SCCS/1400/11

Scientific Committee on Consumer Safety

SCCS

OPINION ON

6-Amino-m-cresol (INCI)
2-Amino-5-methylphenol

COLIPA n° A75

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 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.).

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1. BACKGROUND

Submission I, II and III for 6-amino-m-cresol was submitted by COLIPA[1] in February 1989, in March 1993 and in April 1995 respectively.

The Scientific Committee on Cosmetology (SCC) adopted, at its 46th plenary meeting of 19 February 1991, an opinion with the conclusion, that "Since several studies have shown that this compound has produced positive results in "in vitro" mutagenicity studies, the SCC requires a study for the in vivo induction of UDS."

The SCC confirmed this opinion at the 54th plenary meeting of 10 December 1993.
According to the submission IV, submitted by COLIPA in July 2005, 6-amino-m-cresol is used as a precursor in oxidative hair dyeing products with of maximum concentration on the scalp of 1.5%.

The purpose of the present submission V, submitted by COLIPA in October 2011, is to describe the results of additional studies which provide further perspective on the question of *in vivo* genotoxicity potential of *6-AMINO-m-CRESOL* under conditions relevant to hair dye use. In this submission, additional *in vitro* studies evaluating the dermal and hepatic metabolism of *6-AMINO-m-CRESOL* have been conducted.

2. TERMS OF REFERENCE

Does the Scientific Committee on Consumer Safety (SCCS) consider 6-amino-m-cresol safe for consumers, when used in oxidative hair dye formulations with a concentration on the scalp of maximum 1.5% taking into account the scientific data provided?

[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

6-Amino-m-cresol (INCI name)

Comment
The INCI name is scientifically incorrect. Therefore 2-Amino-5-methylphenol has been used in the whole Opinion

3.1.1.2. Chemical names

6-Amino-3-cresol *	5-Methyl-2-aminophenol
4-Amino-3-hydroxytoluene	2-Amino-5-methylphenol
2-Hydroxy-4-methylaniline	Phenol, 2-amino-5-methyl (CA Index Name, 9CI)
2-Hydroxy-p-toluidine	6-Amino-3-methylphenol (IUPAC)
1-hydroxy-2-amino-5-methylbenzene	

* Scientifically not correct

3.1.1.3. Trade names and abbreviations

Oxygelb
Oxy-Gelb
COLIPA n° A75

3.1.1.4. CAS / EC number

CAS: 2835-98-5
EC: 220-620-7

3.1.1.5. Structural formula

3.1.1.6. Empirical formula

Formula: C_7H_9NO

3.1.2. Physical form

Beige to Red-Brown powder	Summary Submission IV
Beige-Yellow crystalline powder	Ref. 19

3.1.3. Molecular weight

Molecular weight: 123.16 g/mol

3.1.4. Purity, composition and substance codes

Chemical characterisation was performed by NMR.
Purity and impurities in various batches of 6-amino-m-phenol analysed

Sample	95290T001, barrel 9	EFH 010394	LGH 110583/3 barrel II/4 (Fab II/4)	99290T0002 (R99052643)
NMR Content, % w/w	99.5	99.1	99.7	98.9
HPLC purity, area %				
254 nm 290 nm	99.6 99.9	/ 100	/ 100	98.8 98.5 (295nm)
Water content, % w/w	0.02	0.017	0.057	0.1
Sulphated ash, % w/w	<0.1	0.01	0.01	<0.01
2-Amino-4-methylphenol ppm	<1000*	<1000*	<1000*	<1000*
1,2-Diamino-4-methylbenzene, ppm	<20*	<20*	<20*	<59*
4-Methyl-2-nitroaniline, ppm	<10*	<10*	<10*	<23*

*: below detection limit, indicated value is the detection limit
°°: in all batches no significant impurities detected

Comment
Detection limit of 2-amino-4-methylphenol (1000 ppm) is too high

3.1.5. Impurities / accompanying contaminants

See point 3.1.4 on purity, composition and substance codes

3.1.6. Solubility

Water:	5.9 g/L (20°C, pH 7.65)	(Ref. 5)
	4 g/L (pH 7.2)	(Ref. 19)
Acetonitrile:	34 g/L	(Ref. 19)
DMSO:	>100 g/L	(Ref. 19)
Acetone/water (1:1):	33 g/L	(Ref. 19)

Comment
References 5 and 19 state the above results, but methods of analysis and experimental details are not described. It is not known whether EC Method A.6 was used for the determination of water solubility.

3.1.7. Partition coefficient (Log P_{ow})

P_{ow}: 13.8 (calculated)
Log P_{ow}: 1.14 (calculated)

Comment
Log P_{ow} has not been determined by EC Method A.8

3.1.8. Additional physical and chemical specifications

Melting point: 156-159 °C (sublimation) Ref. 4
Boiling point: Not applicable

Flash point:	/
Vapour pressure:	4.1 exp-3 hPa (25 °C) (calculated with MPBPWIN VI.41)
Density:	0.77 g/ml (20 °C) Ref. 4
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm):	

3.1.9. Homogeneity and Stability

The substance is reported to be stable for more than 8 years, if stored dry and protected from light at room temperature.

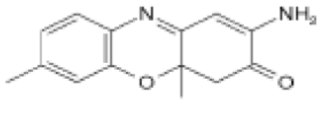
The stability of 2-amino-5-methylphenol in various solutions/suspensions was monitored by HPLC. The solutions were stored at ambient temperature in the absence of light for a period up to 7 days.

DMSO (Approx. 10%):	stable up to 7days, maximum variation < 2%
Acetone/water 1:1 (approx.2.8%):	stable up to 7days, maximum variation 7%
Water (approx. 0.13%) pH7.2	100% at t=0h, 98.2% after 6h, 88.7% after 2d, 49.5% after 7d
PEG400 (approx. 5% solution, pH 8.0):	stable up to 7days, maximum variation< 2%

Ref.: 19

General Comments to physico-chemical characterisation

- Water solubility of 2-Amino-5-methylphenol was described as 5.9 g/L and 4.2 g/L in two different references. The method of determination of water solubility was not described. Apparently EC Method A.6 was not used for the determination of water solubility.
- The Log P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.
- Stability of 2-Amino-5-methylphenol in typical hair dye formulations was not reported
- INCI name is scientifically not correct.
- A product with the trade name Oxygelb Dimer ($C_{14}H_{14}N_2O_2$, molecular weight 242, systematic name 2-amino-4a,7-dimethyl-4,4a-dihydro-3H-phenoxazin-3-one) is formed from 2-amino-5-methylphenol by auto-oxidation and dimerization (Ref. 3, subm V)



3.2. Function and uses

2-amino-5-methylphenol is used as precursor of oxidative hair dye formulations, which are mixed with hydrogen peroxide developer in a ratio 1:1 or 1:3 before application. The maximum on-head concentration of 2-amino-5-methylphenol from the hair dye application is 1.5%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: rat, Wistar
Mouse, CF and CBL
Group size: rat: 6 males and 10 females
Mice, CF1: 10 males and 10 females
Mice, CBL: 10 females
Test substance: 1-hydroxy-2-amino-5-methylbenzene, 10% in aqua dest.
Batch: /
Purity: /
Vehicle: aqua dest.
Dose: Rat, male: 1000, 1250, 1500, 1750 mg/kg bw
Rat, female: 750, 1000, 1250, 1500 mg/kg bw
Mice CF1, male: 600, 900, 1200, 1500 mg/kg bw
Mice CF, female: 750, 1000, 1250, 1500, 1750, 2000 mg/kg bw
Mice CBL, female: 500, 750, 1000, 1250 mg/kg bw
Route: oral, gavage
GLP statement: /
Study period: 1984

The test substance was dissolved in water and administered orally by gavage to groups of male and female rats and mice. Mortality and clinical signs were checked daily during the 14-day observation period. All animals were submitted to a gross necroscopy after termination.

