Scientific Committee on Consumer Safety

SCCS

OPINION ON

2-Amino-5-ethylphenol HCl

COLIPA n° A158

The SCCS adopted this opinion at its 14th plenary meeting

of 27 March 2012

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members

Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Jan van Benthem, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

Contact
European Commission
Health & Consumers
Directorate D: Health Systems and Products
Unit D3 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-SCCS-Secretariat@ec.europa.eu

© European Union, 2012

ISSN 1831-4767 ISBN 978-92-79-30755-3

Doi:10.2772/78892 ND-AQ-12-005-EN-N

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

Prof. J. Angerer
Dr. C. Chambers
Prof. T. Platzek (chairman, rapporteur)
Dr. S.C. Rastogi
Dr. C. Rousselle
Prof. T. Sanner
Dr. J. van Benthem
Prof. M.P. Vinardell
Dr. I.R. White

External experts

Dr. Mona-Lise Binderup National Food Institute, Denmark

Keywords: SCCS, scientific opinion, hair dye, 2-amino-5-ethylphenol HCl, A158, directive 76/768/ECC, CAS 149861-22-3

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 2-amino-5-ethylphenol HCl, 27 March 2012

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

TABLE OF CONTENTS

ACKNOWLEDGMENTS 3

1. BACKGROUND 5

2. TERMS OF REFERENCE 5

3. OPINION 6

4. CONCLUSION 27

5. MINORITY OPINION 28

6. REFERENCES 28

1. BACKGROUND

Submission I for the new hair dye substance 2-Amino-5-ethylphenol HCl (CAS 149861-22-3) was submitted in July 2008 by COLIPA[1].

According to the submission the intended use is as an oxidative hair dye substance with a final concentration on the scalp of up to max 1.0% as 2-amino-5-ethylphenol hydrochloride.

2. TERMS OF REFERENCE

1. *Does the SCCS consider 2-Amino-5-ethylphenol HCl safe for use as an oxidative hair dye with a concentration on-head of maximum up to 1.0 % taken into account the scientific data provided?*

2. *And/or does the SCCS have any further scientific concerns with regard to the use 2-Amino-5-ethylphenol HCl in oxidative hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2-Amino-5-ethylphenol HCl

3.1.1.2. Chemical names

Phenol, 2-amino-5-ethyl-, hydrochloride (CA Index name, 9CI)
2-Amino-5-ethylphenol hydrochloride (IUPAC)
6-Amino-3-ethylphenol hydrochloride

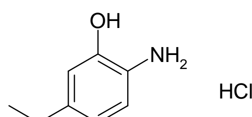
3.1.1.3. Trade names and abbreviations

Subst. Code: A019547
Colipa n°: A158

3.1.1.4. CAS / EC number

CAS: 149861-22-3
EC: /

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_8H_{11}NO.HCl$

3.1.2. Physical form

Beige-yellowish powder

3.1.3. Molecular weight

Molecular weight: 173.64 g/mol

3.1.4. Purity, composition and substance codes

The identification of 2-Amino-5-ethylphenol HCl in the batch RD-CRU 079-16/97-05 was performed by NMR

Comparison of batches of 2-Amino-5-ethylphenol HCl

Description of sample	RD-CRU 079-16/97-05	Lot 07290T0010	Lot 07290T0014	GST079-03/33-08 (in form of the phosphate salt)	GST079-03/52-09 (in form of the phosphate salt)
NMR content (salt) / %, w/w	99.4	-	-	96.5*	98.0
HPLC*** purity / area% 210 nm 254 nm 290 nm	 99.8 99.6 99.9	 100 100 100	 100 100 100	 99.8 99.6 99.6	 99.8 99.9 99.9
HPLC content (w/w)	99.9	98.3	101.6		98.8**
Loss on drying / %, w/w	0.06	0.06	0.02	0.08	0.04
Water content / %, w/w	0.015	0.008	0.0065	0.12	0.05
Residue on ignition / %, w/w	0.83	0.01	0.01	1.68°	0.8°
4-Ethylaniline / ppm	58	138	161	19	Not detectable (LOD 10 ppm)
4-Ethyl-1-nitrobenzene / ppm	Not detectable (LOD 3 ppm)	Not detectable (LOD 15 ppm)	Not detectable (LOD 15 ppm)	17	36
Benzaldehyde / ppm	Not quantifiable (LOQ 0.6 ppm)	-	-	< 1	< 1
2,6-Diamino-3-ethylphenol / ppm	20	Not detectable (LOD 15 ppm)	Not detectable (LOD 15 ppm)	28	750°°
2-Amino-4a,7-diethyl-4,4a,10,10a-tetrahydro-3H-phenoxazin-3-one+ / ppm	Not detectable (LOD 7 ppm)	-	-	64	36
Element screening / ppm	3200 (Na), 54 (Si), 35 (Ca), 150 (Br), 22 (Pb)	16 (Si); 14 (P); 14 (Ca); 40 (Pt)	22 (Si); 20 (Ca); 29 (Pt)	11% (P)	11% (P)

*: The NMR showed small traces of 2-propanol
**: As H_3PO_4 salt
***: Column 250/4 Nucleosil 100-5 C18 Nautilus; CH_3CN:0,02M KH_4PO_4 pH 5,0; 30:70, 1 mL/min, 40 °C
°: Sulphated ash
°° As 2,6-Diamino-3-ethylphenol phosphate (corresponds to ca. 460 ppm free base)
+ This corresponds to oxidative dimeric product of 2-amino-5-ethylphenol

The conversion factor from *2-AMINO-5-ETHYLPHENOL PHOSPHATE* to *2-AMINO-5-ETHYLPHENOL HYDROCHLORIDE* is calculated as follows:

$$\text{Factor} = \frac{\text{Molecular Weight [2-AMINO-5-ETHYLPHENOL-HYDROCHLORIDE]}}{\text{Molecular Weight [2-AMINO-5-ETHYLPHENOL PHOSPHATE]}} = \frac{173.64\ \text{g/mol}}{235.18\ \text{g/mol}} = \underline{0.738}$$

Comment
No documentation was provided for the chemical characterisation of batches Lot 07290T0010, Lot 07290T0014, GST079-03/33-08 and GST079-03/52-09.

3.1.5. Impurities / accompanying contaminants

See 3.1.4

3.1.6. Solubility

Water:	428 g/L (20°C, pH 1.42)	EC Method A.6	ref. 4
Acetone:	> 150 g/L		
DMSO:	> 200 g/L		

3.1.7. Partition coefficient (Log P_{ow})

P_{ow} = 23
Log P_{ow} = 1.37 (pH 7.0, 36°C) (EU - A.8) *(Reference: 2)*

3.1.8. Additional physical and chemical specifications

Particle size distribution:	mean particle diameter: 45.3 µm (CIPAC MT59.1)
pH-value:	1.42 (saturated aqueous solution, 20 °C)
pKa-values:	5.42 and 10.04
Melting point:	218 °C (decomposition)
Boiling point:	225 °C (decomposition)
Density:	1.22 g/cm^3
Vapour pressure:	4.1 exp-8 hPa (20 °C, extrapolated)
Surface tension (in water):	69.9 mN/m (20 °C)
Water solubility:	428 g/l (20 °C)
Flammability (solids):	not highly flammable
Explosive properties:	not explosive
Relative self-ignition temperature:	>400 °C
Oxidising properties:	not oxidising
UV_Vis spectrum (200-800 nm):	/

3.1.9. Homogeneity and Stability

2-Amino-5-ethylphenol hydrochloride solutions used in 13 week oral gavage toxicity study were shown to be homogeneous (variation in top, middle and bottom were 87%-105% of the initial concentration), and these were demonstrated to be stable for 7 days at room temperature (maximum deviation from initial concentration 3.8%).

2-Amino-5-ethylphenol hydrochloride solutions used in prenatal developmental toxicity study were shown to be homogeneous (variation in top, middle and bottom were 89%-103% of the initial concentration), and these were demonstrated to be stable for 7 days at room temperature (deviation from initial concentration <10%).

