be acceptable due to:

- The value of 0.8 was developed at an alternative test site and was not based on Safepharm historical data;
- Tissues may demonstrate batch variability;
- The positive control demonstrated the correct functioning of the assay;
- No equivocal test material results were obtained.

CONCLUSION

The notified chemical is considered to be likely to produce skin irritation when applied neat.

TEST FACILITY

Safepharm Laboratories Ltd (2006b)

B.4. Irritation-eye: Isolated Chicken Eye (ICE) Test

TEST SUBSTANCE

Notified chemical

METHOD

OECD Guidelines not available but this assay has been recently validated by ICCVAM (2006) and ECVAM (ESAC, 2007) as a screening test to identify ocular corrosives and severe irritants as part of a tiered-testing strategy.

Remarks – Method

Method was as per ICCVAM recommended protocol, except that no positive control was included in the study design. However, as the irritancy of the notified chemical was not equivocal this is not considered to invalidate the results obtained.

Concentrations tested: 100% - 30 mg solids,
2% in water, 10% in water – 30 µL

RESULTS

Conc. (%)	Swelling	Max mean scores Opacity	Fluorescein	Irritation Category[1]	Irritation Index[2]
100	25[3]	3.0[3]	3.0	III;IV;IV	145
10	1	0.5	0.5	I;I;I	21
2	0	0.0	0.0	I;I;I	0

1. I =no effect; III= moderate effect; IV= severe effect
2. Irritation index= max mean corneal swelling + max mean opacity (x 20) + mean fluorescin score (x 20)
3. Layer of needle like remains on the cornea.

Remarks – Results

The 2% aqueous solution did not cause any corneal effects. The 10% aqueous solution caused very slight corneal opacity and very slight fluorescein retention by damaged epithelial cells. Undiluted notified chemical caused moderate swelling, severe corneal opacity and severe

	fluorescein retention by damaged epithelial cells. Microscopic examination of the corneas confirmed the observed effects.
CONCLUSION	The notified chemical is severely irritating to the eyes.
TEST FACILITY	TNO (2006)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	2-Amino-5-ethylphenol phosphate Batch: GST 079-03/33-08 99.9% area% at 254 nm by HPLC
METHOD	OECD 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/J 5 females per concentration
Vehicle	DMSO Water/acetone (1:1) mixed with olive oil at ratio of 4:1
Remarks – Method	Two different vehicles were tested in this study. The solvent water/acetone (1:1) mixed with olive oil at ratio of 4:1 is not one of the preferred vehicles listed in the OECD TG. The test item concentrations were chosen due to the maximum solubility in the respective vehicles. The lymph nodes were not pooled in dosing groups, thus allowing individual animal data to be examined. The positive control chosen, p-phenylenediamine, is not one of the controls listed in the OECD TG, and is not a mild sensitiser as usually used. However it is a hair dye chemical known to cause sensitisation in humans during use, which may therefore make it a useful comparison to the notified chemical.

RESULTS

Concentration %	*Proliferative response (Mean DPM)*	*Stimulation Index (Test/Control Ratio)*
DMSO vehicle		
0	547 ± 204	-
0.5	704 ± 143	1.3 ± 0.3
1.5	1143 ± 319	2.1 ± 0.6
5	996 ± 412	1.8 ± 0.8
15	2656 ± 894	4.9 ± 1.6
Acetone/Water/Oil vehicle		
0	777 ± 264	-
0.5	676 ± 170	0.9 ± 0.2
1.5	914 ± 336	1.2 ± 0.4
5	939 ± 577	1.2 ± 0.7
15	992 ± 459	1.3 ± 0.6
Positive Control		
p-phenylenediamine: 1% in DMSO	3695 ± 903	6.6 ± 1.6

Remarks – Results	No mortalities occurred during the study. There were no clinical signs observed. Body weights and weight gains were unaffected by treatment. The positive control produced the appropriate response in DMSO. When DMSO was used as the vehicle there was a 3-fold increase in

isotope incorporation relative to the control group, indicative of induction of skin sensitisation. The EC3 value was calculated to be 8.9%.

When acetone/water/oil was used as the vehicle the notified chemical did not show the potential for induction of skin sensitisation. However as no positive control data in this vehicle was provided, and due to this vehicle not being included in the list of preferred vehicles in the OECD TG, this negative result is questionable.

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

TEST FACILITY MDS Pharma Services (2005)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical
Batch: RD-CRU079-16/97-05
99.1 weight % by NMR
78.3 weight % by HPLC (free base)
99.1 % area % by HPLC (hydrochloride)
99.9 % area % at 254 nm by HPLC

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: Repeated Dose 90-Day Toxicity Study in Rodents.

Species/Strain Rat/Wistar

Route of Administration Oral – gavage

Exposure Information Total exposure days: 90 days;
Dose regimen: 7 days per week;
No recovery period.

Vehicle Bi-distilled water

Remarks – Method Instead of a correction factor of 0.738 a factor of 1.355 was used when determining the doses for the study. The original proposed doses were: 22, 74 and 369 mg/kg bw equivalent to 30, 10 and 500 mg phosphate salt/kg bw
No other major deviations to protocol.
Test item concentrations were determined by HPLC coupled to an UV detector and quantified with the area under the peak and were acceptable.
Under prescribed storage conditions, the formulations used for dosing were stable for 7 days.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10/sex	0	0
low dose (LD)	10/sex	16	0
mid dose (MD)	10/sex	55	1
high dose (HD)	10/sex	272	0

Mortality and Time to Death

No treatment-related mortalities were recorded. One MD male died during blood sampling on Day 90. Precise determination of cause of death could not be determined.

Clinical Observations

No treatment-related effects of toxicological significance could be found in the following parameters: mortality, daily or weekly observations, functional observation battery, ophthalmoscopic examinations and mean daily food consumption. Any observations noted were considered not treatment-related because of low

incidence, not dose-related or were observed at similar incidences as the control animals.

Light discolouration of urine was observed in all MD and HD animals, which was considered related to the notified chemical colour and not of toxicological significance. Salivation was frequently observed in HD animals and was considered due to the taste of the formulation and not of toxicological significance.
HD findings: In males, lower mean body weights from Day 22 and lower mean body weight gain from Day 15.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
HD animals: Mild anaemia with compensatory reticulocytosis in male and female rats including significant reductions in red blood cell count, haemoglobin, haemoglobin concentration distribution width and mean cell haemoglobin concentration, and elevations of mean cell volume, mean cell haemoglobin, methaemoglobin and reticulocyte counts. Shifts in the reticulocyte maturity indices were noted in both sexes from older (low fluorescent) cells to younger (high fluorescent) cells. Also significant elevated bilirubin levels were noted. These findings correlated with histopathological findings.
Urinalysis in males found significantly elevated urine volume, lower density and osmolality. In females significant elevation of nitrite and bilirubin content was recorded.
MD animals: Significant elevations in reticulocyte count in both sexes. Shift in reticulocyte maturity indices was observed in males. Significant increases in mean cell volume and reductions in mean cell haemoglobin concentration and haemoglobin concentration distribution width was seen in females..
LD animals: No test-item related adverse effects were noted.

Effects in Organs
HD animals: Mean absolute and relative spleen weights were elevated in both sexes and correlated with microscopic changes. In both sexes, increased kidney-to-body weight ratios were found, and in males liver-to-body weight ratio was increased.
Yellowish staining of gastrointestinal tissues was considered to be due to the test item. Liver and spleen enlargement was observed in some males. Microscopic changes were found in the kidneys (increased tubular hyaline droplets, lymphoid cell infiltration and tubular basophilia in males; 3 females had renal tubular dilation), spleen (haemopoietic activity was increased and haemosiderin deposits were found in both sexes).
MD animals: Elevated mean absolute liver and kidney weights were observed in females. Haemosiderin deposits and haematopoiesis were increased in female spleens.
LD animals: No test-item related adverse effects were noted.