Results
Clinical signs observed were sedation, tremor, accelerated respiration and exitus. No macroscopic organ changes were noted. The LD_{50} figures were calculated as follows:

Species (sex)	**LD_{50}**
Rats (female)	1225 mg/kg bw
Rats (male)	1375 mg/kg bw
CF1 mice (female)	1225 mg/kg bw
CF1 mice (male)	1020 mg/kg bw
CBL mice (female)	750 mg/kg bw

Ref.: 7

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

Taken from SCC-opinion 1993

Guideline:	/
Species/strain:	Albino Guinea pig (SPF)
Group size:	10 females
Test substance:	1-hydroxy-2-amino-5-methylbenzene
Batch:	/
Purity:	/
Vehicle:	water, thickened with methylcellulose
Dose level:	1% in water, thickened with methylcellulose
Dose volume:	/
GLP:	/
Study period:	1982

The compound as a 1% aqueous solution (thickened with methyl cellulose) was applied on abraded skin area (3x4 cm, washed out after 20 min.) of albino guinea pigs 3 times daily on two consecutive days. A negligible erythema on the first day, not recognizable (only skin area stained) on the second day, was observed; no oedemas and crusts were revealed, during further observation.

Ref.: 8

Comment
The study did not follow a guideline and is not in compliance with GLP.

3.3.2.2. Mucous membrane irritation

Taken from SCC-opinion 1993

Guideline:	/
Species/strain:	Guinea pig, Pirbright SPF
Group size:	10 females
Test substance:	1-hydroxy-2-amino-5-methylbenzene
Batch:	/
Purity:	/
Vehicle:	aqua dest.
Dose level:	1% in aqua dest., pH 4
Dose volume:	0.1 ml
GLP:	/
Study period:	1982

The compound as 1% aqueous solution instilled into one eye (0.1 ml) of 10 female Pirbright white guinea pigs, resulted not irritating after 24 hours observation period (eye reactions evaluated at 0.5, 1, 2, 3, 4, 6, 7 and 24 hours).

Ref.: 9

Comment
The study did not follow a guideline and is not in compliance with GLP.

3.3.3. Skin sensitisation

Taken from SCC-opinion 1993

Guideline:	/
Species/strain:	albino Guinea pig, Pirbright, SPF
Group size:	25 females (15 test, 10 control)
Test substance:	1-hydroxy-2-amino-5-methylbenzene
Batch:	/
Purity:	/
Vehicle:	water

Concentration: 3% suspended in water, thickened with tylose (0.5%)
GLP: /
Study period: 1985

Sensitisation was tested in 15 females Pirbright white Guinea pig treated with 3% in aqueous test suspension of test compound applied epicutaneously without occlusion on abraded flanks, once a day on 5 days/week for 3 weeks, using the method of Magnusson and Kligman. The compound did not show any erythema or oedema 24, 48 and 72 hours after challenge reaction.

Ref.: 4 (subm. I)

Submission IV, 2005

Local Lymph Node Assay (LLNA)

Guideline: OECD 429 (2002)
Species/strain: mouse CBA/J
Group size: 55 females (5 animals/treatment)
Test substance: 6-amino-m-cresol (2-amino-5-methylphenol)
Batch: 99290T0002
Purity: 98.9 area% (HPLC)
Vehicle: vehicle 1: DMSO
vehicle 2: Acetone:water (1:1) mixed with olive oil (3:1)
Concentration: vehicle 1: 0.5, 1.5, 5.0 and 10%
vehicle 2: 0.5, 1.5, 3.0 and 5.0%
Positive control: p-phenylenediamine (PPD), 1% in DMSO
GLP: in compliance
Study period: 7 – 30 November 2004

On days 0, 1 and 2, the animals received 25 μl of the test item formulations, positive control or vehicle on the dorsal surface of each ear. All mice received an intravenous injection of 250 μl of phosphate buffer saline containing 23.6 μCi of ^{3}H-methyl thymidine. Five hours later, the animals were sacrificed and the draining auricular node taken and weighed. Cells were washed twice with PBS and precipitated with ice cold 5% trichloro-acetic acid (TCA), and the pellets were re-suspended in 1 ml TCA and transferred to scintillation cocktail. ^{3}H-methyl thymidine incorporation was measured by liquid scintillation counting.

Results

Treatment	Concentration	Stimulation index
6-amino-m-cresol in DMSO	0.5%	1.3
	1.5%	1.5
	5%	4.2
	10%	8.0
6-amino-m-cresol in acetone/water mixed with olive oil	0.5%	0.9
	1.5%	2.0
	5%	29.3
	10%	33.9
PPD in DMSO	1%	12.5

Results
EC3 values were 3.44% and 1.55% in DMSO and acetone/water (1:1) mixed with olive oil (75%/25%), respectively.

Conclusions
2-amino-5-methylphenol is a strong skin sensitiser under the defined experimental conditions.

Ref.: 11

3.3.4. Dermal / percutaneous absorption

Guideline:	/
Species/strain:	pigmented rat, PVG-strain
Group size:	12 (6 males and 6 females)
Test substance:	[^{14}C]-2-amino-5-methyl phenol hemisulphate
Batch:	/ (non-radio-labelled) c/6459, 58 µCi/mg (radio-labelled)
Purity:	/ (non-radio-labelled) 95% (radiochemical)
Test item:	A: Hair dye formulation containing 15 mg/g test substance B: Test substance dissolved in DMSO (150 mg/ml)
Dose volume:	15 mg/ animal (1.667 mg/cm²)
Method of Analysis:	Liquid scintillation counting
GLP:	/
Study period:	1985

[^{14}C]-2-Amino-5-methylphenol hemisulfate (radiochemical purity 96%) in DMSO (150 mg/ml, 0.1 ml/animal for 24h) and as ingredient of hair dye products (15 mg/g, 1 g mixture/animal for 0.5h) was applied on dorso lumbar region of PVG rats under occlusion (15 mg/animal, 1.667 mg/cm², 190 mCi) At the end of these periods, the plaster and the foil were removed. Animals were kept in individual metabolism cages for collecting urine, faeces and expired air. At 72 hr post-dose animals were killed and tissues removed for radioactivity analysis.

Results
After 72 h, 0.58% (0.41% urine, 0.09% faeces, 0.15% expired air and 0.02% cage washing) of the applied dose of the hair dye product and 14.25% (12.83% urine, 0.82% faeces and 0.60% cages washing) of the solutions in DMSO were excreted. The 82.78% of the applied dose of the hair dye product and 74.48% of the applied dose in DMSO solution were recovered from dressing, washing and application sites. No significant radioactivity levels were found in tissues at 72h after their treatment.

Conclusion
(14C)-2-amino-5-metyl-phenol hemisulfate is absorbed through the intact skin of the rat dissolved in DMSO, but when applied in a hair dye formulation, negligible absorption occurs.

Ref.: 12

Comment of the SCCS
0.58% of the applied dose of 1.667 µg/cm^2 was bioavailable.

The study is not adequate because there are several shortcomings. The study is prior to the OECD 427 guideline (2004). Nevertheless, the number of animals is adequate, the amount applied is not. A detailed review of the data shows that in the methods says that 1 g of the formulation was applied but in the appendix with the individual animals the amount applied seems to be about 0.45 g (each animal different). The methods part says that the reference solution was applied directly to the shaved skin with an automatic pipette (0.1ml) but in the appendix the amount applied is from 0.104 mg to 0.116 mg (not volume). A new study according to the actual standards is required.

New study, submission 2011

Guideline:	OECD 428
Tissue:	human skin (1 breast, 2 abdomen from 3 females) thickness 580-650 µm

Membrane integrity:	/
Diffusion cell:	6-well plate on a Netwell insert, static system (Internal area 0.64 cm^2)
No. of chambers:	12 from 3 donors
Test substance:	[^{14}C]-2-amino-5-methylphenol
Batch:	992990T0002 (non-radio-labelled) CFQ40717 (radio-labelled)
Purity:	99.5% HPLC, 98.1% by NMR (non-radio-labelled) 99% by HPLC (radiochemical)
Test item:	Oxidative hair dye formulation containing 1.5% 2-amino-5-methylphenol
Area dose:	100 mg/cm^2)
Time period:	60 min (3 and 24 hours)
Receptor fluid:	Dulbecco's minimum eagle medium (DMEM) pH 7.21-7.37
Solubility in receptor:	/
Stability:	/
Method of Analysis:	Liquid scintillation counting
GLP:	in compliance
Study period:	December 2009

The dermal penetration of 2-amino-5-methylphenol from a typical oxidative hair dye formulation was investigated in viable human skin obtained from three female donors. The hair dye formulation containing 1.5% 2-amino-5-methylphenol plus reaction partner and hydrogen peroxide was applied to fresh split-thickness skin samples for 60 min (final concentration 0.75%).
Absorption was assessed by collecting receptor fluid samples at 3 h and 24 h post dose. At 24 h post dose, the experiment was terminated by removing the skin sample from the 6-well plate. The well was rinsed with solvent. The skin was then dried and the stratum corneum removed with 15 successive tape strips. The remaining skin was divided into exposed and unexposed skin. The exposed skin sample underwent an extraction procedure. The exposed skin extract was then dried under nitrogen gas and mobile phase was added to the residue. A sub-sample was collected for radioactive measurement and the remainder of the sample was analysed by radio-HPLC analysis. The exposed skin pellet and the unexposed skin were dissolved in Solvable®. The sample for radioactivity measurement were analysed by liquid scintillation counting. Receptor fluid and exposed skin samples were analysed by LC-MSn for metabolite identification and profiling (see section 3.3.9).