General Comments to physico-chemical characterisation

- 2-Amino-5-ethylphenol hydrochloride is used in hair colouring formulations, but it has no EC number.
- A study report on the chemical characterisation of a batch of 2-amino-5-ethanol HCl was submitted. But no documentation was provided on chemical characterisation of 4 other reported batches of 2-amino-5-ethanol HCl/phosphates.
- Stability of 2-amino-5-ethanol HCl in typical hair dye formulations is not reported.

3.2. Function and uses

2-Amino-5-ethylphenol hydrochloride is used as an oxidative hair colouring agent precursor. The intended maximum on-head concentration is 1.0% in oxidative hair dye formulations.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: OECD 423 (2001)
Species/strain: rat, HanRcc:WIST (SPF)
Group size: 2 groups of 3 females
Test substance: 2-amino-5-ethylphenol phosphate (WR 802433)
Batch: GST 079-03/52-09
Purity: 99.9 area% (HPLC)
Vehicle: purified water
Dose levels: 2000 mg/kg bw
Dose volume: 10 mL/kg bw
Route: oral, gavage
GLP: in compliance
Study period: 23 November – 20 December 2005

A single dose acute oral toxicity test was performed in female rats using the acute toxic class method. The test substance dissolved in water was administered at 2000 mg/kg bw to two groups of 3 animals. Animals were observed for 15 days. Body weight of animals was determined just before the administration and on days 1, 8 and 15 during the observation period. All study animals were autopsied after death.

Results
One out of the 6 treated females was euthanized 2.5 h after treatment. The remaining 5 rats survived until the end of the study period. Slight to marked sedation was observed in all treated animals. Ventral or lateral recumbency and cyanosis were noted in all rats. Slight lacrimation, ruffled fur, hunched posture and orange urine were observed in the remaining 5 survival rats. These changes were absent 5 days after the administration. Paleness of skin, muscle twitching and hypothermia were observed in 3 rats. Padding movements were observed in 2 rats and tachypnea in one rats and disappeared 2-hours after administration. Any abnormalities were not observed from the from test day 9 to the end of the study.

The body weights of the animals were within the range commonly recorded for this strain and age. No macroscopic findings were recorded at necropsy, except in the animal killed in extremis in which the fatty tissue was slightly yellow.

Conclusion
The acute toxicity of 2-amino-5-ethylphenol phosphate is low after single oral administration to female rats. The LD50 of 2-amino-5-ethylphenol phosphate is greater than 2000 mg/kg bw, corresponding to 1476 mg/kg bw as hydrochloride salt.

Ref.: 13

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

In Vitro Skin Corrosion: Transcutaneous Electrical Resistance (TER) Assay

Guideline:	According to OECD Guideline no. 430 (2004)
Test system:	*ex vivo* skin discs from rat (1 female; strain BK: Wistar) dorsal skin
Test substance:	2-amino-5-ethylphenol hydrochloride
Batch:	RD-CRU079-16/97-05
Purity:	99.9 area% (HPLC at 254 nm)
Concentrations:	Neat (100%; powder)
Exposure conditions:	Single application on the epidermal surface of three ex vivo rat skin discs for a contact period of 24 h
GLP:	In compliance
Study period:	August 2006

One female rat was used for the study. Following an acclimatisation period of two days the animal was shaved to remove hair from the dorsal surface. The shaved area was washed. Two days later the animal was killed by CO_2 asphyxiation followed by cervical dislocation. The dorsal skin was removed as a single pelt and then mounted, epidermal side uppermost, onto a PTFE tube. The tissue was secured with a rubber "O" ring, excess tissue was trimmed away and the "O" ring/PTFE interface sealed with soft paraffin wax. The tube was supported by a clamp inside a labelled 30 mL glass receptacle containing 10 mL electrolyte solution (154 mM $MgSO_4$).

Two skin discs were taken from the pelt and the TER measured as a quality control procedure. Each disc was confirmed as having a resistance > 10kΩ.

2-Amino-5-ethylphenol hydrochloride was applied as neat substance to the epidermal surface of three skin discs for a contact period of 24 h. At the end of the exposure period, the test material was removed by washing the skin disc with warm tap water until no further test material could be removed. Three positive (36% hydrochloric acid) and negative control (sterile distilled water) discs were also assayed for a contact period of 24 h.

The TER was measured using a Wheatstone Bridge with a low voltage alternating current, the value in Ω/kΩ per skin disc was determined. The mean TER for the skin discs was calculated.

Results
The following results were obtained with the test material 2-amino-5-ethylphenol hydrochloride:
The mean TER was 3.7 kΩ (±1.0), after a contact period of 24 h. At the end of testing the skin discs appeared pale, which indicates damage to the skin. Given these data, no further verification of the results was required.

Conclusion
2-Amino-5-ethylphenol hydrochloride, as a neat substance, was considered to cause skin corrosion.

Ref: 15

Skin Irritation Assay using a Reconstituted Human Epidermis (RHE) Model

Guideline:	/ (but comparable to the RHE models validated by ECVAM in May 2007).
Test system:	*in vitro* Reconstituted Human Epidermis model (SkinEthic; 0.5 cm², cultured for 17 days at air-liquid interface)

Test substance:	2-amino-5-ethylphenol hydrochloride
Batch:	RD-CRU 079-16/97-05
Purity:	99.9 area% (HPLC at 254 nm)
Concentrations:	Neat (100%; powder) and 2 and 10% (w/w) in water
Exposure conditions:	Single application of 16 mg (neat) or 16 µL (2 and 10%) for 15 minutes at room temperature, followed by a rinsing step and a 42 h post-treatment incubation
GLP:	In compliance
Study period:	August 2006

The treatment period was 15 minutes followed by a rinsing step and a 42 ± 2 h post-treatment incubation period. Cell viability was determined by the enzymatic reduction of the yellow MTT tetrazolium salt (3-[4,5-dimethylthiazol-2-y1]-2,5-diphenyl-tetrazolium bromide) to a blue formazan salt (within the mitochondria of viable cells) in the test material treated tissues relative to the negative control).
Histological evaluation of the tissues at the end of the treatment was performed as an additional measure to correlate with cytotoxicity to evaluate irritation for test items which may interfere with MTT.
2-Amino-5-ethylphenol hydrochloride was applied as neat substance and at concentrations of 2% and 10% (w/w) in water. Two endpoints, namely cytotoxicity in the MTT assay, expressed as percent viability of treated cultures in comparison to negative controls, as well as morphological changes identified by histology were evaluated.

Results
The 2% and 10% concentrations of 2-amino-5-ethylphenol hydrochloride did not induce a decrease in cell viability in the MTT assay, resulting in 100.9% viability when applied at 2%, and 106.2% when applied at 10% in water.

2-Amino-5-ethylphenol hydrochloride induced cytotoxicity when applied as neat substance, resulting in 0% viability.

Histological evaluation of the treated tissues did not show any appreciable histological epidermis alteration in comparison to the negative control cultures at 2% and 10%. However, tissue necrosis was observed when 2-amino-5-ethylphenol hydrochloride was applied as neat substance.

The negative and positive controls demonstrate the validity of the assay.

Conclusion
2-Amino-5-ethylphenol hydrochloride is considered as non-irritant (MTT viability > 50%), when tested at 2% and 10% in water. The histological results confirmed the absence of cytotoxicity. When applied as neat substance, 2-amino-5-ethylphenol hydrochloride is classified as irritant. This was confirmed by tissue necrosis observed at histological examination.

Ref: 16

Comment on present status of validation
The skinethic™ reconstituted human epidermis model was validated in 2008. The method has now been adopted as OECD guideline 439 (2010). The above experiment was comparable to the validated protocol but predates it.

3.3.2.2. Mucous membrane irritation

Chicken Enucleated Eye Test (CEET) (Isolated Chicken Eye (ICE) Test)

Guideline:	/ (but in accordance with the test method validated by ECVAM for identification of severe ocular irritants)

Test system:	Isolated chicken eyes (ROSS, spring chickens)
Number of replicates:	Three eyes per test concentration and one eye for negative control
Test substance:	2-amino-5-ethylphenol hydrochloride
Batch:	RD-CRU079-16/97-05
Purity:	99.9 area% (HPLC at 254 nm)
Concentrations:	Neat (100%; powder) and 2 and 10% in water
Exposure conditions:	Single application of 30 mg (neat) or 30 µl (2 and 10% w/w) for 10 seconds
GLP:	Not in compliance
Study period:	August 2006

2-Amino-5-ethylphenol hydrochloride was applied undiluted and as a 2% and 10% aqueous solution to isolated eyes of chickens.