Remarks – Results
The target organs of toxicity were the spleen, kidneys, and liver. Dose relationship was observed with findings increasing from MD to HD groups. The macroscopic changes in the spleen and kidneys correlated with histopathological changes, as well as haematology parameters (for changes in the spleen). All these changes were indicative of a potential for the chemical to cause haemolytic anaemia.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 16 mg/kg bw/day in this study, based on no treatment-related findings of any nature at that dose.

TEST FACILITY RCC Ltd (2008)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	2-Amino-5-ethylphenol phosphate (WR 802433) Batch No.: GST079-03/33-08
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure (Experiment I) and Pre incubation procedure (Experiment II).
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	S9 mix from Phenobarbital/β-naphthoflavone induced rat liver

Concentration Range in Main Test	With and without S9 mix the same concentration ranges were used. a) Experiment I: 33, 100, 333, 1000, 2500, 5000 µg/plate. b) Experiment II: 10, 33, 100, 333, 1000, 2500, 5000 µg/plate.
Vehicle	De-ionised water
Remarks – Method	Since the historical range in the negative and solvent control of strain TA 102 was exceeded (Experiment II, with metabolic activation), this part was repeated (Experiment IIa). Each concentration including controls was tested in triplicate.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Experiment I	5000	5000	5000	Negative
Experiment II/IIa	-	5000	5000	Negative
Absent				
Experiment I	5000	2500	5000	Negative
Experiment II/IIa	-	1000	5000	Negative

NA - not applicable

Remarks – Results	No substantial increase in revertant colony numbers of any of the 5 strains was observed at any dose level, either in the presence or absence of S9 mix. The positive controls produced distinct increases in induced revertant colonies, validating the test.
CONCLUSION	The test substance was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	RCC CCR (2005a)

B.8. Genotoxicity – in vitro Mammalian Cell Gene Mutation Test

TEST SUBSTANCE	2-Amino-5-ethylphenol phosphate (WR 802433) Batch No.: GST079-03/33-08
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species	Mouse
Cell Type/Cell Line	Lymphoma L5178Y cells
Metabolic Activation System	S9 mix from Phenobarbital/β-naphthoflavone induced male Wistar rats
Vehicle	Water
Positive controls	Without S9 mix: Methyl methanesulphonate at 13 µg /mL With S9 mix: Cyclophosphamide at 4.5 µg /mL
Remarks – Method	A range-finding test (pre-test on toxicity, measuring relative suspension growth), and a mutagenicity experiment covering at least 8 analysable concentrations was performed in the absence and presence of S9 mix. Assay performed in 3 independent experiments. First main experiment was performed with and without S9 mix and a treatment period of 4 hours. The experimental part without S9 mix was repeated as IA due to inconsistent cell growth after 24 and 48 h. Experiment IA was repeated due to technical reasons as IB. Second experiment was performed only in absence of S9 mix with a treatment period of 24 hours. An additional experiment IIA was performed to extend the concentration range into the cytotoxic range.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Present				
Test I	48.5*,58.1,69.8*,83.7*,100.5*,120.6*, 144.7*,173.6*,208.3*,250.0*	4	72 h	10-15 days
Test II	-			
Absent				
Test I	58.1*,69.8*,83.7*,100.5*,120.6*,144.7* , 173.6*,208.3*,250*,300*	4	72 h	10-15 days
Test II	4.7,5.6*,11.2*,13.4*,16.1*,19.3*,23.2*, 27.8*,33.3*,40.0*	24	72 h	10-15 days
Test IIA	33.3*,40.0*,48.0*,57.6*,69.1*,83.0*, 99.6,119.5	24	48 h	10-15 days

*Cultures selected for analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in PreliminaryTest	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test I	75	83.7	None	Negative
Test II		-	-	-
Absent				
Test I	300	300	None	Negative
Test II		33.3	None	Negative
Test IIA		69.1	None	Negative

Remarks – Results

The concentration ranges used in the main experiments (I and II) were limited by cytotoxic effects.

A two-fold increase was observed in Test IIA at 83.0 μg/mL. Moderate toxicity also occurred at this concentration (relative total growth =11.4%, and relative cloning efficiency 1 = 17.9%) and the absolute value of the mutation frequency (190 mutants per 10^6 cells) remained within the historical range of negative and solvent controls.

Therefore no biologically relevant increase in mutant colony numbers was observed in the main experiments, with or without metabolic activation.

Solvents used as negative controls produced no genotoxic effects. Appropriate reference mutagens were used as positive controls and showed an increase in induced mutant colonies, validating the methodology.

CONCLUSION

The test substance did not induce mutations in mouse lymphoma L5178Y cells treated in vitro under the conditions of the test.

TEST FACILITY

RCC CCR (2005b)

B.9. Genotoxicity – in vitro Micronucleus Assay in Human Lymphocytes

TEST SUBSTANCE

2-Amino-5-ethylphenol phosphate (WR 802433)
Batch No.: GST079-03/33-08

METHOD

OECD TG 487 (2004, Draft guideline)

Species	Human (females)
Cell Type	Peripheral lymphocytes
Metabolic Activation System	S9 liver fraction from Arochlor 1254 induced animals
Vehicle	DMSO

Positive controls	In absence of S9: 4-Nitroquinoline 1-oxide: 1.25 to 5 µg/mL (clastogenic control) and Vinblastine: 0.04 to 0.08 µg/mL (aneugenic control) In presence of S9: Cyclophosphamide: 3.125 to 12.5 µg/mL
Remarks – Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*PHA** Treatment Period (hrs)*	*Exposure Period (hrs)*	*Harvest Time (hrs after initiation)*
Present				
Test 1	66.21*, 82.77*, 103.5, 129.3*, 161.7, 202.1, 252.6, 315.7, 394.7, 493.3, 616.7	24	3	72
Test II	83.89, 104.9, 131.1*, 163.8, 204.8*, 256.0*, 320.0, 400.0	48	3	96
Absent				
Test 1	12.32, 15.39*, 19.24, 24.05*, 30.07, 37.58*, 46.98, 58.73, 73.41	24	20	72
Test II	27.25, 32.06*, 37.71, 44.37*, 52.20, 61.41*, 72.25, 85.00	48	20	96

*Cultures selected for metaphase analysis.
** PHA = Phytohaemagglutin (stimulates cells to divide)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present			
Test I	129.3	188.2	Negative
Test II	256.0	>400.0	Genotoxic effect
Absent			
Test I	37.58	224.0	Genotoxic effect
Test II	61.41	>100.0	Genotoxic effect

* Approximately 60% reduction in replication index (RI)

Remarks – Results

In Test I in the absence of metabolic activation a significantly increased frequency of micronucleated binucleate (MNBN) cells was observed at the 2 highest concentrations analysed, with a dose-response evident.

In Test II the frequencies of MNBN cells were significantly elevated in the presence and absence of metabolic activation (S9). In the presence of S9 all concentrations analysed showed frequencies above historical negative control values. In the absence of S9 the highest concentration analysed showed a frequency above historical control values and a clear dose response was evident.

As no further investigation was undertaken it was not determined whether the effect seen was due to clastogenicity or aneugenicity of the test substance.

The positive control markedly induced mutant colonies, and both the positive and vehicle controls were within the historical control data of the performing laboratory, demonstrating the validity and sensitivity of the test.

CONCLUSION

The test substance induced micronuclei in cultures of human peripheral blood lymphocyte cells treated in vitro under the conditions of the test.