Results

Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [^{14}C]-2-amino-5-methylphenol in Test Preparation (0.75%, w/w) to Human Split Thickness Skin

	Cell Number and Donor Number													
	Cell 1 0315	Cell 2 0315	Cell 3 0315	Cell 4 0315	Cell 7 0316	Cell 8 0316	Cell 9 0316	Cell 10 0316	Cell 13 0320	Cell 14 0320	Cell 15 0320	Cell 16 0320	Mean	SD
Skin Wash 60 min	134.05	115.21	132.80	128.00	114.96	119.88	112.04	130.62	114.71	109.46	126.37	106.11	120.35	9.63
Tissue Swab 60 min	0.40	0.60	0.22	4.45	0.15	3.26	0.36	9.76	1.64	2.24	1.53	1.50	2.17	2.73
Pipette Tips 60 min	0.13	0.07	0.23	0.04	0.06	0.07	0.06	0.10	0.02	0.06	0.20	0.03	0.09	0.07
Dislodgeable Dose 60 min	134.57	115.88	133.25	132.49	115.17	123.21	112.47	140.48	116.37	111.76	128.10	107.64	122.62	10.82
Tissue Swab 24 h	0.00	0.01	0.00	0.01	0.00	0.01	0.04	0.01	0.10	0.00	0.01	0.01	0.02	0.03
Donor Chamber Wash	0.28	0.12	0.10	0.15	0.11	0.29	0.07	0.75	0.60	0.24	0.22	2.59	0.46	0.70
Total Dislodgeable Dose	134.86	116.00	133.36	132.64	115.28	123.51	112.57	141.24	117.08	112.01	128.32	110.24	123.09	10.61
Unexposed Skin	0.01	0.03	0.03	0.03	0.00	0.00	0.14	0.01	0.08	0.01	0.01	0.03	0.03	0.04
Stratum Corneum 1-5	0.03	0.07	0.16	0.14	0.08	0.29	0.19	0.26	0.32	0.09	0.40	0.30	0.19	0.12
Stratum Corneum 6-10	0.04	0.01	0.01	0.02	0.05	0.04	0.04	0.05	0.17	0.04	0.33	0.05	0.07	0.09
Stratum Corneum 11-15	0.04	0.01	0.01	0.00	0.02	0.02	0.01	0.03	0.15	0.01	0.01	0.01	0.03	0.04
Stratum Corneum	0.10	0.10	0.18	0.16	0.15	0.36	0.24	0.34	0.64	0.14	0.74	0.37	0.29	0.21
Total Unabsorbed	134.97	116.12	133.56	132.83	115.43	123.87	112.95	141.59	117.80	112.15	129.07	110.64	123.42	10.60
Exposed Skin	0.03	0.26	0.07	0.05	0.06	0.10	0.24	0.11	0.20	0.04	0.04	0.04	0.10	0.08
Receptor Fluid	0.05	0.11	0.12	0.13	0.06	0.17	0.97	0.21	0.45	0.05	0.06	0.13	0.21	0.26
Receptor Chamber Wash	0.00	0.01	0.01	0.01	0.00	0.02	0.29	0.01	0.01	0.00	0.00	0.00	0.03	0.08
Total Absorbed	0.05	0.11	0.13	0.14	0.07	0.19	1.27	0.22	0.46	0.05	0.06	0.14	0.24	0.34
Dermal Delivery	0.08	0.37	0.20	0.19	0.13	0.29	1.51	0.33	0.66	0.09	0.10	0.18	0.34	0.40
Mass Balance	135.05	116.49	133.76	133.02	115.56	124.16	114.46	141.92	118.46	112.24	129.17	110.82	123.76	10.48

Distribution of Radioactivity (μg equiv/cm²) at 24 h Post Dose Following Topical Application of [^{14}C]-2-amino-5-methylphenol in Test Preparation (0.75%, w/w) to Human Split Thickness Skin

	Cell Number and Donor Number													
	Cell 1 0315	Cell 2 0315	Cell 3 0315	Cell 4 0315	Cell 7 0316	Cell 8 0316	Cell 9 0316	Cell 10 0316	Cell 13 0320	Cell 14 0320	Cell 15 0320	Cell 16 0320	Mean	SD
Skin Wash 60 min	1028.09	883.57	1018.55	981.67	853.87	890.41	832.16	970.18	1122.63	1071.26	1236.74	1038.45	993.97	118.57
Tissue Swab 60 min	3.06	4.58	1.67	34.14	1.09	24.18	2.69	72.48	16.05	21.94	14.93	14.69	17.63	20.22
Pipette Tips 60 min	0.97	0.57	1.78	0.30	0.46	0.52	0.47	0.72	0.17	0.57	1.96	0.30	0.73	0.57
Dislodgeable Dose 60 min	1032.12	888.72	1021.99	1016.11	855.41	915.11	835.32	1043.38	1138.85	1093.76	1253.63	1053.45	1012.32	122.37
Tissue Swab 24 h	0.02	0.05	0.04	0.05	0.03	0.05	0.29	0.05	0.99	0.03	0.05	0.11	0.15	0.28
Donor Chamber Wash	2.17	0.89	0.77	1.15	0.82	2.18	0.52	5.60	5.90	2.38	2.16	25.31	4.15	6.90
Total Dislodgeable Dose	1034.30	889.66	1022.80	1017.31	856.26	917.34	836.13	1049.03	1145.75	1096.17	1255.85	1078.87	1016.62	124.23
Unexposed Skin	0.07	0.20	0.22	0.20	0.01	0.03	1.05	0.06	0.80	0.07	0.07	0.32	0.26	0.33
Stratum Corneum 1-5	0.21	0.57	1.22	1.07	0.58	2.18	1.39	1.96	3.15	0.86	3.89	2.98	1.67	1.17
Stratum Corneum 6-10	0.28	0.11	0.09	0.15	0.38	0.29	0.27	0.38	1.62	0.35	3.26	0.53	0.64	0.92
Stratum Corneum 11-15	0.27	0.05	0.05	0.02	0.11	0.17	0.09	0.21	1.47	0.12	0.10	0.13	0.23	0.39
Stratum Corneum	0.77	0.73	1.36	1.24	1.08	2.64	1.76	2.55	6.24	1.33	7.25	3.63	2.55	2.15
Total Unabsorbed	1035.14	890.59	1024.38	1018.75	857.34	920.01	838.94	1051.64	1152.80	1097.57	1263.16	1082.82	1019.43	125.76
Exposed Skin	0.26	1.97	0.52	0.41	0.44	0.76	1.81	0.80	1.95	0.36	0.39	0.40	0.84	0.67
Receptor Fluid	0.35	0.83	0.91	0.97	0.47	1.30	7.23	1.56	4.40	0.46	0.57	1.29	1.69	2.05
Receptor Chamber Wash	0.04	0.04	0.08	0.08	0.03	0.12	2.18	0.07	0.14	0.03	0.02	0.05	0.24	0.61
Total Absorbed	0.39	0.87	0.99	1.04	0.50	1.42	9.41	1.63	4.55	0.49	0.59	1.34	1.93	2.61
Dermal Delivery	0.65	2.84	1.51	1.45	0.94	2.18	11.22	2.43	6.50	0.85	0.98	1.74	2.77	3.09
Mass Balance	1035.78	893.43	1025.89	1020.20	858.28	922.19	850.16	1054.07	1159.30	1098.42	1264.14	1084.56	1022.20	124.79

The dermal delivery was 0.34 ± 0.40% (2.77 ± 3.09 μg/cm^2) of the applied dose.