Approximately 7 weeks old chickens (obtained from slaughter animals for human consumption) were used as eye-donors. Within 2 h of slaughter, eyes were enucleated and placed in a superfusion apparatus. Eyes with a corneal thickness deviating more than 10% of the average, or eyes that were unacceptably stained with fluorescein, or eyes that showed any other signs of damage were not used. Nine test eyes (3 per test concentration) and one negative control were selected for testing.
After an equilibration period of 45-60 minutes, the corneal thickness of the eyes was measured once more to determine the zero reference value for corneal swelling calculations. At time t = 0, i.e. immediately after the zero reference measurement, the following procedure was applied for each test eye: the clamp holding the test eye was placed on paper tissues outside the chamber with the cornea facing upwards. Next, three corneas were treated with 30 mg neat 2-amino-5-ethylphenol hydrochloride or 30 µL of a 2%, or a 10% aqueous solution of 2-amino-5-ethylphenol hydrochloride. After an exposure period of 10 seconds, the corneal surface was rinsed thoroughly with 20 mL of isotonic saline of ambient temperature. After rinsing, each eye in the holder was returned to its chamber. The control eye was treated with saline only. The eyes were examined at 0, 30, 75, 120, 180 and 240 minutes after treatment. All examinations were carried out with the slit-lamp microscope. At 30 minutes the fluorescein retention was scored.
Ocular irritation was evaluated using the endpoints of corneal thickness (swelling), corneal opacity and fluorescein retention.
After the final examination, the test eyes and the control eye were preserved in a neutral aqueous phosphate-buffered solution of 4% formaldehyde. The tissues selected were embedded in paraffin wax, sectioned at 5 µm and stained with Periodic Acid Schiff (PAS) for histological examination.

Results
Neat 2-amino-5-ethylphenol hydrochloride caused moderate corneal swelling (25%), severe corneal opacity and severe fluorescein retention by damaged epithelial cells. In addition, a layer of needle-like remains of the test substance was observed on the cornea.

2-Amino-5-ethylphenol hydrochloride tested at 10% caused only very slight corneal opacity and very slight fluorescein retention, while 2% did not cause any corneal effects.

The Irritation Indices calculated were 0, 21 and 145 for the 2%, 10% and 100% (neat substance) treatment groups, respectively.

Microscopic examination of the corneas treated with the neat test sample generally confirmed the effects observed by slit-lamp examination, i.e. revealing moderate erosion and slight vacuolisation of the epithelium. No abnormalities were observed in the stroma and endothelium.

No abnormalities were observed during the microscopic examination of the corneas treated with 2-amino-5-ethylphenol hydrochloride at 10%, which demonstrated, that the effects of corneal opacity and very slight fluorescein retention were not accompanied by cell damage.

No abnormalities were observed in the microscopic examination of the corneas treated with 2-amino-5-ethylphenol hydrochloride at 2%.

Conclusion
On the basis of the results obtained in the CEET, 2-amino-5-ethylphenol hydrochloride tested at 2% and 10% was identified as not irritating to the eyes. 2-Amino-5-ethylphenol hydrochloride tested as a neat substance was identified as severely irritating to eyes.

Ref: 19

Comment
The CEET (ICE) is a screening method for hazard identification and not for risk assessment. The method has now been adopted as OECD guideline 438 (2009) for eye corrosivity. No fully validated alternative methods for eye irritation exist.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD Guideline no. 429 (2002)
Species:	Mouse, strain CBA/J
Group size:	5 females per test concentration
Test substance:	2-amino-5-ethylphenol phosphate In DMSO and in water/acetone (1:1) mixed with olive oil at a ratio of 3:1
Batch:	GST 079-03/33-08
Purity:	99.9 area% (HPLC at 254 nm)
Concentrations:	0.5, 1.5, 5.0 and 15.0% in DMSO 0.5, 1.5, 5.0 and 15.0% in acetone/water/olive oil
Route:	Dermal
Dosing schedule:	Once daily on three consecutive days
GLP:	In compliance
Study period	April 2005

25 µl of 0 (vehicle only), 0.5, 1.5, 5.0 and 15.0% 2-amino-5-ethylphenol phosphate in DMSO as well as 25 µl of 0 (vehicle only), 0.5, 1.5, 5.0 and 15.0% 2-amino-5-ethylphenol phosphate in a mixture of aqua/acetone (1:1) with olive oil (3:1) were applied to the surface of the ear of five female mice per group for three consecutive days. As an appropriate positive control for hair dye precursors, p-phenylenediamine (PPD) at 1% in DMSO was investigated in parallel under identical test conditions.
Animals were checked for morbidity/mortality at least once daily. Observation for clinical signs was done before and once per day after dosing. Body weight was determined at day -1 and day 5.
At day 5, the mice received an intravenous injection of 250 µl solution containing 22.6 µCi of [H3] methyl thymidine. Approximately 5 h later, the mice were sacrificed, and the draining auricular lymph nodes were removed and collected in PBS. After preparing a single cell suspension for each mouse, cells were precipitated by 5% trichloro-acetic acid (TCA), and the radioactivity was determined (incorporation of [H3] methyl thymidine in the pellets) by means of liquid scintillation counting as disintegration per minute (dpm).

Results
The positive control PPD at a concentration of 1% in DMSO gave a mean stimulation index of 6.6.

In DMSO, 2-amino-5-ethylphenol phosphate was positive in the local lymph node assay, as there was more than 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle. The mean stimulation indices were 1.3, 2.1, 1.8 and 4.9 at the concentrations of 0.5%, 1.5%, 5.0% and 15.0%, respectively. The EC3 value was 8.9% in DMSO.
In acetone/water/olive oil, 2-amino-5-ethylphenol phosphate was negative in the local lymph node assay, as there was less than a 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle. The mean stimulation indices were 0.9, 1.2, 1.2 and 1.3 at the concentrations of 0.5%, 1.5%, 5.0% and 15.0%, respectively. An EC3 value could therefore not be calculated.

Conclusion
2-amino-5-ethylphenol phosphate is a moderate skin sensitiser under the defined experimental conditions in DMSO, with an EC3 value of 8.9% (corresponding to 6.6% HCl salt) and is not a skin sensitiser under the defined experimental conditions in acetone/water mixed with olive oil.

Ref: 23

Conclusion skin sensitisation
Considering the calculated EC3 value of the phosphate salt of 2-amino-5-ethylphenol in DMSO, the EC3 value of the hydrochloride salt was calculated by using the correction factor related to the different free base content of the phosphate and the hydrochloride salt.

EC3 value phosphate-salt: 8.9%
8.9% = 8.9 g/100 ml x 0.738 (correction factor) = 6.6 g /100 ml = 6.6%
EC3 value hydrochloride-salt: 6.6%

Based on this calculation, 2-amino-5-ethylphenol hydrochloride is considered as a moderate skin sensitiser.

3.3.4. Dermal / percutaneous absorption

Percutaneous absorption *in vitro*

Guideline:	OECD 428 (2004)
Tissue:	Porcine back or flank skin 3 donors (frozen/thawed; thickness: ≤ 1000 µm)
Method:	Diffusion Teflon-chambers
No. of chambers:	Two experiments with 6 chambers each (five for the formulation containing the dye stuff and one for the blank formulation)
Membrane integrity	Tritiated water
Test substance:	2-amino-5-ethylphenol hydrochloride
Batch no:	RD-CRU 079-16/97-05
Purity :	99.9 area% (HPLC at 254 nm)
Test substance:	2-Amino-5-ethyl[U-14C]phenol hydrochloride
Batch no:	CFQ14698 Batch 1
Radiochemical purity:	99.3%
Chemical identity:	HPLC profile and retention time of the labelled product identical with that of the standard compound
Concentration:	1.0 mg/cm², tested as part of an oxidative hair dye formulation
Dose	100 mg cm² of formulation (A158 mixed equimolar with A154)
Receptor	PBS
Stability in receptor	Stable at 3 days
Solubility in receptor	388 mg/ml
Detection	liquid scintillation
GLP:	In compliance

Study period August 2006

The cutaneous absorption of 1.0% 2-amino-5-ethylphenol hydrochloride in a typical hair dye formulation in the presence of hydrogen peroxide and a reaction partner (A154) was investigated in vitro, using pig skin preparations. Two independent experiments were performed with 6 diffusion cells per experiment. For calculations, the mean value of all valid skin samples (n=9) was used.