TEST FACILITY

Covance Laboratories Ltd (2004)

B.10. Genotoxicity – in vivo Mouse Bone Marrow Micronucleus Test

TEST SUBSTANCE
2-Amino-5-ethylphenol phosphate (WR 802433)
Batch No.: GST 079-03/33-08
Purity: 99.6 area% (HPLC at 254 nm)

METHOD
OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain: Mouse/NMRI

Route of Administration: Oral – gavage

Vehicle: Deionised water

Remarks – Method: Doses selected based on findings in pre-experiment on toxicity. Males and females were administered 2-amino-5-ethylphenol phosphate by oral gavage at doses of 1250 and 2000 mg/kg bw, respectively. Ruffled fur, eyelid closure, prone position, and reduction of spontaneous activity occurred within 2 hours of administration. Urine was coloured orange. No effects were seen by 6 hours or later. No deaths occurred at this dose. Mortality was observed in females at the next dose of 1500 mg/kg bw.
Females only were used as they were most sensitive to the test item in pre-experiments. The gavage volume administered was 10 mL/kg/bw.

For animals treated with the vehicle or 1250 mg/kg bw (the highest dose) 6000 PCEs were scored per animal due to the data for one animal needing to be confirmed. These scores were adjusted to /2000 PCEs to allow better comparison with other treated animals.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time Hours*
Negative control	5 females	0	24
1	5 females	312.5	24
2	5 females	625	24
3	5 females	1250	24
4	5 females	1250	48
CP	5 females	40	24

CP=cyclophosphamide

RESULTS

Doses Producing Toxicity: 625 mg/kg bw (prone position, reduction of spontaneous activity) and 1250 mg/kg bw (prone position, reduction of spontaneous activity, two mortalities occurred within 6 hours of administration).

The mean number of PCEs was not substantially decreased after treatment, when compared to the vehicle and historical controls.

Genotoxic Effects: The number of micronuclei per 2000 PCEs was not significantly increased after treatment at any of the test item doses, in comparison to the negative vehicle controls.

Remarks – Results: Although the test substance was not found to be cytotoxic to bone marrow, the coloured urine in the highest group tested indicates that the test substance was sufficiently bioavailable and so is likely to have reached the target organ. This is supported by the ADME study (see below) conducted on the notified chemical, which shows rapid and near complete absorption of the chemical from the gut into the blood stream, followed by extensive metabolism and excretion via the urine.

The positive control (CP) markedly induced micronuclei, and both the

positive and vehicle controls were within the historical control data of the performing laboratory, demonstrating the validity and sensitivity of the test.

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

TEST FACILITY RCC CCR (2004)

B.11. Genotoxicity – in vivo Comet Assay

TEST SUBSTANCE 2-Amino-5-ethylphenol phosphate
Batch No.: GST 079-03/33-08
Purity: 99.6 area% (HPLC at 254 nm)

METHOD In-house protocol
Species/Strain Rat/Sprague Dawley
Route of Administration Oral – gavage
Vehicle Deionised water
Remarks – Method A range finding test was conducted administering 1000 mg/kg bw on two consecutive days to 2 male and 2 female rats. This dose was chosen based on the Maximum Tolerated Dose in the in vivo micronucleus study (1250 mg/kg bw). Observations after dosing included decreased movement and lethargy, but all animals had recovered within 1 hr after dosing. Male rats experienced an average body weight loss of 7.75 g, while the female rats lost an average of 1.01 g.

In the Comet Assay each animal (5 males/dose) received two administrations of the test substance (24 and 4 h before sacrifice). After sacrifice portions of the liver, duodenum and urinary bladder were removed and mechanically minced in a solution of 10% v/v DMSO, 20 mM Na_2EDTA, at pH 7.60. Portions of each organ were also reserved for histopathology.

The minced cell suspensions were then mixed with 0.5% agarose and layered onto slides pre-coated with 1% agarose. Slides were then lysed for at least 1 hour (2.5 M NaCl, 100mM Na_2EDTA, 10 mM Tris base, pH 10, with 10% v/v DMSO and 1% v/v Triton X-100). After rinsing duplicate slides per sample were treated with alkaline electrophoresis buffer (30 mM NaOH, 1 mM Na_2EDTA, pH >13) for 20 minutes and then electrophoresed for 40 minutes at 0.7 V/cm, 300 mA and 1 to 10°C. After electrophoresis slides were neutralised, dipped in alcohol and air dried.

Prior to analysis the slides were stained with SYBR Gold™. After staining, 100 cells (50 cells /slide) per sample were scored using an automated image analysis system (Komet GLP Image Analysis System Version 6.0.2.3) to determine % Migrated DNA, tail length and Olive Tail Moment (OTM, = the distance between the centre of gravity of the DNA distribution in the tail and the centre of gravity of the DNA distribution in the head x the fraction of DNA in the tail).

Low Molecular Weight (LMW) DNA Diffusion Analysis was used to determine the extent of cytotoxicity in the samples. One slide per sample was removed from the lysing solution after 1.05 to 1.12 hours, neutralised, dipped in alcohol and air dried. Slides were then stained with SYBR Gold™ and scored visually using the following categories: I = condensed DNA; II = diffused DNA.

Group	Number and Sex of Animals	Dose mg/kg bw	Sacrifice Time
Vehicle control	5 males	0	4 h after 2nd dose
1	5 males	250	4 h after 2nd dose
2	5 males	500	4 h after 2nd dose
3	5 males	1000	4 h after 2nd dose
Positive control (EMS)	5 males	125	4 h after single dose

EMS = ethylmethanesulfonate

RESULTS

Organ and Dose Group	Mean % Migrated DNA	Mean OTM	Mean % Cells with LMW DNA (Cytotoxicity)
Liver			
Vehicle control	7.4	1.3	14.8
1	6.6	0.9	19.8
2	9.1	1.5	16.0
3	6.9	1.1	12.0
Positive control (EMS)	16.9	4.0*	26.6*
Urinary Bladder			
Vehicle control	17.0	4.7	15.4
1	24.3	7.6	19.6
2	17.8	5.0	12.6
3	16.8	4.6	15.6
Positive control (EMS)	31.1	9.6*	20.2*
Duodenum			
Vehicle control	19.8	5.3	14.8
1	22.5	6.5	16.4
2	22.9	7.2*	23.4*
3	26.7	9.0*	28.0*
Positive control (EMS)	34.0	10.5*	18.4

* Indicates results that were a statistically significant ($p < 0.05$) change relative to the vehicle control.

Doses Producing Toxicity

At doses of 500 and 1000 mg/kg bw a significant, dose-dependent increase in the % duodenal cells with LMW DNA (compared to control samples) was observed. In addition histopathological analysis found subacute inflammation in the duodenum of 1 of the animals from the 1000 mg/kg bw dose group.

Clinical observations of animals in these groups included decreased movement, hunched posture and lethargy.

Genotoxic Effects

At doses of 500 and 1000 mg/kg bw a significant increase in DNA migration (as measured by the OTM) was observed in cells of the duodenum (Significance: p=0.049 at 500 mg/kg bw; p=0.004 at 1000 mg/kg bw).

Remarks – Results

The test substance was found to increase the DNA migration in duodenal cells of animals treated at the highest two doses, but not in liver or urinary bladder cells. Cytotoxicity, as an increase in the % cells with LMW DNA, was also observed in the duodenum at these doses. Subacute inflammation of the duodenum was observed in the histopathological analysis of one of the animals from the high dose group. The test substance is known to be corrosive, and therefore may have caused irritation and damage to the duodenal cells at the concentrations dosed (2.5% and 5%). As a result the study authors

considered the increase in DNA migration to be induced by cytotoxicity and not a specific genotoxic effect.