The concentration of 0.75% used in this study is half the maximum concentration of use but the applicant justified this based on a time of exposure of 60 min, double of the typical time period of on head exposure.

Ref.: 3 (subm V)

Comment

The integrity of the membrane has not been reported. The dose applied is too high. The variability in dermal delivery is too large, i.e. SD is higher than the mean.

Mean + 2SD (2.77 + 2 x 3.09 = 8.95 μg/cm²) should be considered as dermal delivery. As the concentration of 2-amino-5-methylphenol in the test formulation is half of the maximum use concentration, dermal absorption of 2 x 8.95 = 17.90 μg/cm² should be used to calculate MoS.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Guideline:	/
Species/strain:	rat, Wistar BOR:WisW (SPF/TNO)
Group size:	120 (4 groups of 15 males and 15 females)
Test substance:	Oxygelb
Batch:	23080
Purity:	/
Vehicle:	0.5% carboxymethylcellulose
Dose levels:	0, 50, 250, 500 mg/kg bw/d
Dose volume:	1 ml/100 g of bw
Route:	oral
Administration:	gavage
GLP:	in compliance
Study period:	1985

Oxygelb as 0.5% in carboxymethylcellulose, was administered orally by stomach tube at doses of 0, 50, 250 and 500 mg/kg bw/d to 15 males and 15 females rats per dose (1 ml/100 g bw) for 4 weeks. The animals were checked daily for mortality and clinical signs. A functional observation battery was conducted in all animals before the first exposure and in week 4 in 5 animals per sex and dose. An ophthalmological and an auditory function investigation were carried out in 10 animals per sex and dose before treatment and in week 4. Body weight, water and food consumption were measured weekly. Urinalysis was performed prior to first treatment and in week 4. Blood sampling and clinical biochemistry were done prior to the first treatment and on the day of termination. All animals were subjected to necroscopy. A number of organs were weighed and selected tissues were stored for further analysis. Some organs of 10 animals of both sexes of the control and high dose animals were examined histopathologically.

Results

<u>500 mg/kg bw/d</u>

A moderate reduced activity during the 1st treatment week and later a moderate increased activity for 10 minutes post treatment were observed. Reduced body weight gain was observed during week 1 and 2 in females. In addition, a significant increase in water consumption and an increased urine excretion (yellow-orange discoloured) was noted. Some significant alterations of haematology and clinical chemistry values were recorded: reduction in erythrocytes, haemoglobin, haematocrit and iron in males and females; increase in reticulocytes in males and females and MCV and prothrombin time in females.

At autopsy significant increases in liver, kidney and spleen weights were found and dark discoloured spleens.

<u>250 mg/kg bw/d</u>

A slightly increased activity for 10 minutes post treatment was noted during the 3rd and 4th week. In addition, water consumption as well as urine excretion (yellow-orange discoloured) were increased. Significant alterations of haematology and clinical chemistry values were observed (reduction in erythrocytes and haemoglobin in males and females and iron in females; increase in reticulocytes and haematocrit in males and females). At autopsy increases in liver, kidney and spleen weights were found.

<u>50 mg/kg bw/d</u>

No mentionable findings were reported.

No significant histopathological alterations were observed at all doses.

Ref.: 13

Conclusion

The oral dose of 50 mg/kg bw/d represents the NOAEL.

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

Guideline: /
Species/strain: rat, Wistar Bor: WISW/TNO (SPF)
Group size: 10 males and 10 females
Test substance: 1-hydroxy-2-amino-5-methylbenzol
Batch: /
Purity: 98%
Vehicle: 10% suspension in 5% Gummi Arabicum
Dose levels: 800 (reduced to 500 from week 6 onwards) mg/kg bw/d
Stability: /
Route: oral
Administration: gavage
GLP: in compliance
Study period: 13 July – 21 October 1981

The compound (98% purity) as 10% suspension in 5% gum Arabic was administered orally by stomach intubation for 90 days to 10 male and 10 female albino rats at the dose of 800 mg/kg bw/d which due to clinical signs was reduced in the sixth week to 500 mg/kg bw/d. 2 rats died during the treatment.

Results
Food consumption, body weight and body weight gain were significantly reduced in both sexes. Urine analysis revealed sedimentation of tyrosine crystals. Relative and absolute weights of the liver, kidney and spleen were increased. Elevation of bilirubin (both sexes) and reduction of iron concentrations (males) were observed. No macroscopic and histopathological effects were detected. No NOAEL could be identified.

Ref.: 14

Comment
This study is considered inadequate since it does not conform to a guideline, the batch number is not known and only one dose group was used.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Studies with 2-amino-5-methylphenol

Bacterial Reverse Mutation Assay

Guideline: OECD 471 (1997)
Species/Strain: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102
Replicates: triplicate cultures
Test substance: 2-amino-5-methylphenol
Batch: 99290T0002
Purity: 98.8 area% (HPLC at 254 nm)
Solvent: DMSO
Concentration: 0, 100, 316, 1000, 2500 and 5000 µg/plate
Treatment: direct plate incorporation method with 48 to 72 h incubation without and with metabolic activation

GLP:	/
Study period:	11 October 2005 – 14 October 2005

2-amino-5-methylphenol was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. The prescribed maximum concentration of the OECD guideline, 5000 µg/plate, was used as the top concentration. The test was performed according the direct plate-incorporation method. Toxicity was evaluated on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Appropriate negative and positive controls were included.

Results
Clear evidence of toxicity was observed as a thinning or complete suppression of the bacterial background lawn at concentration of and above 2500 µg/plate in all tester strains except for TA102. In this strain the toxic effects started at a concentration of 1000 µg/plate. A biologically relevant and concentration dependent increase in revertant colonies was observed in TA100 both in the absence and presence of S9-mix. No increase above the historical range of solvent controls was observed in the other tester strains used both in the absence and presence of S9-mix.

Conclusion
Under the experimental conditions used, 2-amino-5-methylphenol was mutagenic in *Salmonella* strain TA100 in this bacterial gene mutation tests both in the absence and the presence of S9 metabolic activation.

Ref.: 22

Comment
As a clear positive result in TA100 was found, it was not considered necessary to perform a repeat experiment under modified conditions.

In vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1997)	
Species/strain:	mouse lymphoma L5178Y cells	
Replicates:	duplicates in 2 independent experiments	
Test substance:	2-amino-5-methyl-phenol	
Batch:	00290T0001	
Purity:	97.8% (HPLC)	
Solvent:	culture medium	
Concentrations:	initial experiment:	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10 and 25 µg/ml without S9-mix
		0.5, 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/ml with S9-mix
	verification experiment:	10, 20, 40, 60, 80, 100, 120, 140, and 160 µg/ml without S9-mix
Treatment:	4 h treatment both without and with S9-mix; expression period 72 h and a selection period of 11-14 days	
GLP:	in compliance	
Study period:	19 June 2001 – 30 November 2001	

2-Amino-5-methyl-phenol 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 1500 µg/ml in the absence of S9-mix 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, followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation and a selection growth 11-14 days. Toxicity was measured in the main experiments as percentage suspension and relative 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 a low large/small colonies ratio (<4) was associated with clastogenic effects and/or chromosomal aberrations. Negative and positive controls were in accordance with the OECD guideline.

Results
Only in the verification experiment and not in the initial experiment the appropriate level of toxicity (about 10-20% survival after the highest concentration) was reached.
A concentration dependent and biologically relevant increase in the mutant frequency was observed in both experiments with metabolic activation as compared to the controls. An increased occurrence of small colonies was found indicating a mutagenic or clastogenic effect of 2-amino-5-methyl-phenol. Without metabolic activation a biologically relevant increase in the mutant frequency was not found; all mutant values found were within the range of the historical control data.

Conclusion
Under the experimental conditions used, 2-amino-5-methyl-phenol was mutagenic in this mouse lymphoma assay using the *tk* locus as reporter gene. The increased occurrence of small colonies indicated a clastogenic rather than a mutagenic effect of 2-amino-5-methyl-phenol.