400 mg of the formulation (= 100 mg/cm²), containing 1.0% 2-amino-5-ethylphenol hydrochloride, was applied to the skin samples (= 1.0 mg of test item/cm²) for 60 minutes and subsequently washed off with water and shampoo. The determination of the amount of 2-amino-5-ethylphenol hydrochloride in the washings (= amount dislodgeable from the skin surface) was performed by measuring the radioactivity by means of scintillation counter. At 16, 24, 40, 48, 64 and 72 h, the content of 2-amino-5-ethylphenol hydrochloride was determined in the receptor fluid by the same method. At termination of the experiment, the skin was heat-treated and the "upper skin" (stratum corneum and upper stratum germinativum) was mechanically separated from the "lower skin" (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the radioactivity was quantified by means of scintillation counting.

Results

	Skin	Integrity-Test	1)		2)		3)		4)		1) + 2) + 3) + 4)	
	No (series)	$^{3}H_2O$ Permeation (4 hours cumulative)	Receptor fluid (72 hours cumulative)		Lower skin (72 hours cumulative)		Upper skin (72 hours cumulative)		Rinsing solution (after 60 minutes)		Total***	
		[% Dose]	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]**	[µg/cm²]	[% Dose]
Application of 100 mg 1 % 2-Amino-5-ethylphenol hydrochloride in a typical hair dye formulation* per 1 cm² skin	2 (1)	1.4	0.916	0.096	0.228	0.024	2.045	0.214	907.39	94.83	953.74	99.68
	4 (1)	1.4	1.003	0.106	0.470	0.050	3.139	0.333	877.01	93.09	939.55	99.73
	6 (1)	1.6	2.277	0.239	0.394	0.041	4.714	0.494	854.82	89.58	907.98	95.15
	8 (1)	1.4	2.547	0.266	0.422	0.044	4.405	0.461	876.69	91.65	927.48	96.96
	10 (1)	1.4	0.457	0.048	0.520	0.054	4.347	0.454	907.52	94.74	954.97	99.70
	12 (1)	1.8	0.877	0.093	0.558	0.059	4.724	0.498	900.56	95.03	959.04	101.20
	2 (2)	1.0	1.763	0.184	0.585	0.061	2.329	0.243	908.19	94.73	954.12	99.52
	4 (2)****	3.5	1.405	0.150	0.566	0.060	3.175	0.338	863.82	92.01	930.11	99.07
	6 (2)	1.6	2.149	0.224	0.743	0.077	5.231	0.545	926.31	96.50	974.51	101.52
	8 (2)****	2.1	3.769	0.395	1.029	0.108	4.475	0.469	869.01	91.15	924.92	97.02
	10 (2)	1.2	0.992	0.103	0.515	0.053	3.314	0.342	896.03	92.59	933.15	96.43
	12 (2)****	2.1	1.143	0.118	0.586	0.060	4.180	0.432	926.28	95.62	963.51	99.47
Mean		**1.708**	**1.442**	**0.151**	**0.493**	**0.051**	**3.805**	**0.398**	**894.95**	**93.64**	**944.95**	**98.88**
± S.D		0.653	0.749	0.078	0.142	0.015	1.136	0.119	21.71	2.11	19.92	2.19
(n)		(12)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(9)

*vehicle: (typical hair dye formulation as detailed in Annex III was mixed 1:1 with peroxide solution, see 4.3); **Corrected for individual applied dose; *** Total is corrected for losses on tips; **** Outlier: not considered for the calculation of the mean (with the exception for the integrity test)

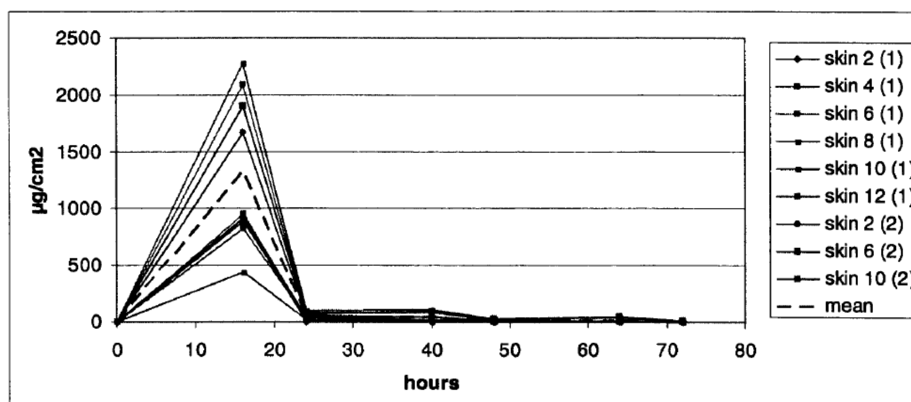
The mass balance of the test substance resulted in values of 95.15 to 101.52% recovery for all (9) skin samples with acceptable integrity.

The majority of the test substance was in the rinsing solutions (894.95 ± 21.71 µg/cm²). Small amounts of 2-amino-5-ethylphenol hydrochloride were found in the upper skin (3.805 ± 1.136 µg/cm²), in the lower skin (0.493 ± 0.142 µg/cm²) and in the fractions of the receptor fluid collected within 72 h (1.442 ± 0.749 µg/cm²).

Amount of 2-amino-5-ethylphenol hydrochloride in:	**µg/cm² (mean ± S.D, n=9)**			**%* (mean ± S.D, n=9)**		
Receptor fluid (72 h)	1.442	±	0.749	0.151	±	0.078
Lower skin (72 h)	0.493	±	0.142	0.051	±	0.015

Upper skin (72 h)	3.805	±	1.136	0.398	±	0.119
Rinsing solution (after 60 min)	894.95	±	21.71	93.64	±	2.11
Total balance (recovery)**	944.95	±	19.92	98.88	±	2.19

* Corrected for individual applied dose; ** Total is corrected for losses on tips



With respect to the receptor fluid, 2-amino-5-ethylphenol hydrochloride was detectable predominantly within the first fractions collected during the 72 h experimental period (fractions 0-16 h). The small amounts of the test substance detected after 16 h indicate that 2-amino-5-ethylphenol hydrochloride is not available from a potential reservoir in the skin (depot).

Under the assumption that a depot effect is absent, a maximum amount of 1.935 ± 0.762 µg/cm² of 2-amino-5-ethylphenol hydrochloride is considered as biologically available (n=9, three donors; receptor fluid + lower skin; 1.442 µg/cm² + 0.493 µg/cm²)

Ref: 26

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (30 days) oral toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) toxicity (oral, dermal)

Guideline: OECD 408
Species/strain: Wistar rats
Group size: 10 animals per sex and dose and a recovery group
Test substance: 2-Amino-5-Ethylphenol HCl in bi-distilled water
Batch: RD-CRU 079-16/97-05
Purity: 99.9%
Dose level: 0, 16, 55 and 272 mg/kg bw/day
Route: Oral, gavage
Exposure period: 90 days
GLP: in compliance
Date: June 2006 – September 2006

2-Amino-5-Ethylphenol HCl in bi-distilled water was administered, by gavage, once daily to Wistar rats (10/sex/per dose) for 90 days. The test substance was administered at dosage levels of 0, 16, 55 and 272 mg/kg bw/day. The control group received the vehicle (bi-distilled water) only. The animals were sacrificed at the end of the study. All animals were

observed daily for mortality, clinical signs and water consumption. Body weights, clinical signs and food consumption were recorded periodically. Ophthalmological examination was performed prior to treatment and to sacrifice. Haematology and clinical chemistry was conducted at the end of the treatment period. Organ weights were measured and macroscopy and histopathology was performed, on all animals.

Results
No mortality and no clinical signs of toxicity were observed on functional tests, ophthalmoscopic examinations and food consumption. Orange discoloration of the urine was observed in males and females treated at the dose of 272 and 55 mg/kg bw/day. It was not considered as an adverse effect.