In positive control (EMS) rats the comet tail length was markedly increased for liver, stomach and urinary bladder epithelial cells, demonstrating the validity and sensitivity of the test for detection of genotoxic effects.

CONCLUSION The notified chemical was not genotoxic in this in vivo Comet assay under the conditions of the test.

TEST FACILITY Helix3 Inc. (2006)

B.12. Developmental toxicity

TEST SUBSTANCE Notified chemical
Batch: RD-CRU 079-16/97-05
Purity: 99.9 area% (HPLC at 254 nm)

METHOD OECD 414 Prenatal Developmental Toxicity
Species/Strain Rat/ Wistar HanRCC:Wist (SPF)
Route of Administration Oral – gavage
Exposure Information Exposure period: Days 6 through 20 of gestation
Dose regimen: Daily
Vehicle Highly purified water
Remarks – Method No significant protocol deviations. GLP compliant.

RESULTS

Group	Number of Animals	Dose mg/kg bw/day	Mortality
1	22 females	0	0
2	22 females	22	0
3	22 females	74	0
4	22 females	369	0

Mortality and Time to Death

No mortalities were recorded. All dams survived to scheduled necropsy.

Effects on Dams

No treatment related effects were observed in dams treated at the two lowest doses (22 and 74 mg/kg bw).

A number of effects were noted in dams treated at the highest dose (369 mg/kg bw). Ventral recumbency and sedation were observed from Day 9 until end of treatment. Sedation was less severe over the last 4 days of treatment. Many dams had blue extremities for 2-3 days starting from Day 9 or 10 of the gestation period. The study authors considered this to be non-adverse since it was a transient occurrence. However it may be related to the known anaemic effects of the test substance (effects seen in 90 Day Repeat Dose Study). Yellow Discolouration of bedding was also noted, and is likely to be related to the staining properties of the test substance. Average food consumption was significantly decreased over the entire period (20.7%) compared to the vehicle controls. Average body weight gain was significantly decreased from Days 7-21 post coitum. Average weight gains were 33.3% in the high dose group compared to 46.8% in the vehicle control group.

Statistically significant increases in pre-implantation loss and corresponding decrease in the number of implantation sites as a percentage of corpora lutea was noted in dams treated at the highest dose. There were also statistically significant increases in post-implantation loss and foetal resorptions (6.1% foetal resorptions as percentage of implantation sites compared to 0.3% in controls).

Effects on Foetus

No treatment related effects on foetuses were observed at the two lowest doses (22 and 74 mg/kg bw).

At the highest does, a number of treatment related effects were observed. Average foetal body weights were significantly reduced compared to the controls (4.2 grams vs. 5.0 grams on a litter basis, respectively). Visceral examinations showed increased incidence of thyroid enlargement and increased incidence of malpositioned origin of common carotid artery.

Skeletal and cartilage examinations found incidences of splits in the cartilage of the sternebrae, not found in the foetuses of other dose groups. There was also a statistically significant increase of non ossified and incompletely ossified bones on both a litter and foetus basis, particularly in the cervical vertebral bodies, and distal and proximal phalanges of the fore and hind limbs. Also increased incidence of wavy ribs and misshapen bones (sternebra, scapula, humerus, radius, ulna and femur), a well as incidences of bipartite/irregular ossification or fusion of the sternebrae.

Remarks – Results

The effects observed in animals treated at the highest dose were considered to be related to the test item as they were outside the range of historical controls and/or were only observed at the highest dose.

Effects on reproductive and developmental endpoints were only observed in the presence of significant maternal toxicity.

CONCLUSION

The NOAEL was determined to be 74 mg/kg bw/day, for both maternal and foetal toxicity.

TEST FACILITY RCC Ltd (2007)

B.13. Pharmacokinetic/toxicokinetic: Percutaneous absorption *in vitro*

TEST SUBSTANCE	Notified chemical Batch: RD-CRU 079-16/97-05 Purity: 99.9 area% (HPLC at 254 nm) and 2-Amino-5-ethyl [U-^{14}C]phenol hydrochloride Batch: CFQ14698 Batch 1 Radiochemical purity: 99.3%
METHOD	OECD 428: OECD Guideline for the testing of chemicals. Skin absorption: *In vitro* method. OECD Environmental Health and Safety Publications, series on testing and assessment No. 28 Guidance Document for the conduct of skin absorption studies.

STUDY DESIGN AND OBJECTIVE

Cutaneous absorption of 1% 2-Amino-5-ethylphenol hydrochloride (notified chemical) in a typical hair dye formulation in the presence of hydrogen peroxide and a reaction agent (i.e. under oxidative conditions) was investigated in pig skin preparations, which were continuously rinsed with physiological receptor fluid at 32°C. Two independent experiments were performed with 6 diffusion cells per experiment (2 samples each from 3 different donors). The mean value of all valid skin samples (n=9) in contact with 1% notified chemical were used for calculations.

Integrity of skin preparations was determined by examining penetration characteristics with tritiated water.

After checking skin integrity 400 mg of formulation (100 mg/cm^2) containing 1% notified chemical, was applied to the skin samples (= 1 mg notified chemical/cm^2) for 60 mins and then washed off with shampoo and water. Amount of notified chemical in washings was determined by scintillation counting. The amount of notified chemical in the receptor fluid was determined at 16, 24, 40, 48, 64 and 72 hrs by the same method. At termination, skins were heat-treated and the upper layers (stratum corneum and upper stratum

germinativum) were separated from the lower layers (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and radioactivity quantified by scintillation counting.

RESULTS

Skin integrity: 1.0 to 3.5% of applied dose was found after 4 hours in the receptor fluids, which were within the limits of acceptance (≤ 2.0%) for 9 skin samples used for determination, while remaining 3 samples (> 2.0%) were not used for calculations.

Amount of test substance in:	μg/cm^2 (n = 9)		% of applied dose* (n = 9)	
	Mean ± SD	Range	Mean ± SD	Range
Receptor fluid (72 hrs)	1.442 ± 0.749	0.457-2.547	0.151 ± 0.078	0.048-0.266
Lower skin (72 hrs)	0.493 ± 0.142	0.228-0.743	0.051 ± 0.015	0.024-0.077
Upper skin (72 hrs)	3.805 ± 1.136	2.045-5.231	0.398 ± 0.119	0.214-0.545
Rinsing solution (60 mins)	894.95 ± 21.71	854.82-926.31	93.64 ± 2.11	89.58-96.50
Total balance (recovery)**	944.95 ± 19.92	907.98-974.51	98.88 ± 2.19	95.15-101.52

* Corrected for individual applied dose

** Total is corrected for losses on application tips

The majority of the notified chemical was found in the rinsing solutions (0.895 ± 0.022 mg/cm^2). Small amounts were found in the upper skin (3.805 ± 1.136 μg/cm^2), the lower skin (0.493 ± 0.142 μg/cm^2) and in fractions of receptor fluid collected within 72 hours (1.442 ± 0.749 μg/cm^2). The mass balance of the test substance found 95.15 to 101.52% recovery for all 9 skin samples with acceptable integrity. With respect to the receptor fluid the notified fraction was predominantly detectable within the first fraction collected (fractions 0-16 hours).

The study authors conclude that, under the assumption that a depot effect is absent, a maximum amount of 1.935 ± 0.762 μg/cm^2 (0.202%) of notified chemical is considered biologically available (n=9, three donors; receptor fluid + lower skin).

REMARKS – RESULTS

While the study appears to have followed the OECD TG 428 it does not take into account the SCCP guidance for dermal absorption studies conducted on cosmetic ingredients (SCCP, 2006a). In particular:

- Not enough chambers were tested (the SCCP recommends 6 cells per donor);
- The amount applied (100 mg/cm^2) is much higher than the amount recommended in the SCCP Guidance (20 mg/cm^2);
- The separation method for the skin layers resulted in 'upper skin' which consisted of both stratum corneum and part of the epidermis. This therefore includes bioavailable chemical, as well as chemical that will be sloughed off with the stratum corneum. The result for systemically available chemical using only the lower skin and receptor fluid is therefore an underestimation.