Ref.: 16

In vitro Micronucleus Test

Guideline:	draft OECD 487 (2004)	
Species/strain:	human lymphocytes from 2 female donors	
Replicates:	duplicate cultures in two independent experiments	
Test item:	6-amino-m-cresol (2-amino-5-methylphenol)	
Batch:	99290T0002	
Purity:	98.8% (HPLC)	
Solvent:	DMSO	
Concentrations:	experiment 1:	8.590, 13.42, 26.21 µg/ml without S9-mix 34.68, 43.35, 54.18, 67.73 µg/ml with S9-mix
	experiment 2:	17.18, 21.47, 26.84 µg/ml without S9-mix 25.00, 50.00 µg/ml with S9-mix
Treatment:	20 h treatment without S9-mix, harvest time 48 h after the start of treatment 3 h treatment with S9-mix, harvest time 48 h after the start of treatment	
GLP:	in compliance	
Study period:	5 April 2004 – 27 August 2004	

2-amino-5-methylphenol 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-methylphenol. The test concentrations for micronucleus analysis were selected by evaluating the effect of 2-amino-5-methylphenol on the replication index. The highest concentration should produce approximately 60% decrease in replication index. Micronuclei were analysed at three or four concentration levels. Negative and positive controls were in accordance with the draft OECD guideline.

Results
In both experiments in the presence of S9-mix biologically relevant increases in lymphocytes with micronuclei were not observed. In the absence of S9-mix in experiment 1 an increase in lymphocytes with micronuclei was only seen at the highest concentration tested (26.21 µg/ml) whereas in experiment 2 a concentration dependent and biologically relevant increase in lymphocytes with micronuclei was observed. In isolation the result of experiment 1 would have been considered of questionable biological importance 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.

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

Ref.: 17

In vitro alkaline Comet assay

Guideline:	/
Species/strain:	V79 cells
Replicates:	duplicate cultures in 2 independent experiments
Test item:	2-amino-5-methylphenol
Batch:	99290T0002
Purity:	98.8 area% (HPLC at 254 nm)
Solvent:	DMSO
Concentrations:	experiment 1: 308, 616 and 1232 µg/ml without and with S9-mix experiment 2: 25, 50, 75, 100 and 150 µg/ml without S9-mix
Treatment:	3 h treatment without and with S9-mix
GLP:	/
Study period:	14 November 2005 – 30 November 2005

2-amino-5-methylphenol has been investigated for induction of DNA damage in V79 cells using the Comet assay. Liver S9 fraction from Aroclor™ 1254-induced rats was used as the exogenous metabolic activation system. The initial concentrations were chosen based on experience with similar compounds. The highest concentration applied in this experiment was 1232 µg/ml, corresponding to the maximum concentration of 10 mM recommended by OECD for *in vitro* genotoxicity tests.
Cells were treated for 3 h without and with S9-mix and harvested immediately after treatment. Electrophoresis was performed for 30 min at 25V, corresponding to approximately 1.1 V/cm, at 300 mA. DNA was stained with the fluorescence dye SYBR Gold. For the evaluation of Comets the % tail DNA (= tail intensity) was used as assessment parameter. 50 cells per slide and one slide per sample were scored (100 cells total per concentration level). Cytotoxicity was measured as relative cell density and cell viability.
Appropriate negative and positive controls were included.

Results
In experiment 1 in the absence of metabolic activation excessive cytotoxicity was observed. Cell viability at the highest concentration in the absence of metabolic activation was decreased to 0%. In the presence of metabolic activation a decrease to 94.2% was

reported. Therefore, in experiment 2, a different concentration range was employed. In experiment 2, cell viability at the highest concentration was reduced to 89.9%.
The result of experiment 1 in the absence of metabolic activation was not considered acceptable due to excessive cytotoxicity. However, in the repeat experiment in the absence of metabolic activation and in experiment 1 in the presence of metabolic activation a concentration dependent and biologically relevant increase in the amount of DNA in the tail was observed. The % tail DNA values were clearly outside the historical range for the solvent control.

Conclusion
Under the experimental conditions used, 2-amino-5-methylphenol was genotoxic in this *in vitro* alkaline Comet assay with V79 cells.

Ref.: 15

Studies with 2-acetylamino-5-methylphenol, a metabolite of 2-amino-5-methylphenol

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102
Replicates:	triplicate cultures in 3 independent experiments
Test substance:	2-acetylamino-5-methylphenol
Batch:	MOR0323/1
Purity:	99.5 area% (HPLC)
Solvent:	DMSO
Concentration:	experiment 1: 100, 316, 1000, 2500 and 5000 µg/plate experiment 2: 100, 316, 1000, 2500 and 5000 µg/plate; all strains except TA102 experiment 2: 50, 100, 200, 400, 800, 1600 µg/plate strain; TA102 only experiment 3: 100, 200, 400, 800, 1600 and 3200 µg/plate; TA102 only
Treatment:	experiment 1: direct plate incorporation method with 48 to 72 h incubation without and with S9-mix experiment 2 and 3: pre-incubation method with 60 min pre-incubation and 48 to 72 h incubation without and with S9-mix
GLP:	/
Study period:	18 October 2005 – 7 November 2005

2-Acetylamino-5-methylphenol, a metabolite of 2-amino-5-methylphenol, was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. The 3 experiments were performed both in the presence and absence of S9-mix using triplicate cultures. The prescribed maximum concentration of the OECD guideline, 5000 µg/plate, was the top concentration. However, in experiment 2 and 3 (TA102 only) different intervals between the test concentrations were used for strain TA102. Experiment 1 was performed according the direct plate-incorporation method; experiment 2 and 3 according the pre-incubation method with 60 min pre-incubation. Toxicity was evaluated on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Appropriate negative and positive controls were included.

Results
In experiment 1 and 2, toxic effects were not observed in all tester strains except TA102 used both without and with S9-mix. For strain TA102, in experiment 1 toxic effect was observed at a concentration of 2500 µg/plate and above. In experiment 2 where the maximal concentration for TA 102 was reduced to of the 1600 µg/plate toxicity did not occur

whereas in experiment 3 toxic effects were again found at the highest concentration tested 3200 µg/plate.
A biologically relevant increase in revertant colonies was not observed in any of the strains tested at any concentration level in the absence or presence of S9-mix in all 3 experiments.

Conclusion
Under the experimental conditions used 2-acetylamino-5-methylphenol was not mutagenic in this gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 23

Comment
Although the authors stated that there was no toxicity found in the assay with TA102, the reduction in the number of revertant colonies found at the highest concentrations tested pointed to toxicity of 2-acetylamino-5-methylphenol in TA102.

In vitro **Micronucleus Test**

Guideline:	draft OECD 487 (2004)	
Species/strain:	human lymphocytes from 2 healthy, non-smoking female donors	
Replicates:	duplicate cultures in two independent experiments	
Test item:	2-acetylamino-5-methylphenol	
Batch:	MORO323/1	
Purity:	99.5% (HPLC)	
Solvent:	DMSO	
Concentrations:	experiment 1:	600, 900 and 1200 µg/ml without S9-mix 150, 400 and 700 µg/ml with S9-mix
	experiment 2:	700, 1100 and 1300 µg/ml without S9-mix 100, 700 and 1200 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:	28 April 2006– 1 June 2007	

2-Acetylamino-5-methylphenol 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 (range-finder and experiment 1) or 48 h after mitogen stimulation by phytohaemagglutinin (range-finder and 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). Test concentrations were based on the results of a cytotoxicity range-finder; cultures of human peripheral blood lymphocytes were treated with a range of 10 increasing concentrations of 2-acetylamino-5-methylphenol up to 1652 µg/ml, the prescribed maximum concentration of the OECD guideline. The test concentrations were selected by evaluating the effect of 2-acetylamino-5-methylphenol 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
No precipitation was observed in any 2-acetylamino-5-methylphenol treated culture.

In the absence of metabolic activation, in both experiments the number of lymphocytes with micronuclei was generally elevated compared to those observed in the concurrent solvent controls. However, with the exception of single cultures at 900 and 1200 µg/ml in experiment 1, all frequencies of binuclear cells with micronuclei fell within the normal range of the historical controls. These positive findings were, therefore, considered of no biological relevance.
In the presence of metabolic activation, treatment with 2-acetylamino-5-methylphenol resulted in concentration-dependent and statistically significant increases in the number of cells with micronuclei compared to concurrent solvent controls. However, in experiment 1, with the exception of one single culture, the frequencies of binuclear cells with micronuclei fell within the range of the historical controls and were therefore considered not biologically relevant. In experiment 2 the frequencies of lymphocytes with micronuclei were at the 2 highest concentrations outside the range of the historical controls.