At the dose of 272 mg/kg bw/day, lower mean body weights were observed in male rats but not in females. Changes in haematological parameters indicated mild anaemia with compensatory reticulocytosis in male and female rats. Significantly elevated bilirubin levels were noted in males and females. Changes observed in the urinalysis parameters of rat males included significantly elevated urine volume with lower density and osmolarity. In the females, a significant elevation of nitrite and the bilirubin content was found. Mean absolute and relative spleen weights were elevated in male and female with microscopic changes (increase in hemosiderin deposits). Relative kidney weights were also increased in males and females as well as relative liver weight in males only. Increased presence of tubular hyaline droplets and increased lymphoid cell infiltration and tubular basophilia were observed in male rats. Corneal opacities were also noted in four males and seven females.

At the dose of 55 mg/kg bw/day, signs of slight anaemia were observed in male and female rats (significant elevation in the reticulocyte counts, increased mean cell volume and reduced mean cell haemoglobin concentration).

Conclusion
As no test item-related effects were observed at the dose of 16 mg/kg bw/day, this dose was considered as the NOAEL.

Ref.: 27

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)	
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102	
Replicates:	triplicate cultures in two independent experiments	
Test substance:	ethyl-oxygelb-phosphat	
Batch:	GST079-03/33-08	
Purity:	99.6 area% (HPLC)	
Vehicle:	deionised water	
Concentration:	experiment 1:	33, 100, 333, 1000, 2500 and 5000 μg/plate, without and with S9-mix
	experiment 2:	10, 33, 100, 333, 1000, 2500 and 5000 μg/plate, without and with S9-mix
Treatment:	experiment 1:	direct plate incorporation method with at least 48 incubation without and with S9-mix

	experiment 2:	pre-incubation method with 60 minutes pre-incubation and at least 48 h incubation, without and with S9-mix
GLP:	in compliance	
Study period:	16 September 2004 – 2 February 2005	

Ethyl-oxygelb-phosphat was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment for toxicity with strains TA98 and TA100. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. The pre-experiment is reported as part of experiment 1 since evaluable plates at five concentrations or more in all strains used were obtained. Experiment 1 was performed according to the direct plate-incorporation test, experiment 2 with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
No visible reduction of the background growth was observed without and with S9-mix in all strains used in experiment 1 but it was reduced at higher concentrations in all strains used in the pre-experiment and experiment 2.
Clear evidence of toxicity, as a reduction in the number of revertants, was observed in TA102 at concentration of 2500 µg/plate and above (experiment 1) and at 1000 µg/plate and above (experiment 2), at 2500 µg/plate and above in all other tester strains in experiment 2 without S9-mix, and at 5000 µg/plate in experiment 1 (without and with S9-mix) and experiment 2 (with S9-mix) for all remaining tester strains.
A biologically relevant increase in revertant colonies was not observed in any of the five tester strains used at any concentration level, neither in the presence nor in the absence of metabolic activation.

Conclusion
Under the experimental conditions used, ethyl-oxygelb-phosphat was not mutagenic in this bacterial gene mutation tests

Ref.: 28

In vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1997)	
Species/strain:	mouse lymphoma L5178Y cells	
Replicates:	single cultures in 3 independent experiments	
Test substance:	ethyl-oxygelb-phosphat	
Batch:	GST079-03/33-08	
Purity:	99.6 area% (HPLC)	
Vehicle:	deionised water	
Concentration:	experiment I:	58.1, 83.7, 100.5, 120.6, 144.7, 173.6, 208.3, 250.0 and 300.0 µg/ml, without S9-mix 48.5, 69.8, 83.7, 100.5, 120.6, 144.7, 173.6, 208.3 and 250.0 µg/ml, with S9-mix
	experiment II:	5.6, 11.2, 13.4, 16.1, 19.3, 23.2, 27.8, 33.3 and 40.0 µg/ml, without S9-mix
	experiment IIA:	33.3, 40.0, 48.0, 57.6, 69.1 and 83.0 µg/ml, without S9-mix
Treatment:	experiment I:	4 h treatment both without and with S9-mix; expression period 72 h and a selection period of 10-15 days

	experiment II:	24 h treatment without S9-mix; expression period 48 h and a selection period of 10-15 days
GLP:	in compliance	
Study period:	17 August 2004 – 30 May 2005	

Ethyl-oxygelb-phosphat was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test for toxicity with concentrations up to 2400 µg/ml (± 10 mM), the prescribed maximum concentration of the OECD guideline, measuring suspension growth relative to the concurrent vehicle control cell cultures. In the main tests, cells were treated for 4 h both without and with S9-mix (experiment I) or 24 h without S9-mix (experiment II), followed by an expression period of 72 h or 48 h, respectively, to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as percentage suspension and total growth of the treated cultures relative to the concurrent vehicle control cell cultures. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. An increased occurrence of small colonies indicated by an increased small/large colonies ratio was associated with clastogenic effects. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test strong toxic effects were observed at 300 µg/ml and above in the absence and at 75 µg/ml and above in the presence of S9-mix. Following continuous treatment the cell growth was strongly reduced at 37.5 µg/ml and above. Precipitation was observed at 600 µg/ml (4 h treatment) or 300 µg/ml (24 h treatment) and above in the absence of S9-mix and at 1200 µg/ml and above in the presence of S9-mix.
No precipitation was observed in the main experiments. In experiment I the appropriate level of toxicity (about 10-20% survival after the highest concentration) was reached. Experiment IIA was performed since in experiment II this level of toxicity was not obtained.
In the presence of metabolic activation, a biologically relevant increase in the mutant frequency was not observed as compared to the controls. In the absence of metabolic activation, a concentration dependent increase in the mutant frequencies with an increase in the ratio small/large colonies pointing to a clastogenic effect, was observed. However, all mutant values found were within the range of the historical control data. Exclusively, in experiment IIA at the highest concentration tested (83.0 µg/ml) an increase in the mutant frequency just inside the range of the historical control data was found. Although the threshold of twice the mutant frequency of the corresponding solvent control was slightly exceeded, this increase was considered not biologically relevant.

Conclusion
Under the experimental conditions used, ethyl-oxygelb-phosphat was not mutagenic in this mouse lymphoma assay using the *tk* locus as reporter gene.

Ref.: 29

In vitro Micronucleus Test

Guideline:	according recommendations of the International Workshop on Genotoxicity Testing and principles for testing described in OECD 473	
Species/strain:	human lymphocytes from 2 healthy, non-smoking female donors	
Replicates:	duplicate cultures in two independent experiments	
Test item:	2-amino-5-ethylphenol	
Batch:	GST 079-03/33-08	
Purity:	99.6 % (HPLC)	
Vehicle:	DMSO	
Concentrations:	experiment 1:	15.39, 24.05 and 37.58 µg/ml without S9-mix

		66.21, 82.77 and 129.3 µg/ml with S9-mix
	experiment 2:	32.06, 44.37 and 61.41 µg/ml without S9-mix
		131.1, 204.8 and 256.0 µg/ml with S9-mix
Treatment:	experiment 1:	24 h PHA stimulation, 20 h treatment without S9-mix or 3 h with S9-mix, harvest time 48 h after the start of treatment
	experiment 2:	48 h PHA stimulation, 20 h treatment without S9-mix or 3 h with S9-mix, harvest time 48 h after the start of treatment
GLP:	in compliance	
Study period:	5 April 2004– 21 June 2004	

2-Amino-5-ethylphenol has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in cultured human peripheral blood lymphocytes. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Treatment of lymphocytes commenced approximately 24 h (experiment 1) or 48 h after mitogen stimulation by phytohaemagglutinin (experiment 2). In the absence of S9-mix lymphocytes were treated for 20 h, in the presence of S9-mix for 3 h; cells were harvested 48 h after the beginning of treatment. The final 27-28 h of incubation was in the presence of cytochalasin B (final concentration 6 µg/ml). Cultures of human peripheral blood lymphocytes were treated with a range of about 16 increasing concentrations of 2-amino-5-ethylphenol. The test concentrations for micronucleus analysis were selected by evaluating the effect of 2-amino-5-ethylphenol on the replication index. The highest concentration should produce approximately 60% decrease in replication index. Negative and positive controls were in accordance with the draft OECD guideline.