It may be possible to try and derive a worst case dermal absorption value from the maximum absorption achieved in an individual cell (since not enough chambers were used) by combining the upper skin, lower skin and receptor fluid values. This would give an absorption value of 8.123 μg/cm^2 (0.846% of applied dose).

CONCLUSION

Due to several shortcomings in the study a definitive dermal absorption value cannot be determined, although an indicative worst case value under oxidative conditions is 8.123 μg/cm^2 (0.846%).

TEST FACILITY Cosmital SA (2006)

B.14. Pharmacokinetic/toxicokinetic: ADME Study

TEST SUBSTANCE Notified chemical
Batch: RD-CRU 079-16/97-05
Purity: 99.9 area% (HPLC at 254 nm)
and
2-Amino-5-ethyl [U-^{14}C]phenol hydrochloride

	Batch: CFQ14698 Batch 1 Radiochemical purity: 99.3
METHOD	OECD 417: Toxicokinetics (1984) and 427: Skin absorption: in vivo method. (2004) EC Directive 88/302/EEC B.36 Toxicokinetics
Species/Strain	Rat/Wistar (female)
Route of Administration	IV (Group 1) Oral – gavage (Group 2) Dermal (Groups 3 and 4)
Vehicle	IV administration: phosphate buffer (PBS) Oral administration: Milli-Q water Dermal administration, LD group: Milli-Q water and 81905108B (water based vehicle mimicking use conditions, i.e. including typical ingredients found in hair dye formulations – non-oxidative conditions) Dermal administration, HD group: dimethylsulphoxide (DMSO)
Dose	IV: 75 mg/kg bw (containing approximately 10 MBq/kg bw of radioactivity) Oral: 370 mg/kg bw (containing approximately 12 MBq/kg bw of radioactivity) Dermal: 20 mg/mL (2% w/v, 0.5 h exposure) or 100 mg/mL (10% w/v, 24 h exposure) containing approximately 1.5 or MBq/kg bw of radioactivity (approximately equal to 16 or 80 mg/kg bw and 0.2 and 1 mg/cm^3)

STUDY DESIGN AND OBJECTIVE

Eight groups of rats were used: 4 for mass balance (n= 4/group) and 4 for toxicokinetics (n=6/group). In dermal administration groups, animals were collared to prevent ingestion. For the dermal low dose group, a water based vehicle mimicking end use conditions of the notified chemical under non-oxidative conditions, was used (81905108B). The design of the dermal high dose was to achieve a relatively high bioavailability (ie. 24 h exposure, occlusive conditions, DMSO as vehicle) compared to standard application conditions for hair dye formulations (30 mins exposure, unoccluded, water based vehicle mimicking use conditions).

Animals in mass balance groups were housed individually in metabolism cages to obtain a total ^{14}C-radioactivity balance per animal. Urine and faeces were collected in 0-8, 8-24, 24-48, 48-72 and 72-96 h intervals. Animals were euthanized 96 h after dose administration and tissues and organs collected. Total radioactivity was determined by LSC. For metabolism evaluation, selected faeces and urine samples were pooled per group and the metabolic profile was investigated by LC- PDA -RAD-MS (Liquid Chromatography-Photodiode Array- Radioactivity- Mass Spectrometry).
In the toxicokinetic groups, blood was sampled from each rat at 0.25, 0.5, 1, 2, 4, 8, 24, 48 and 72 h after dosing. Total radioactivity and 2-amino-5-phenol hydrochloride equivalents were determined.

RESULTS

No mortalities occurred.
After IV administration, all animals displayed lethargy (moderate to severe) and ventro-lateral recumbency. These observations were gone within 10 minutes. Animals also had piloerection, hunched posture and yellowish/orange urine on Day 1. Piloerection was noted for the remainder of the study.
After oral administration, all animals displayed moderate lethargy, hunched posture, piloerection, ptosis and orange/yellow urine on Day 1.
Some HD dermal animals had orange/yellow urine on Day 2.

Oral absorption was calculated using urine data and also using plasma data. The oral absorption was high, being 101% when calculated from the urine data and 60%, when calculated from the plasma data.
Dermal absorption in the low and high dose groups was 3.2% or 6.0 μg/cm^2 and 63% or 670 μg/cm^2, respectively. The amount retained in the application skin site may also become systemically available. The skin residue dose was therefore considered potentially absorbed and potentially systemically available. Therefore the absorbed fraction and the potentially absorbed fraction were combined and termed the 'total potentially absorbed fraction'. This was 5.0% (9.0 μg/cm^2) and 63% (680 μg/cm^2) of the applied dose in the low and high dose dermal groups respectively. Differences in absorption between the dermal dosing groups were due to dosing vehicle, concentration dose and duration of exposure (24 vs. 0.5 h). When calculated from

the plasma data, the absorption was 2 and 56% in the low and high dose groups, respectively.

Oral absorption was rapid with T_{max} of 0.5 h. Dermal absorption was also rapid with a T_{max} of 0.5 h in both groups. Large interindividual variation was observed in the plasma concentrations after dosing; in addition in the low dose group plasma concentrations were below LOQ from 2 h onwards. The AUC for the low dose group was calculated for up to 2 hours. Dose normalised C_{max} and AUC values were calculated but high variability was observed, which may affect the interpretation of these values. The dose-normalised exposure was not equal between the dermal low and high groups, probably due to the longer exposure period for the dermal high dose group.

Urine was the most important route of excretion of 2-amino-5-ethylphenol hydrochloride. Urinary excretion accounted for 82% of the IV dose, 83% of the oral dose, 2% after low dermal dosing and 57% after high dermal dosing. Urinary excretion was highest during the first 24 h, thereafter a decreasing excretion rate was noted with increasing time intervals. Faecal excretion accounted for 11% of the IV dose, 11% after the oral dose, 1% after low dermal dosing and 4% after high dermal dosing.

At termination, average total remaining radioactivity in blood, carcass plus tissues ranged between 0.8 and 2.5% indicating no major accumulation of radioactivity. In the IV and oral groups no tissues had residual concentrations greater than blood. In the dermal groups, concentration equivalents greater than in the blood were observed only in the treated skin: 1.8% and 0.8% in the low and high groups, respectively, related to the applied dose.

Blood concentrations were approximately 10-fold higher than plasma concentrations, indicating distribution of the notified chemical (or metabolites) into the red blood cells. Plasma concentrations were in the same order of magnitude for groups 1, 2 and 4; they were below the LOQ in low dermal dose group (group 3).
The average total recovery in all dose groups was between 94 and 101% of the applied dose.

Results are summarised in the following tables:

Mass balance data

Group No.	Test item dose level/concentration	Dosing route	Absorption %	Excretion via urine/faeces (%)
1	75 mg/kg bw	IV	100	82/11
2	370 mg/kg bw	Oral	101	83/11
3	16 mg/kg bw; 20 mg/mL; 0.2 mg/cm^2	Dermal low	3*	2/1
4	80 mg/kg bw; 100 mg/mL; 1 mg/cm^2	Dermal high	63*	57/4

* without skin residue

Toxicokinetic data

Group No.	Test item dose level/concentration	Dosing route	F_{abs} %	C_{max} mg/kg	AUC_{last} hr*mg/kg
5	75 mg/kg bw	IV	NA	NA	270
6	370 mg/kg bw	Oral	60	96.8	828
7	16 mg/kg bw; 20 mg/mL; 0.2 mg/cm^2	Dermal low	2	1.04	1.04
8	80 mg/kg bw; 100 mg/mL; 1 mg/cm^2	Dermal high	56	39.8	173

F_{abs}: absolute oral/dermal bioavailability, calculated as (AUC_{last} po or dermal / AUC_{last} IV)*(dose IV/dose po or dermal)*100%
NA: not applicable

Seven (7) potential metabolite peaks of test item were qualitatively detectable in urine samples and 3 potential metabolites (one not identified) in plasma extracts. Sulfation, glucuronidation and acetylation were identified as the major metabolic pathways.