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

Ref.: 7 (Submission V)

In vitro alkaline Comet assay

Guideline:	/
Species/strain:	V79 cells
Replicates:	duplicate cultures
Test item:	2-acetylamino-5-methylphenol
Batch:	MOR0323/1
Purity:	99.5 area% (HPLC at 254 nm)
Vehicle:	DMSO
Concentrations:	413, 826 and 1652 µg/ml without and with S9-mix
Treatment:	3 h treatment without and with S9-mix
GLP:	/
Study period:	14 November 2005 – 30 November 2005

2-acetylamino-5-methylphenol, a metabolite of 2-amino-5-methylphenol in human keratinocytes after exposure to 2-amino-5-methylphenol, has been investigated for induction of DNA damage in V79 cells using the Comet assay. Liver S9 fraction from Aroclor™ 1254-induced rats was used as the exogenous metabolic activation system. The initial concentrations were chosen based on experience with similar compounds. The highest concentration applied in this experiment was 1652 µg/ml, corresponding to the maximum concentration recommended by OECD for *in vitro* genotoxicity tests.
Cells were treated for 3 h without and with S9-mix and harvested immediately after treatment. Electrophoresis was performed for 30 min at 25V, corresponding to approximately 1.1 V/cm, at 300 mA. DNA was stained with the fluorescence dye SYBR Gold. For the evaluation of Comets the % tail DNA (= tail intensity) was used as assessment parameter. 50 cells per slide and one slide per sample were scored (100 cells total per concentration level). Cytotoxicity was measured as relative cell density and cell viability.
Appropriate negative and positive controls were included.

Results
2-acetylamino-5-methylphenol caused moderate cytotoxic effects in V79 cells both in the absence and the presence of metabolic activation. Cell viability at the highest concentration was decreases to 91.9 and 94.1% in the absence and presence of metabolic activation, respectively.

Both in the absence and the presence of metabolic activation a biologically relevant increase in the amount of DNA in the tail was not found. The % tail DNA values were clearly within the historical range for the solvent control.

Conclusion
Under the experimental conditions used, 2-acetylamino-5-methylphenol was not genotoxic in this *in vitro* alkaline Comet assay with V79 cells.

Ref.: 15

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

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

Guideline:	OECD 474 (1997)
Species/strain:	Crl:CD® (SD)BR rats
Group size:	5 animals/sex/dose group/harvest point
Test substance:	5-methyl ortho-aminophenol
Batch:	99290T0002
Purity:	/
Vehicle:	2.5% aqueous hydroxypropylcellulose (HPC)
Dose level:	0, 100, 200, 400 mg/kg bw
Route:	intraperitoneal injection
Sacrifice times:	24 h and 48 h (control and high dose only) after treatment
GLP:	in compliance
Study period:	1 July 2002 – 24 July 2002

5-methyl ortho-aminophenol has been investigated for induction of micronuclei in the polychromatic erythrocytes of male and female rats. Test doses were based on the results of a dose range-finding study on acute toxicity. Male and female rats were treated intraperitoneally with 400 mg/kg bw 5-methyl ortho-aminophenol and examined for toxic signs and/or mortality immediately after treatment and at 1, 24 and 48 h after treatment.
In the main experiment mice were exposed intraperitoneally to 0, 100, 200, 400 mg/kg bw. Bone marrow cells were collected 24 h or 48 h (control and high dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Negative and positive controls were in accordance with the OECD guideline.

Results
In the dose range-finding study both male and female rats survived the dose level of 400 mg/kg bw. Clinical observations included cyanosis, hypoactivity, flattened posture, irregular respiration, pale body, lateral and sternal recumbency, few faeces and/or squinted eyes.
In the micronucleus test, one female of the 400 mg/kg bw group died. At the 400 mg/kg bw dose level clinical effects included irregular respiration, hypoactivity, recumbency, paleness, salivation, cyanosis, squinted eyes, flattened posture, urine staining, tremors/convulsions, few faeces, piloerection and/or no urine. At the 200 mg/kg bw dose level, irregular respiration and hypoactivity were noted immediately and 1 h after dosing. The rats returned to normal by the next observation interval.
A decrease in the PCE/NCE ratio was only observed at 400 mg/kg bw at the 48 h bone marrow sampling time. However, the clinical signs reported indicate systemic distribution and thus bioavailability of 5-methyl ortho-aminophenol.
In both male and female rats a biologically relevant and dose dependent increase in the number of cells with micronuclei was observed. Exposure to 400 mg/kg bw resulted in increases outside the range of the historical control values for females from the 24 h sacrifice group and for males and females from the 48 h sacrifice group. In males at 400 mg/kg bw at 48 h the increase was also statistically significant. In females non of the increases were statistically significant. But, based on the magnitude of the induction of

micronuclei in females exposed to 400 mg/kg bw at both sampling times, the increase in the number of cells with micronuclei was considered to be biologically relevant.

Conclusions
Under the experimental conditions used 5-methyl ortho-aminophenol did induce an increase in the number of bone marrow cells with micronuclei and, consequently, 5-methyl ortho-aminophenol is genotoxic (clastogenic and/or aneugenic) in bone marrow cells of rats.

Ref.: 18

In Vivo Unscheduled DNA Synthesis (UDS) Test

Guideline:	draft OECD 486 (1991)
Species/strain:	male Wistar HanIbm: WIST (SPF) rats
Group size:	5 rats/group
Test substance:	A 75
Batch:	EFH 010394
Purity:	/
Vehicle:	carboxymethylcellulose, 0.5% aqueous solution
Dose level:	0, 150 and 1500 mg/kg bw
Route:	orally
Sacrifice times:	2 h and 16 h after dosing
GLP:	in compliance
Study period:	20 April 1994 – 15 June 1994

A 75 was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on a pre-experiment for toxicity measuring acute toxic symptoms at intervals of 1 h and 24 h after oral administration of 1200, 1500 and 2000 mg/kg bw. In the main experiment the highest dose was 1500 mg/kg bw. The animals were starved before treatment.
Hepatocytes for UDS analysis were collected by perfusion with 0.05% w/v collagenase approximately 2 h (high dose only) and 16 h after administration of A 75. The quality of the actual performed perfusion was determined by the trypan blue dye exclusion method. Three cultures were established for each animal. At least 90 minutes after plating the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 12-14 days.
UDS was reported as net grains per nucleus: the nuclear grain count subtracted with the number of grains in a nuclear sized area adjacent to each nucleus. Increased net grain ccounts should be based on enhanced nuclear grain counts rather then on decreased cytoplasmic grain counts. Unscheduled synthesis was determined in 50 randomly selected hepatocytes on 2 replicate slides per rat. Only one positive control in accordance with OECD guideline has been used.

Results
In the pre-experiment for toxicity at 2000 mg/kg bw one rat died within 24 h after treatment. Whereas at 1500 mg/kg bw exclusively brown coloured urine was reported, at 1200 mg/kg bw toxic reactions were seen: reduction of spontaneous activity, abdominal position, eyelid closure and piloerection. On the basis of these data 1500 mg/kg bw was estimated to be a suitable dose.
The viability of the hepatocytes determined by means of the trypan blue dye exclusion assay were in the range of the historical laboratory control data except for 2 rats of which the viability of the hepatocytes was substantially decreased.
In the main test, one rat from the 1500 mg/kg bw group died within 16 h after treatment. The remaining rats showed toxic reactions like piloerection and eyelid closure. The urine and inner organs of these animals were coloured at the 16 h sacrifice time. A biological relevant

increase in mean net nuclear grain count as compared to the untreated control was not found in hepatocytes of any treated animal both for the 2 h and the 16 h treatment time.