Results
In experiment 1 in the presence of S9-mix biologically relevant increases in lymphocytes with micronuclei were not observed. In the absence of S9-mix frequencies of lymphocytes with micronuclei were observed that were statistically significantly and concentration-dependently elevated compared to concurrent vehicle controls for the two highest concentrations tested. In isolation these results of experiment 1 would have been considered equivocal but as clear concentration-dependent increases in lymphocytes with micronuclei were found in experiment 2, the result from experiment 1 was considered biologically relevant as well.
In experiment 2, both in the absence and presence of S9-mix statistically significant increases in the number of lymphocytes with micronuclei were found. For the treatment without S9-mix the increase was clearly concentration-dependent.

Conclusion
Under the experimental conditions used 2-amino-5-ethylphenol induced an increase in lymphocytes with micronuclei and, consequently, is genotoxic (clastogenic and/or aneugenic) in cultured human peripheral blood lymphocytes.

Ref.: 30

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

***In vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline:	OECD 474 (1997)
Species/strain:	female NMRI mice
Group size:	5 animals/sex/dose
Test substance:	ethyl-oxygelb-phosphat
Batch:	GST079-03/33-08
Purity:	99.6 area% (HPLC)
Vehicle:	deionised water
Dose level:	0, 312.5, 625 and 1250 mg/kg bw

Route: single oral dose
Sacrifice times: 24 h and 48 h (high dose only) after treatment
GLP: in compliance
Study period: 30 March 2004 – 21 September 2004

Ethyl-oxygelb-phosphat has been investigated for induction of micronuclei in the polychromatic erythrocytes of female mice. Test doses were based on the results of a pre-test for toxicity. Male and female mice were treated orally with doses between 500 and 2000 mg/kg bw and examined for acute toxic symptoms and/or mortality at 1, 2-4, 6, 24, 30 and 48 h after treatment. In the main experiment female mice were exposed orally to 0, 312.5, 625 and 1250 mg/kg bw. The mice were examined for acute toxic symptoms and/or mortality at 1, 2-4, 6 and 24 h after treatment. Bone marrow cells were collected 24 h or 48 h (highest dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test all mice survived the dose levels up to 2000 mg/kg bw except for 2 female mice which died at 1500 mg/kg bw. Clinical observations observed included: reduction in spontaneous activity, abdominal position, eyelid closure, ruffled fur and apathy. Mice treated with doses of 1500 mg/kg bw and above had yellow coloured urine.
In the micronucleus test, 2 mice treated with 1250 mg/kg bw died. The remaining mice and those of the 625 mg/kg bw group showed a reduction of spontaneous activity and abdominal position as well as coloured urine (1250 mg/kg bw).
A decrease in the PCE/TE ratio was not observed at both sampling times. However, the clinical signs reported, particularly the coloured urine, indicated systemic distribution and thus bioavailability of ethyl-oxygelb-phosphat. A biologically relevant and dose dependent increase in the number of cells with micronuclei was not observed at any sampling time and dose level of ethyl-oxygelb-phosphat.

Conclusions
Under the experimental conditions used ethyl-oxygelb-phosphat did not induce an increase in the number of bone marrow cells with micronuclei and, consequently, ethyl-oxygelb-phosphat is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

In vivo alkaline single cell gel electrophoresis (comet) assay in mice

Guideline: /
Species/strain: male sprague Dawley rats
Group size: 5 male rats/dose
Test substance: 2-amino-5-ethylphenol phosphate
Batch: GST079-03/33-08
Purity: 99.6 area% (HPLC)
Solvent: Water (dH_2O)
Dose levels: 0, 500 and 1000 mg/kg bw/day
Treatment: orally on 2 consecutive days
Sacrifice time: 4 ± 0.5 after the last treatment
Organs studied: liver, duodenum and urinary bladder
GLP: in compliance
Study date: 18 July 2005 – 10 August 2005

2-Amino-5-ethylphenol phosphate has been investigated for the induction of DNA damage in the alkaline single cell gel electrophoresis (comet) assay in various tissues of rats. Test concentrations were based on the results of a dose range finder test in which 2 rats/sex were treated with 1000 mg/kg bw/day on 2 consecutive days, based on the MTD of 1250

mg/kg bw as determined in an earlier experiment. Rats were observed 1 h after each treatment and at the end of each treatment day.
In the main experiment male rats were exposed orally on 2 consecutive days to 0, 500 and 1000 mg/kg bw/day. One h and 4 h (after the second application only) after each treatment and at the end of each treatment day rats were observed for mortality and clinical signs of stress. Tissues were collected 4 ± 0.5 h after the last treatment. Following exsanguination, the liver, duodenum and urinary bladder were collected. Cells for analysis were obtained with a lysis procedure at pH=10. Electrophoresis was performed at 0.7 V/cm, 300 ± 10 mA and 1°-10° C at the start of electrophoresis. Parts of these tissues were used for histopathological examination.
Cytotoxicity was studied by low molecular weight (LMW) DNA diffusion analysis; cells with extensive DNA degradation associated with cell death exhibit a highly diffuse pattern of DNA compared to the condensed pattern associated with the high molecular weight DNA. Per organ 100 nuclei were examined for Olive tail moment (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 magnified with the fraction of DNA in the tail). Relevant positive controls were included in the experiments.

Results
In the range finder test, immediately after dosing most rats showed lethargy with decreased movements. However, all animals recovered within 2 h. In the main test, identical clinical effects were observed in a number of rats.
Cytotoxicity, measured as the percentage of cells with LMW DNA, was statistically significantly and dose-dependently increased by 2-amino-5-ethylphenol phosphate treatment in the duodenum but not in the liver and urinary bladder.
A dose dependent and statistically significant increase in DNA migration (Olive tail moment) was observed in cells of the duodenum, but not in the liver and urinary bladder.
Slides from the duodenum of all treated groups and the negative control group were evaluated histopathogically. Oral administration of 2-amino-5-ethylphenol phosphate resulted in a possible treatment related microscopic finding of subacute inflammation at 1000 mg/kg/bw/day. Based on the cytotoxicity found at the same doses at which an increased migration was found, the clinical signs observed in these animals and the subacute inflammation, the increase in DNA migration is most likely due to cytotoxicity instead of genotoxicity. Consequently the increase in DNA migration in cells of the duodenum is considered not biologically relevant.

Conclusion
Under the experimental conditions used, 2-amino-5-ethylphenol phosphate did not induce a biologically relevant increase in DNA migration in cells of the liver, duodenum and urinary bladder of rats and, consequently, 2-amino-5-ethylphenol phosphate is not genotoxic in this Comet assay with rats.

Ref.: 34

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline: OECD 414

Species/strain: Wistar rat
Group size: 22 pregnant females per dose group
Test substance: 2-Amino-5-Ethylphenol hydrochloride in highly purified water
Batch: RD-CRU 079-16/97-05
Purity: 99.9%
Dose levels: 0, 22, 74, 369 mg/kg bw/day
Route: oral gavage
Dosing period: days 6 through day 20 of gestation
GLP: In compliance
Date: May 2006 – November 2006

2-Amino-5-Ethylphenol hydrochloride was administered, by gavage, to 3 groups of 20 or 21 pregnant Wistar rats; the control group received highly purified water. Administration was performed daily at dosage levels of 0, 22, 74, or 369 mg/kg bw (based on a dose-range finding study) from day 6 to 20 of gestation. All mated females were sacrificed at day 21 post coitum. The animals were observed for clinical signs and individual body weights were recorded daily. Food consumption was measured for the day-intervals 0-3, 3-6, 6-9, 9-12, 12-15, and 15-18. Immediately following sacrifice, the uterus was removed, weighed and the number of (non)viable foetuses, early and late resorptions and the number of total implantations and corpora lutea was recorded. A macroscopic examination of the organs was carried out. All foetuses were individually weighed and sexed and gross external malformations were examined. One half of the foetuses were examined for skeletal defects and variations of the ossification process by Alizarin Red staining and one half was evaluated for visceral anomalies.

Results
Females from the high dose group (369 mg/kg bw/day) showed clinical signs such as ventral recumbency and sedation which were considered related to the treatment. Transient blue extremities during 2-3 days following the start of the treatment were noted but not considered related adverse. Food consumption and body weight gains were also significantly reduced in this group.