Summary table: proposed metabolites detected in urine.

Rad Rt*	m/z**	MS Rt***	Metabolic reaction	Interdosing comparison (%)			
				IV	Oral	Dermal low	Dermal high
5.7-5.9 (ret 1)	210 (227, 268)	5.7	Acetylation at N or O, carboxylic acid group at ethyl group	4.2	trace[1]	-	5.7
9.4-9.6 (ret 2)	372	9.1	Acetylation at N or O, hydroxylation (ethyl group or ring), glucuronic acid conjugate	3.2	5.6	-	4.6
11.6-11.7 (ret 4)	314	11.4	Glucuronidation at O or N	13.7	16.7	-	3.9
12.1 (ret 6)	276 (292, 310)	11.8	Acetylation at N or O, hydroxylation (ethyl group or ring), sulfation at hydroxyl group	1.6	trace[1]	-	5.1
13.7-14.0 (ret 11)	218	13.8	Sulfation at N or O	59.8	77.8[2]	trace[1]	65.3

1. Metabolite was only observed with mass spectrometry analysis
2. Additional metabolites with m/z 299 (glucose conjugate and m/z 300 (acetylated-cysteine conjugate) contribute to the RAD peak

* = radioactivity retention time
** = mass-to-charge ratio
*** = mass spectrometry retention time

Sulfation was the major route of metabolic conversion of 2-amino-5-ethylphenol hydrochloride after all routes of administration. Glucuronidation (4-17%) and acetylation (7-16%) only observed in combination with other pathways, were also important pathways. A greater proportion of acetylation was observed after dermal absorption.

Summary table: proposed metabolites detected in plasma.

Rad Rt*	m/z**	MS Rt***	Metabolic reaction	Interdosing comparison (%)			
				IV 0.5 h	Oral 1h	Dermal low 0.25 h	Dermal high 1 h
1.5-1.7 and 13.8-14.1 (ret 1 and 2)	218	13.9	Sulfation at N or O	75.9	100	trace [1]	100
18.0-18.1 (ret 4)	138	17.7	Parent compound (2-amino-5-ethylphenol hydrochloride)	13.5	trace [1]	trace [1]	trace [1]
-	180	19.7	Acetylation at N or O	trace [1]	trace [1]	trace [1]	trace [1]
29.5-29.7 (ret 6)	271	29.3	Not identified	5.9	trace [1]	-	trace [1]

1. Metabolite was only observed with mass spectrometry analysis

In the plasma, sulfation was the major metabolic route after all three routes of administration, corresponding to

the urine observations.

CONCLUSION
2-Amino-5-ethylphenol hydrochloride was extensively absorbed, readily distributed into all organs, extensively metabolised and excreted via urine. Dermal absorption was high after the 24 h exposure period and low after the 30 min exposure period. Sulfation of the parent compound was the major metabolic reaction. No major qualitative differences were observed between the oral and dermal routes of administration.

TEST FACILITY Notox BV (2007)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Municipal sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Titration
Remarks - Method	There were no significant deviations from the standard protocol. The $ThCO_2$ for the test substance is 2.02 mg CO_2/mg test substance and for the reference compound (sodium benzoate) is 2.13 mg CO_2/mg reference substance. A test concentration of 20 mg/L, corresponding to a carbon content of 11 mg C/L in the test vessel was used.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation (average of replicates)*	*Day*	*% Degradation*
1	1	1	3
4	1	4	29
25	10	14	100
28	11	28	100

Remarks - Results	There was no significant degradation of the test substance in the 10 day window and after the 28 day test period. In comparison, the reference compound reached the pass level of 60% within the 10 day window and 100% degradation after 14 days. The biodegradation of the reference substance was not inhibited by the notified chemical in the toxicity control test.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Dr. U. Noack-Laboratorien (2006f)

C.1.2. Bioaccumulation

Bioaccumulation data were not provided. On the basis of the low partition coefficient, the high water solubility, and the instability in water, the notified chemical is not expected to bioaccumulate.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-Static, 96h EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – Semi-Static, 96h
Species	Zebra fish (*Danio rerio*)
Exposure Period	96 h
Auxiliary Solvent	None
Water Hardness	56 – 97 mg $CaCO_3$/L

Analytical Monitoring: RP-HPLC (UV)

Remarks – Method: The test was performed under semi-static conditions with daily renewal of the test media. A preliminary range finding study was carried out. The tests were performed with a normal photo period at pH 7.5 ± 0.3 in a temperature range of 21.7 ± 0.7ºC. Effects or symptoms of toxicity were also provided over 96 h at the various concentration levels which included: lethargy, fish on its side, slow escape reflex and missing escape reflex.

The concentrations of the notified chemical in the test solutions were determined by a validated isocratic, reversed-phase HPLC (UV) method which employed 20% acetonitrile/80% phosphate buffer as the mobile phase.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual (initial measured concentration)*		*2 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	< LOQ	7	0	0	0	0	0
0.040	0.047	7	0	0	0	0	0
0.090	0.098	7	0	0	0	0	0
0.210	0.198	7	0	0	0	14	14
0.450	0.426	7	0	0	29	100	100
1.000	0.951	7	0	14	100	100	100

LC50: >0.951 mg/L at 24 h
0.484 mg/L at 48 h
0.255 mg/L at 72 h
0.255 mg/L at 96 h (95% CI: 244 – 266 mg/L)

NOEC (or LOEC): NOEC = 0.198 mg/L at 96 h (the mortality of 1/7 fish was considered to not be significant)

Remarks – Results: The range finding study indicated 100% mortality at 1 mg/L after 48 h. At the lowest test concentration (0.1 mg/L) effects including hectic swimming and hyperventilation in all fish were observed up to 48 h, but normal behaviour after 72 and 96 h.

In the definitive test, all effect levels were based on the initial measured concentrations of the test substance due to the 24 h old test substance concentrations being < LOQ (except for the test carried out at the highest concentration). The acceptable LOQ value was 0.030 mg/L rather than that listed as 0.090 mg/L. Hence the lowest concentration of 0.047 mg/L could be accurately quantified. No explanation was provided why the concentrations of the test substance were < LOQ after 24 h. However, indications from the experimental protocols for some physico-chemical and ecotoxicological effects tests demonstrate that the notified chemical is photochemically unstable. As the fish test was conducted using a normal photo period, some photodegradation of the notified chemical over 24 hours can be expected. Also, in water at pH 7 and 20ºC, the half-life of the notified chemical is 48 hours in the dark, which indicates degradation of the notified chemical can also occur during the dark phase of each 24 hour illumination cycle.

Normal behaviour was observed for all fish over 96 hours for the control test. At 0.426 mg/L there was 100% mortality after 72 h. Prior to this timepoint, non-lethal effects were observed including all fish showing slow escape reflex after 2 h, and after 48 h, 3/7 fish were lethargic and 2/7 fish were lying on their sides (while 2/7 fish were dead).