Conclusions
Under the experimental conditions used, A 75 did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 20

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:	/
Species/strain:	rat, albino Sprague-Dawley
Group size:	23 mated females; 26 at 200 mg/kg bw/d
Test substance:	1-hydroxy-2-amino-5-methylbenzene
Batch:	23005
Purity:	/
Vehicle:	distilled water
Dose levels:	0, 5, 50 and 200 mg/kg bw/d
Dose volume:	10 ml/kg bw
Route:	oral, gavage
Administration:	daily for 10 consecutive days from day 6 to 15 of gestation
Positive control:	vitamin A, 15 mg/kg bw in rape oil
GLP statement:	/
Study period:	28 October – 30 December 1981

1-Hydroxy-2-amíno-5-methylbenzene in distilled water (10 ml/kg bw) was administered orally by gastric intubation at doses of 5, 50 and 200 mg/kg bw/d to 23-26 pregnant Sprague-Dawley rats from day 8 to 15 of gestation. A concurrent negative control received the vehicle, a further concurrent positive control was treated with 15 mg/kg bw/d Vitamin A. Animals were observed daily for clinical signs during the dosing period. Body weight was recorded on days 0, 6, 15, and 19 of gestation. At day 19 the study was terminated and the animals subjected to necropsy. The common sectio parameters were recorded. Skeletal and visceral abnormalities were registered.

Results
No clinical signs of toxicity were observed in the dams. Body weights and body weight gain were similar to controls. No embryotoxicity or teratogenicity was observed. The NOAEL of both embryo- and maternal toxicity was 200 mg/kg bw/d.

Ref.: 25

3.3.9. Toxicokinetics

ADME study in rats

Guideline:	OECD 417 (1984); OECD percutaneous absorption *in vivo* (draft 2000)
Species/strain:	rat: Sprague-Dawley Crl: CD Br (outbred)
Group size:	40 females 4 per dose level (groups 1 to 4, mass balance) 6 per dose level (groups 5 to 8, kinetics)
Test substance:	6-amino-m-cresol (Oxygelb, WR 23080) (2-amino-5-methylphenol) 2-amino-5-methyl[U-^{14}C]phenol
Batch:	99290T0002 CFQ13827 batch 1 (radiolabelled)
Purity:	98.8%
Radiochemical purity:	97.9% (HPLC)
Vehicle:	i.v.: PEG400/0.9% saline 40:60 Oral: PEG400 Dermal: acetone/water 1:1
Dose levels:	i.v.: 25 mg/kg bw (group 1 and 5) Oral: 25, 400 mg/kg bw (group 2, 3, 6 and 7) Dermal: 30 mg in 1 ml, exposed area on the shaved back skin 10 cm^2 (group 4 and 8)
Route:	oral, gavage
Administration:	single dose
GLP statement:	in compliance
Study period:	13 May – 8 October 2004

Absorption, distribution, metabolism and excretion of ^{14}C- 2-amino-5-methylphenol was investigated in Sprague-Dawley rats after a single oral, intravenous or dermal dose. Eight groups were used: four groups for the mass balance study and four groups for toxicokinetics. The doses used were: 25 mg/kg bw intravenously, 25 or 400 mg/kg bw orally, 10 mg/kg bw (0.3 mg/cm^2, 30 mg/ml) dermally. The vehicles were PEG400/0.9% saline 40:60 intravenously, PEG400 orally, acetone/water 1:1 dermally. The design for the dermal application was chosen to achieve a high bioavailability for comparison of the metabolite profiles.

In the mass-balance groups (1-4) urine and faeces were collected in 0-8, 8-24, 24-48, 48-72 and 72-96 hr intervals. Total radioactivity in urine, faeces, tissues and organs was determined. Selected urine and faeces samples were pooled per group and the metabolite profile was investigated. In the toxicokinetic groups (5-8) blood was sampled alternatively from several rats per time point at 0.25, 0.5, 1, 2, 4, 8, 24 and 48 h after dosing.

Results
The average total recovery of radioactivity in groups 1 to 4 was between 92 and 98% of the applied dose. The mean oral absorption was 99% (25 mg/kg bw) and 96% (400 mg/kg bw) with T_{max} values of 0.26 and 0.51 h, C_{max} values of 25.2 and 117 mg/kg bw and AUC_{∞} values of 48.8 and 967 h x mg/kg bw, respectively. The dose-normalised C_{max} value was 3 times lower for the high dose group compared to the low dose group. The increased T_{max} and the decreased dose-normalised C_{max} point to a slower absorption in the high dose group. Urinary excretion accounted for 91 and 84% while faecal excretion was 4.7 and 8.4% of the administered dose, respectively.

The dermal absorption was 5.1% (0.019 mg/cm^2) from excretion, cage-wash, carcass and unexposed skin and 6.8% (0.026 mg/cm^2) when adding skin residue dose. T_{max} was 0.25 h and C_{max} was 0.62 mg/kg bw while an AUC_∞ value of 1.69 h x mg/kg bw was calculated. Urinary excretion accounted for 3% while faecal excretion was 0.7% of the administered dose.

Urine samples were analysed by two methods, one for profiling only (HPLC-RAD) and one for metabolite identification (LC-PDA-RAD-MS). Up to 9 radioactivity peaks were observed, the profile of the dermal group showed a different spectrum (one peak missing, 2 additional peaks). In addition to the unchanged test compound, after po and i.v. application, analytical data suggest that oxidised and N-acetylated derivatives were found while after dermal application no metabolites could be identified because of the low amounts available.

Conclusion
2-Amino-5-methylphenol administered orally was well absorbed, readily distributed, extensively metabolised and excreted mainly via urine. Metabolism resulted in oxidised and N-acetylated derivatives. After dermal application absorption was 5.1% (0.019 mg/cm^2) from excretion, cage-wash, carcass and unexposed skin and 6.8% (0.026 mg/cm^2) when adding skin residue dose. Excretion took place mainly via urine but elimination was slower compared to oral administration.

Ref.: 24

Comments
Only female rats were used. Identification and quantification of metabolites in urine and serum were apparently hampered by interferences with PEG-400 oligomers. No conjugates of 2-amino-5-methylphenol were found in urine.

Bioavailability across the intestinal barrier *in vitro*

Guideline:	/
Species/strain:	human intestinal epithelial cell line TC-7
Test substance:	6-amino-m-cresol (2-amino-5-methylphenol)
Batch:	99290T0002
Purity:	98.8 area% (HPLC)
Vehicle:	
Dose levels:	50 µM in HBSS buffer containing 1% DMSO
Incubation time:	60 min
Reference compounds:	atenolol, propanolol, ranitidine, vinblastine
GLP statement:	Not in compliance but quality assurance statement included
Study period:	2 - 23 November 2004

The bioavailability of 2-amino-5-methylphenol across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells *in vitro*. The permeability from the apical (A, pH 6.5) to the basolateral (B, pH 7.4) side was investigated at 37 °C in 96-well Multiscreen plates with shaking for a 60 min contact time. Analysis of the donor (apical) and receiver (basolateral) samples was done by means of HPLC-MS/MS, and the apparent permeability coefficient (P_{app}) was calculated for two independent experiments. ^{14}C-mannitol (4 µM) was used to demonstrate the integrity of the cell monolayer. Only monolayers with a mannitol permeability of < 2.5 x 10^{-6} cm/sec were used. Propranolol and ranitidine were used to validate the experimental conditions.
According to the laboratory's classification system, a low permeability is considered for test items revealing a P_{app} < 2 x 10^{-6} cm/sec. A P_{app} of 2 - 20 x 10^{-6} cm/sec and a P_{app} ≥ 20 x 10^{-6} cm/sec classify a substance to have a medium or a high permeability, respectively. Ranitidine, which has a 50 % absorption in humans, was used as low permeability reference compound, as recommended by FDA.

Results

The figures for the reference substances propranolol (P_{app} = 47.8 x 10^{-6} cm/sec), a high permeability reference compound with about 100 % absorption in humans, and ranitidine (P_{app} = 0.4 x 10^{-6} cm/sec) with an absorption of about 50 % in humans, were well within the typical range of 20 – 60 x 10^{-6} cm/sec and < 2 x 10^{-6} cm/sec, respectively. 2-Amino-5-methylphenol (96 % recovery) revealed a P_{app} of 129.9 x 10^{-6} cm/sec and thus was classified to be of high permeability, indicating a complete absorption from the gastro-intestinal tract. As the absorption from the gastro-intestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption 2-amino-5-methylphenol after oral administration.