At 369 mg/kg bw/day, there was a significant increase in pre-implantation loss and a decrease in the number of implantation sites as a percentage of corpora lutea. An increase in post-implantation loss and foetal resorption was also observed in this dose group. As a consequence, a reduced number of foetuses per dam was also observed in this dose group. The foetuses from this dose group had also a reduced body weight and an increase in visceral malformations such as enlarged thyroid and malpositioned origin of the common carotid artery or in skeletal malformations such as delayed in ossification of bones were reported. There were increased incidences of malformed bones and splits in the cartilage of the sternebrae. Enlarged thyroids and malpositioned origin of the common carotid artery occurred in this group.

At 22 and 74 mg/kg bw/day, no maternal clinical signs, feed consumption and body weight gain change of sign of toxicity were reported. No variations in pregnancy and litter data were reported in these 2 dose groups.

Conclusion
The NOEL for maternal effects and for developmental effects on foetuses were both considered to be 74 mg/kg bw/day.

Ref.: 35

Comments
Based on the clinical effects on dams and the resorption and malformations on foetuses at the dose of 369 mg/kg bw/day, the SCCS considered the NOAEL for maternal effects and for developmental effects on foetuses to be 74 mg/kg bw/day.

3.3.9. Toxicokinetics

Guideline: OECD 417 (Toxicokinetics);
OECD 427 (Skin Absorption: in vivo method);
EC 88/302/EC, Annex V, B36 "Toxicokinetics"
Species/strain: Wistar rat
Group size: 4 females per group for ADME and 6 females per group for TK
Test substance: 2-Amino-5-Ethylphenol hydrochloride
Batch: Radiolabelled 2-Amino-5-Ethylphenol hydrochloride: CFQ14698 Batch 1
Non radiolabelled 2-Amino-5-Ethylphenol hydrochloride: RD-CRU 079-16/97-05
Purity: Radiolabelled 2-Amino-5-Ethylphenol hydrochloride: 99.3%
Non Radiolabelled 2-Amino-5-Ethylphenol hydrochloride: 99.9%
Route:
Dose levels:

Group No	Dosing route	Dose Level/Concentration
1 ADME	iv	75 mg/kg bw
2 ADME	oral	370 mg/kg bw
3 ADME	dermal	16 mg/kg bw; 20 mg/mL; 0.2 mg/cm² – 0.5h
4 ADME	dermal	80 mg/kg bw; 100 mg/mL; 1 mg/cm² – 24h
5 TK	iv	75 mg/kg bw
6 TK	oral	370 mg/kg bw
7 TK	dermal	16 mg/kg bw; 20 mg/mL; 0.2 mg/cm²– 0.5h
8 TK	dermal	80 mg/kg bw; 100 mg/mL; 1 mg/cm²– 24h

Vehicle **Dermal exposure group 3 and 7**: Water Milli-Q and Off-white cream (representative of human use conditions):
Lanette O 8.4%
Dusoran MD 1%
Sodium Laurylethersulfate 12% Benz 28%
EDTA 0.12%
Sodium sulphite 0.48%
Ascorbic Acid 0.36%
Water: 71.98%
Ammonia: 5.46%
Dermal exposure group 4 and 8: Dimethylsulfoxide (DMSO to increase dermal absorption)
Intravenous injection group 1 and 5: Phosphate Buffered Saline (PBS)
Oral exposure group 2 and 6: Water Milli-Q
Dosing period: GLP: In compliance
Date: September 2006 – October 2006

Absorption, Distribution, Metabolism and Elimination (ADME) and Toxicokinetics (TK) of 2-Amino-5-Ethylphenol hydrochloride was studied in Wistar rat following single dermal, intravenous and oral exposure. ADME was studied in 4 groups of 4 female rats receiving 2-Amino-5-Ethylphenol hydrochloride as a single dose either by iv, dermal or oral routes. TK was studied in 4 groups of 6 female rats receiving as a single dose 2-Amino-5-Ethylphenol hydrochloride also either by iv, dermal or oral routes.

In the ADME groups, urine and faeces were collected at different times: 0-8, 8-24, 24-48, 48-72 and 72-96hr. Animals were euthanized 96hr after dose administration and several

tissues and organs were collected and analysed. Metabolite profile in pooled urine and faeces samples were determined.

In the TK groups blood were sampled 0.25, 0.5, 1, 2, 4, 8, 24, 48 and 72h after dosing and equivalent concentrations of Amino-5-Ethylphenol hydrochloride were determined.

Results

Group No	Dosing route	Dose Level	Absorption %	Excretion % urine/faeces	F abs %	Cmax mg/kg	AUC last
1 ADME	iv	75 mg/kg bw	100	82/11			
2 ADME	oral	370 mg/kg bw	101	83/11			
3 ADME	dermal	16 mg/kg bw	3	2/1			
4 ADME	dermal	80 mg/kg bw	63	57/4			
5 TK	iv	75 mg/kg bw			na	na	270
6 TK	oral	370 mg/kg bw			60	96.8	828
7 TK	dermal	16 mg/kg bw			2	1.04	1.04
8 TK	dermal	80 mg/kg bw			56	39.8	173

The average total radioactivity in all dose groups was between 94 and 101% of the applied dose.

The oral absorption of Amino-5-Ethylphenol hydrochloride is high and fast. The dermal absorption is strongly dependent on the vehicle, the concentration dose and the duration of exposure. Dermal absorption was also fast with a Tmax of 0.5 hours. Large inter-individual variations were observed.

Amino-5-Ethylphenol hydrochloride is mainly excreted by urine whatever the route of exposure. No major accumulation of radioactivity was observed after 96 hours.

Blood concentrations in all groups were around 10 times higher than in plasma concentrations, indicating distribution of the test substance into the red blood cells.

3 potential metabolites were detected in plasma, among them 2 were identified and 7 metabolites were detected in urine. Sulfation, glucuronidation and acetylation were the major metabolic pathways whereas cysteine and glucose conjugation, carboxylation and hydroxylation were of minor importance.

Conclusion
Amino-5-Ethylphenol hydrochloride administered orally was extensively absorbed, largely distributed and extensively metabolized and excreted in urine. No major qualitative differences in the metabolite profile between the oral and dermal routes of administration were observed.

The NOAEL derived from the 90 days oral toxicity study can be used to calculate the MoS without any adjustment.

Ref.: 36

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

INCI Name

Oxidative / Non oxidative conditions

Absorption through the skin	**A**	**=**	**2.70 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.56 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.026 mg/kg bw/d**
No Observed Adverse Effect Level (90 day study, oral route, rat)	**NOAEL**	**=**	**16 mg/kg bw/d**

MOS	**=**	**614**

3.3.14. Discussion

Physico-chemical properties
2-Amino-5-ethylphenol hydrochloride is used as an oxidative hair colouring agent pre-cursor. The intended maximum on-head concentration is 1.0% in oxidative hair dye formulations.
2-Amino-5-ethylphenol hydrochloride is used in hair colouring formulations, but it has no EC number. A study report on the chemical characterisation of a batch of 2-amino-5-ethanol HCl was submitted. But no documentation was provided on chemical characterisation of 4 other reported batches of 2-amino-5-ethanol HCl/phosphates. Stability of 2-amino-5-ethanol HCl in typical hair dye formulations is not reported.

Irritation, sensitisation
2-Amino-5-ethylphenol hydrochloride is considered as non-irritant to the skin when tested at 2% and 10% in water. 2-Amino-5-ethylphenol hydrochloride, as a neat substance, was considered to cause skin corrosion
2-Amino-5-ethylphenol hydrochloride tested as a neat substance was identified as severely irritating to eyes.
2-Amino-5-ethylphenol hydrochloride is considered as a moderate skin sensitiser.

Dermal absorption
Under the assumption that a depot effect is absent, a maximum amount of 1.935 + 0.762= 2.70 µg/cm² of 2-amino-5-ethylphenol hydrochloride is considered as biologically available.