CONCLUSION	The notified chemical is very toxic to fish
TEST FACILITY	Dr. U. Noack-Laboratorien (2006g)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Semi-static, 48 h EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – Semi-static, 48 h
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	253 – 263 mg $CaCO_3$/L
Analytical Monitoring	RP-HPLC (UV)
Remarks - Method	The test was performed under semi-static conditions with renewal at 24 h. The test concentration range was determined from a preliminary range finding study. The reference substance used was potassium dichromate. There were no other significant deviations from the standard protocol other than a 24 h dark cycle due to the photosensitivity of the notified chemical. The analytical method was the same as that used for the fish toxicity test.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual (initial measured results)*		*24 h*	*48 h*
Control	< LOQ	20	0	0
1.25	1.22	20	0	0
2.50	2.40	20	0	1
5.00	4.95	20	5	8
10.0	9.83	20	15	20
20.0	19.6	20	16	20

EC50	6.46 mg/L at 24 hours (95% CI: 5.62 – 7.24) 5.30 mg/L at 48 hours (95% CI: 5.04 – 5.55)
NOEC	2.50 mg/L at 48 hours (immobilisation rates ≤ 10% were judged to not be significant)
Remarks - Results	A yellow colouration, due to the formation of the oxidative dimer, was observed in the test vessels. After 24 h exposure, the concentrations of notified chemical in the test solutions had declined to 30 – 93% relative to the nominal initial concentrations. The EC50 values were calculated by sigmoidal dose-response regression based on the nominal concentrations. The EC50 value of the reference substance, potassium dichromate, after 24 h (1.95 mg/L; 95% CI: 1.71 – 2.16 mg/L) was within the prescribed concentration range of 1.0 – 2.5 mg/L.
CONCLUSION	The notified chemical is toxic to invertebrates
TEST FACILITY	Dr. U. Noack-Laboratorien (2006h)

C.2.3. 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	Green algae (*Desmodesmus subspicatus*)
Exposure Period	72 hours
Concentration Range	Nominal: 0.040, 0.100, 0.210, 0.470, 1.030, 2.270, 5.000 mg/L Actual: 0.047, 0.111, 0.215, 0.472, 1.05, 2.32, 5.03 mg/L
Auxiliary Solvent	None
Water Hardness	0.24 mmol Ca+Mg/L
Analytical Monitoring	Cell density was measured using the chlorophyll a fluorescence method (excitation at 435 nm and emission at 685 nm). The same RP-HPLC (UV) method used in the fish and daphnia tests was used to measure the concentrations of the notified chemical in the test solutions and controls.
Remarks - Method	The study was performed under static conditions with an initial cell density of ~ 10^4 cells/mL in the OECD growth media. Standard protocol was followed using a 24 h/day light regime at the standard intensity and a pH range 8.2 ± 0.3. Three replicates were tested for each of the test substance concentrations and six replicates for the control. A range finding test was carried out using dye solutions to determine the inhibition effects of the test substance on cell growth caused only by lack of light. The reference substance used in the definitive test was potassium dichromate. The E_bC50 and E_rC50 values after 72 hours were calculated by sigmoidal-dose-response regression analyses. NOEC values were determined by calculation of statistical significances of biomass integrals and growth rates using ANOVA and Dunnett's test. A recovery test was performed whereby the affected algae were allowed to grow for a further 4 days (after the definitive test was completed) in fresh medium.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
0.890 (95% CI: 0.852 – 0.930)	0.215	2.007 (95% CI: 1.90 – 2.12)	0.472

Remarks – Results

The range finding test indicated that inhibition effects on cell growth were caused by the test substance. The biomass in the control cultures increased exponentially by a factor of ~ 40 within the 72 hour test period. The CV of average specific growth rates during the whole test period in the replicate control cultures of 10% using the mean nominal value exceeded the OECD limit of 7% for this algal species. However, it is noted that there is no difference between the section-by-section growth rate and the average growth rate indicating constant exponential growth. The study was considered valid.

After 72 h exposure, the notified chemical was < LOQ except for the test with the highest concentration (i.e. 5% of the nominal initial concentration). Some lowering is expected since it is performed under static conditions, however, a significant decrease is observed, probably due to the photosensitivity and oxidative instability of the notified chemical in water under neutral and static exposure conditions. The effect values were based on the initial measured concentrations but it is noted that the concentration range was not within ± 20%. In this case the geometric mean of the initial measured value and end value was not evaluated since an actual end value was not reported (only < LOQ). The

	toxicity of the reference substance to green algae was determined over 72 h and an E_bC50 value of 0.31 mg/L (95% CI: 0.30 – 0.32 mg/L) and an E_rC50 value of 0.65 mg/L (95% CI: 0.57 – 0.73 mg/L) were obtained (acceptable range not provided). The results of the recovery test indicated that the inhibition effects were reversible following exposure up to 5.03 mg/L of the notified chemical.
CONCLUSION	The notified chemical is toxic to algae.
TEST FACILITY	Dr. U. Noack-Laboratorien (2006i)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Municipal sewage sludge (Sarstedt)
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 320, 1000 mg/L Actual: Not provided
Remarks – Method	Standard protocol was followed except copper (II) sulphate pentahydrate was used as the reference substance. Other OECD validity criteria were satisfied. The study was considered to be valid. The range-finding study was used to determine the nominal concentrations for the final study.
RESULTS	
IC50	186 mg/L
Remarks – Results	The IC50 of the reference substance was determined by probit analysis and had a value of 120 mg/L which is within the acceptable range (52 – 157 mg/L). The EC20 for the notified chemical is 21 mg/L.
CONCLUSION	The notified chemical is not toxic to activated sludge from municipal sewage treatment plants at concentrations < 21 mg/L.
TEST FACILITY	Dr. U. Noack-Laboratorien (2006j)

BIBLIOGRAPHY

Cosmital SA (2006) cutaneous absorption of 1% 2-amino-5ethylphenol hydrochloride (WR 803665) in a typical hair dye formulation in the presence of hydrogen peroxide and reaction partner 1-hydroxyethyl 4,5-diamino pyrazole sulfate (A154, WR18247) through pig skin *in vitro* (Study No. KP 166, 1 December 2006) Marly, France, Cosmital SA/Rd-EPSK (Unpublished report submitted by notifier).

Covance Laboratories Ltd (2004) 2-Amino-5-ethylphenol phosphate (WR 802433): Induction of micronuclei in cultured human peripheral blood lymphocytes (Study No. 213/39, 30 September 2004) North Yorkshire, England, Covance Laboratories Ltd (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006a) Notified Chemical: Water Solubility (Flask Method) (Study No. CWF107441, 28 July 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006b) Notified Chemical: Hydrolysis as a function of pH (Study No. CPH107444, 15 August 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006c) Notified Chemical: Partition co-efficient (*n-octanol/water*) using HPLC-method (Study No. COH107442, 7 August 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006d) Notified Chemical: Estimation of the Adsorption Coefficient (K_{oc}) on Soil and Sewage Sludge using HPLC-method (Study No. CAH107441, 27 July 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006e) Sieve Analysis (Study No. CPG107441, 5 July 2006) Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier)

Dr.U.Noack-Laboratorien (2006f) Notified Chemical: Ready Biodegradability Modified Sturm Test (Study No. AST107441, 14 August 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006g) Notified Chemical: Fish (Zebrafish), Acute Toxicity Test, Semi-Static, 96 h (Study No. FAZ107441, 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006h) Notified Chemical: Acute Immobilisation Test (Semi-Static, 48 h) to *Daphnia magna* (Study No. DAI107441, 5 July 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006i) Notified Chemical: Alga, Growth Inhibition Test with *Desmodesmus subspicatus*, 72 h (Study No. SSO107441, 28 June 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

Dr.U.Noack-Laboratorien (2006j) Notified Chemical: Respiration Inhibition Test with Activated Sludge (Study No. BBR107441, 14 June 2006). Kathe-Paulus Strasse 1, D-31157, Sarstedt, Germany, Dr. U. Noack-Laboratorien (Unpublished report submitted by notifier).