General toxicity
The acute toxicity of 2-amino-5-ethylphenol phosphate is low after single oral administration to female rats. The LD50 of 2-amino-5-ethylphenol phosphate is greater than 2000 mg/kg bw, corresponding to 1476 mg/kg bw as hydrochloride salt.
In a subchronic toxicity study in rats signs of slight anemia were observed at 55 mg/kg bw/day. As no test item-related effects were observed at the dose of 16 mg/kg bw/day, this dose was considered as the NOAEL (2-amino-5-ethylphenol HCl).
Based on the clinical effects on dams and the resorption and malformations on foetuses at the dose of 369 mg/kg bw/day, the SCCS considered the NOAEL for maternal effects and for developmental effects on foetuses were both considered to be 74 mg/kg bw/day.

Amino-5-Ethylphenol hydrochloride administered orally was extensively absorbed, largely distributed and extensively metabolized and excreted in urine. No major qualitative differences in the metabolite profile between the oral and dermal routes of administration were observed.
The NOAEL derived from the 90 days oral toxicity study can be used to calculate the MoS without any adjustment.
No further reproductive toxicity studies were provided.

Mutagenicity
Overall, the genotoxicity of 2-amino-5-ethylphenol phosphate is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy.
2-amino-5-ethylphenol phosphate did not induce gene mutations in a gene mutation test in bacteria nor in a mouse lymphoma assay in mammalian cells. However in an *in vitro* micronucleus test an increase of cells with micronuclei was observed.
The positive findings from the *in vitro* tests were not confirmed in *in vivo* tests. In an *in vivo* micronucleus test, 2-amino-5-ethylphenol phosphate exposure did not result in an increase in bone marrow cells with micronuclei and in a Comet assay an increase in DNA migration in cells of the liver, duodenum and urinary bladder was not observed.
Consequently, on the basis of these tests, 2-amino-5-ethylphenol phosphate can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of 2-amino-5-ethylphenol HCl in oxidative hair dye formulations at a maximum on-head concentration of 1.0% does not pose a risk to the health of the consumer, apart from its sensitisation potential.

5. MINORITY OPINION

Not applicable

6. REFERENCES

Submission I, 2008

1. Damkröger, G.; Identity and purity test of Ethyloxygelb-Chlorid (A019547; WR 803665); WELLA Service GmbH; 21.12.2006
2. Geffke, T.; Partition coefficient (n-octanol / water) using HPLC-method; NOACK; 2006
3. Lange, J.; Sieve analysis; NOACK; 2006
4. Lange, J.; Water solubility (Flask method); NOACK; 2006
5. Lange, J.; Melting point / melting range; NOACK; 2006
6. Möller, M.; Boiling point A.2. (OECD 103); SIEMENS AG; 2006
7. Möller, M.; Vapour pressure A.4. (OECD 104); SIEMENS AG; 2006
8. Lange, J.; Surface tension; NOACK; 2006
9. Lange, J.; Flammability of solids; NOACK; 2006
10. Möller, M.; Explosive properties A.14.; SIEMENS AG; 2006
11. Möller, M.; Auto-flammability (Solids - determination of relative self-ignition temperature) A.16.; SIEMENS AG; 2006
12. Geffke, Th.; Oxidising properties of solids; NOACK; 19.07.2006
13. Arcelin, G.; 2-Amino-5-ethylphenol phosphate (WR 802433): Acute oral toxicity study in rats; RCC; 2006
14. Tenberken-Poetzsch, B.; Analysis report 2007/1784; WELLA AG; 2007
15. Warren, N.; Transcutaneous electrical resistance assay with 2-Amino-5-Ethylphenol Hydrochloride (WR 803665); SAFEPHARM; 2006
16. Warren, N.; 2-Amino-5-ethylphenol hydrochloride (WR 803665): Skin irritation assessment in vitro using the SkinEthic reconstituted human epidermal model; SAFEPHARM; 2006
17. Kandárová, H.; Liebsch, M.; Schmidt, E.; Genschow, E.; Traue, D.; Spielmann, H.; Meyer, K.; Steinhoff, C.; Tornier, C.; De Wever, B.; Rosdy, M.; Assessment of the skin irritation potential of chemicals by using the SkinEthic reconstructed human epidermal model and the common skin irritation protocol evaluated in the ECVAM skin irritation validation study. ALTERN. LAB. ANIM. 34: 393-406; 2006.
18. Faller, C.; Aeby, P.; Goebel, C.; in vitro Assessment of Skin Irritation Potential of Hair Dyes using a Reconstituted Human Epidermis (RHE) Model. 6th World Congress on Alternatives & Animal Use in the Life Sciences: P2-2023, 225; Tokio, August 21-25, 2007.
19. Prinsen, M. K.; Chicken enucleated eye test with 2-Amino-5-ethylphenol hydrochloride (WR 803665); an ex vivo alternative to the Draize eye irritation test with albino rabbits; TNO; 2006
20. Prinsen, M. K.; Koeter, H.B.W.M; Justification of the Enucleated Eye Test with Eyes of Slaughterhouse Animals as an Alternative to the Draize Eye Irritation Test with Rabbits; FD Chem. Toxic.: Vol. 31., No. 1., 69-76, 1993.
21. Prinsen, M. K.; The Chicken Enucleated Eye Test (CEET): A practical (Pre)Screen for the Assessment of Eye Irritation/Corrosion Potential of Test Materials; FD Chem. Toxic.: Vol. 34., No. 3., 291-296, 1996.
22. Warbrick, E. V.; Dearman, R. J.; Lea, L. J.; Basketter, D. A.; Kimber, I.; Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations; J. APPL. TOXICOL.; 19, 255-260; 1999
23. Ravel, G.; 2-Amino-5-ethylphenol phosphate WR 802433 - Local lymph node assay; MDS PHARMA SERVICES; 2005
24. Contact sensitisation: Classification according to potency; ECETOC; 1-29; 2003

25. Goettel, O.; Conversion factor from 2-Amino-5-ethyl-phenol-chloride; COSMITAL SA; 2006
26. Sieber, T. P.; Cutaneous absorption of 1% 2-Amino-5-ethylphenol hydrochloride (WR 803665) in a typical hair dye formulation in the presence of hydrogen peroxide and reaction partner 1-Hydroxyethyl 4,5-Diamino pyrazol sulfate (A154, (WR18247) through pig skin in vitro; COSMITAL SA; 2006
27. Braun, W. H.; 2-Amino-5-Ethylphenol Hydrochloride (WR 803665): 13-week oral (gavage) toxicity study in Wistar rats; RCC; 2008
28. Sokolowski, A.; Salmonella Typhimurium reverse mutation assay with Ethyl-Oxygelb-Phosphat (WR 802433); RCC-CCR; 2005
29. Wollny, H.-E.; Cell mutation assay at the thymidine kinase locus (TK +/-) in mouse lymphoma L5178Y cells with Ethyl-Oxygelb-Phosphat (WR 802433); RCC-CCR; 2005
30. Whitwell, J.; 2-Amino-5-ethylphenol phosphate (WR 802433): Induction of micronuclei in cultured human peripheral blood lymphocytes; COVANCE; 2004
31. Honarvar, N.; Micronucleus assay in bone marrow cells of the mouse with Ethyl-Oxygelb-Phosphat (WR 802433); RCC-CCR; 2004
32. Hartmann, A.; Agurell, E.; Beevers, C.; Brendler-Schwaab, S.; Burlinson, B.; Clay, P.; Collins, A.; Smith, A.; Speit, G.; Thybaud, V.; Tice, R. R.; Recommendations for conducting the in vivo alkaline comet assay; MUTAGENESIS; 18, 45-51; 2003
33. Sasaki, Y. F.; Sekihashi, K.; Izumiyama, F.; Nishidate, E.; Saga, A.; Ishida, K.; Tsuda, S.; The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database; CRIT. REV. TOXICOL.; 30, 629-799; 2000
34. Vasquez, M.; 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); HELIX3 INC.; 2006
35. Whitlow, S.; Flade, D.; 2-Amino-5-ethylphenol hydrochloride (WR 803665): Prenatal developmental toxicity study in the Han Wistar rat; RCC; 2007
36. Wenker, M.A.M.; Absorption, distribution, metabolism and excretion of 2-Amino-5-ethylphenol hydrochloride (WR 803665) in the Wistar rat; NOTOX; 2007
37. König, P.; Data base search for references for Trade name: Ethyl-oxygelb-phosphat (WR 802433), and Trade name: Ethyloxygelb-chloride(A019547) (WR 803665); WELLA SERVICE GMBH; 2006