ESAC (2007) ESAC statement on the conclusions of the ICCVAM retrospective study on organotypic in vitro assays as screening tests to identify potential ocular corrosives and severe irritants as determined by US EPA, EU(R41) and UN GHS classifications in a tiered testing strategy, as part of a weight of evidence approach. European Centre for the Validation of Alternative methods (ECVAM) Scientific Advisory Committee (27 April 2007)

ESAC (2008) Statement on the scientific validity of in vitro tests for skin irritation testing. European Centre for the Validation of Alternative methods (ECVAM) Scientific Advisory Committee (5 November 2008)

Helix3 Inc. (2006) Comet assay dose range finder and analysis of DNA damage induced by *in vivo* exposure of Sprague-Dawley rats to 2-amino-5-ethylphenol phosphate (WR 802433) (Study No. HX009-001, 31 August 2006) Morrisville, USA, Helix3 Inc. (Unpublished report submitted by notifier).

Hueber-Becker F, Nohynek GJ, Dufour EK, Meuling WJA, de Bie ATHJ, Toutain H, Bolt HM (2007) Occupational exposure of hairdressers to [^{14}C]-*para*-phenylenediamine-containing oxidative hair dyes: A mass balance study. Food and Chemical Toxicology, **45**:160-169.

ICCVAM (2006) In vitro ocular toxicity test methods for identifying severe irritants and corrosives Nov 2006, Interagency Coordinating Committee on the Validation of Alternative Methods, downloaded on 16 March 2009 from http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm

Kandarova H, Liebsch M, Schmidt E, Genschow E, Traue D, Spielmann H, Meyer K, Steinhoff C, Tornier C, De Wever B, Rosdy M (2006) Assessment of the skin irritiation potential of chemicals using the SkinEthic reconstructed human epidermal model and the common skin irritation protocol evaluated in the ECVAm skin irritation validation study. ATLA, **34**: 398-406.

Lee H-S, Lin Y-W (2009) Permeation of hair dye ingredients, *p*-phenylenediamine and aminophenol isomers, through protective gloves. Ann. Occup. Hyg. **53**(3): 289-296.

Lind ML, Boman A, Sollenberg J, Johnsson S, Hagelthorn G, Meding B (2005) Occupational dermal exposure to permanent hair dyes among hairdressers. Ann. Occup. Hyg. **49**(6): 473-480.

Lind ML, Johnsson S, Meding B, Boman A (2007) Permeability of hair dye compounds *p*-phenylenediamine, toluene-2,5-diaminesulfate and resorcinol through protective gloves in hairdressing. Ann. Occup. Hyg. **51**(5): 479-485.

McCall PJ, Swann RL, Laskowski DA, Unger SM, Vrona SA & Dishburger HJ (1980) Estimation of Chemical Mobility in Soil from Liquid Chromatographic Retention Times. Bulletin of Environmental Contamination and Toxicology, **24** (1): 190 195.

MDS Pharma Services (2005) 2-Amino-5-ethylphenol phosphate WR 802433 – Local lymph node assay (Study No. AA24527, 1 April 2005) Saint Germain sur l'Arbesle, France, MDS Pharma Services (Unpublished report submitted by notifier).

Nixon R, Roberts H, Frowen K, Sim M (2006) Knowledge of skin hazards and the use of gloves by Australian hairdressing students and practising hairdressers. Contact Dermatitis, **54**: 112-116.

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

Notox BV (2007) Absorption, distribution, metabolism and excretion of 2-amino-5-ethylphenol hydrochloride (WR 803665) in the Wistar rat (Study No. 462713, 4 December 2007) The Netherlands, Notox B.V (Unpublished report submitted by notifier).

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia

RCC-CCR (2004) Micronucleus assay in bone marrow cells of the mouse with Ethyl-oxygelb-phoshat (WR 802433) (Study No. 825700, 21 September 2004) Rossdorf, Germany, RCC Cytotest Cell Research GmbH (Unpublished report submitted by notifier).

RCC-CCR (2005a) Salmonella Typhimurium Reverse Mutation Assay with Ethyl-oxygelb-phoshat (WR 802433) (Study No. 845101, 2 February 2005) Rossdorf, Germany, RCC Cytotest Cell Research GmbH (Unpublished report submitted by notifier).

RCC-CCR (2005b) Cell mutation assay at the thymidine kinase locus (TK$^{+/-}$) in mouse lymphoma L5178Y cells with Ethyl-oxygelb-phoshat (WR 802433) (Study No. 845102, 30 May 2005) Rossdorf, Germany, RCC Cytotest Cell Research GmbH (Unpublished report submitted by notifier).

RCC Ltd (2006a) 2-Amino-5-ethylphenol phosphate (WR 802433): Acute oral toxicity study in rats (Study No. A39644, 7 April 2006) Itingen, Switzerland, RCC Ltd (Unpublished report submitted by notifier).

RCC Ltd (2007) Notified chemical: Prenatal developmental toxicity study in the Han Wistar rat (Study No. A17785, 13 August 2007) Fullensdorf, Switzerland, RCC Ltd (Unpublished report submitted by notifier).

RCC Ltd (2008) Notified chemical: 13-Week oral (gavage) toxicity study in Wistar rats (Study No. A17730, 22 January 2008) Itingen, Switzerland, RCC Ltd (Unpublished report submitted by notifier).

Ripin D H and Evans D A (2009) pKa's of Inorganic acids, Oxo-Acids, Nitrogen Acids, etc. <http://www.chem.fsu.edu/~mcquade/evans_pKa_table1.pdf>. Accessed 2009 Feb 26.

Safepharm Laboratories Ltd (2006a) Notified chemical: Transcutaneous electrical resistance assay (Study No. 2269/0043, 14 December 2006) Derbyshire, UK, Safepharm Laboratories Limited (Unpublished report submitted by notifier).

Safepharm Laboratories Ltd (2006b) Notified chemical: Skin irritation assessment *in vitro* using the SkinEthic Reconstituted human epidermal model (Study No. 2269/0039, 6 December 2006) Derbyshire, UK, Safepharm Laboratories Limited (Unpublished report submitted by notifier).

SCCP (2005) Opinion on Exposure to reactants and reaction products of oxidative hair dye formulations, Scientific Committee on Consumer Products (SCCP/0941/05)

SCCP (2006a) Opinion on Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients – updated March 2006, Scientific Committee on Consumer Products (SCCP/0970/06)

SCCP (2006b) Memorandum on Hair Dye Substances and their Skin Sensitising Properties, Scientific Committee on Consumer Products (19 December 2006)

SCCP (2007) Opinion on Sensitivity to hair dyes – consumer self testing (SCCP/1104/07)

SCHER/SCCP/SCENIHR (2009) Risk assessment methodologies and approaches for genotoxic and carcinogenic substances. Scientific Committee on Health and Environmental Risks / Scientific Committee on Consumer Products / Scientific Committee on Emerging and Newly Identified Health Risks (21 January 2009).

Sparc Calculator v4.2 (2007) Sparc Online Calculator pKa. < http://ibmlc2.chem.uga.edu/sparc/>. Accessed 2009 Feb 20.

TNO (2006) Chicken Enucleated Eye Test with 2-Amino-5-ethylphenol hydrochloride (WR 803665); an alternative to the Draize eye irritation test with rabbits (Study No. 6815/04, 25 October 2006) Zeist, the Netherlands, TNO Quality of Life (Unpublished report submitted by notifier).

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.

Wella Analytical (2007) Analysis Report 2007/1784 – Potentiometric titration of Ethyloxygelb-chloride (WR 803665) and Ethyloxygelb-phosphate (WR 802433).