Ref.: 26

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

Kinetic analyses with human recombinant NAT1 and NAT2 enzymes

Guideline:	/
Test System:	Yeast lysates containing recombinant human NAT1 and NAT2 isoenzymes
Recombinant human isoenzymes:	1) NAT1 4 encoded by the *NAT1*4* allele, associated with rapid acetylators 2) NAT1 14B encoded by the *NAT1*14B* allele, associated with slow NAT1 acetylators. *NAT1*14B* possesses a G560A single nucleotide polymorphism (SNP) in the *NAT1* coding region resulting in the amino acid change R187Q. 3) NAT2 4 encoded by the *NAT2*4* allele, associated with rapid acetylators 4) NAT2 5B encoded by the *NAT2*5B* allele, associated with slow NAT2 acetylators. *NAT2*5B* possesses three SNPs in the *NAT2* coding region: T341C (I114T), C481T (silent) and A803G (K268R).
Test substance:	1) WR23080, A000640 (Oxygelb, R0301), (termed 2-amino-5-methylphenol (A075) in the study), free base
Batch:	99290T0002, R99052643
Purity:	98.9 weight% (NMR; date of documented analysis 28.1.2000; claimed expiration date 5.12.2010)
Cofactor:	acetyl coenzyme A, 1 mM, (saturating concentration)
Reference substance:	1) p-aminobenzoic acid (PABA) (Sigma, lot number 066H5037)
Purity:	not documented
	2) sulfamethazine sodium salt (SMZ) (Sigma, lot number 057F0009
Purity:	not documented

Method of analysis:	H-NMR (identity of A075 and assay); N-acetylation activity was determined by spectrophotometric determination of coenzyme A.
GLP statement:	No
Study period:	May – June 2009

Human NAT1 and NAT2 were recombinantly expressed in yeast cells. The coding regions of the isoenzymes *NAT1*4*, *NAT1*14B*, *NAT2*4*, and *NAT2*5B* were amplified by polymerase chain reaction (PCR) and purified PCR products were ligated with the plasmid pESP-3 obtained from Stratagene (La Jolla, California). Constructs were ultimately transformed into competent *Schizosaccaromyces pombe* and expressed following the instructions provided by the manufacturer (Stratagene). Total cell lysates were prepared from the yeast and centrifuged at 13,000xg for 20 minutes. Supernatants were collected, aliquoted, and stored at -80ºC until used for enzymatic assays.

Yeast lysates containing the human enzyme were incubated with variable concentrations of 2-amino-5-methylphenol in combination with a saturating concentration of the cofactor acetyl coenzyme A. *p*-Aminobenzoic acid (PABA) and sulfamethazine (SMZ) were used as reference controls. PABA is a highly selective substrate for human NAT1 and SMZ is a highly selective substrate for human NAT2. All N-acetyltransferase assays were conducted at lysate protein concentrations and over time periods in which the reactions were linear.

Apparent Michaelis-Menten kinetic parameters were calculated for 2-amino-5-methylphenol, PABA and an SMZ. Apparent Km values were used to assess the affinity of 2-amino-5-methylphenol as a substrate for human NAT1 and NAT2, and Vmax/Km values were calculated to assess the catalytic efficiencies.

Results
2-Amino-5-methylphenol was found to be an excellent substrate for both recombinant human NAT1 and NAT2 with affinity and catalytic activity comparable to PABA for NAT1 and comparable to SMZ for NAT2. Similar Km values (0.3 – 0.5 mM) were found for 2-amino-5-methylphenol both with recombinant human NAT1 and NAT2 and both with rapid and slow acetylating isoenzymes. Both the NAT1 and NAT2 acetylation polymorphisms were clearly expressed by N-acetylation of 2-amino-5-methylphenol with approximately 10-fold higher catalytic efficiencies in rapid than in slow acetylators both with recombinant human NAT1 and NAT2.

Conclusion
From these results it can be expected that 2-amino-5-methylphenol would be metabolized in humans via N-acetylation by both NAT1 and NAT2, whereas PABA is metabolized primarily by NAT1 and SMZ is metabolized primarily by NAT2. The relative importance of NAT1 versus NAT2 in the human metabolism of 2-amino-5-methylphenol will depend on the relative tissue expression of the two enzymes, the route of exposure to 2-amino-5-methylphenol and the presence of other enzymes, such as UDP-glucuronosyltransferase and sulfotransferase enzymes, in the tissue which may compete with N-acetyltransferases. With respect to tissue distribution, NAT1 would be important in human skin since it is highly expressed in that tissue whereas NAT2 is not expressed in skin but is expressed in liver. The high catalytic activity of NAT1 for 2-amino-5-methylphenol and its high expression in skin suggests that this compound can be expected to be readily metabolized via N-acetylation in human skin. However, the polymorphic differences between rapid and slow acetylators in catalytic activities of NAT1 for 2-amino-5-methylphenol and possible differences in the individual expression of NAT1 in human skin should be taken into account.

Ref.: 1, Submission V

Metabolic stability, metabolite profile and species comparison in primary hepatocytes of human, rat and mouse

Guideline:	/
Species/strain:	human, rat and mouse hepatocytes in suspension culture
Test substance:	2-amino-5-methylphenol (Oxygelb, 23080)
Batch:	99290T0002
Purity:	98.8 area% (HPLC)
Test concentration:	10 µM
Incubation time:	4 h
Method of analysis:	HPLC-MS/MS
GLP statement:	in compliance
Study period:	19 March – 1 April 2003

2-Amino-5-methylphenol (10 µM, 1.23 µg/ml) was incubated 4 h at 37 °C with hepatocytes pooled from three male humans, male Sprague-Dawles rats and male ICR/CD-1 mice (approximately 1 x 10^6 cells/ml). Three lots of human hepatocytes (NAT2 genotypes 5*/7*, wt/5*, wt/6*) were pooled to yield an average rapid metabolizer phenotype. The reaction was stopped by acetonitrile, protein was pelleted by 3000 g centrifugation and the supernatant was analysed. As marker reactions the routinely used coumarin 7-hydroxylation (representing CYP 2A6) and 7-ethoxycoumarin O-deethylation (CYPs 1A, 2A and 2B) were determined. In addition, chlorzoxazone 6-hydroxylation (CYP 2E1), p-aminobenzoic acid N-acetylation (NAT1 and NAT2) and sulfamethazine N-acetylation (functional state of NAT) were analysed. The metabolic stability and metabolite profile of 2-amino-5-methylphenol was determined by LC/MS/MS analytics.

Results
2-Amino-5-methylphenol showed a rapid rate of metabolism in hepatocytes of all species in the order human ≈ mouse > rat (82.5, 82.8 and 62.4% assumed to be metabolised within 1.5 h under the experimental conditions). Metabolites formed indicated an intensive phase II metabolism, sulfate ester and glucuronide being the major metabolites. 2-Amino-5-methylphenol appeared not to be acetylated by human and mouse hepatocytes and acetylation by rat hepatocytes remained questionable.

Conclusion
The metabolism of 2-amino-5-methylphenol is similar for human, rat and mouse primary hepatocytes. The test substance was extensively metabolized by sulfate and glucuronide conjugation.

Ref.: 27

Comment
Human hepatocytes only from males were used. The pooled hepatocytes from 3 donors represent an average rapid metabolizer phenotype (NAT2). Metabolic conversion was determined by the decrease of the parent substrate in the hepatocyte suspensions. The conjugates formed were determined in a semi-quantitative manner.

***In vitro* metabolism of 2-amino-5-methylphenol in human hepatocytes**

Guideline:	/
Test system 1:	Suspended hepatocyte test system: human hepatocytes from CellzDirect, lots HuP88, HuP90, and HuP94 Cell Density: 10^6 cells/ml, 100,000 cells/well
Test system 2:	Plated hepatocyte test system: Human hepatocytes from CellzDirect, lots Hu4242 and Hu 4224 Cell Density: 700,000 cells/ml, 350,000 cells/well

Test Substance:	6-Amino-m-cresol (2-amino-5-methylphenol)
Batch:	Non-radiolabeled 6-amino-m-cresol: 99290T0002 [^{14}C]-2-amino-5-methylphenol: Suspended hepatocyte test system: CFQ40755, Batch B1, specific activity 473 µCi/mg Plated hepatocyte test system: CFQ13827, Batch 1, specific activity 998 µCi/mg
Purity:	CFQ40755, Batch B1: Radiochemical purity: 98.9% by HPLC, 97.8% by TLC CFQ13827, Batch 1: Radiochemical purity: 97.9% by HPLC, 97.4% by TLC Non-labelled 6-amino-m-cresol: 99.7 area% by HPLC (210 nm), 98.1% by NMR
Test concentrations:	Suspended hepatocytes: 1, 10, 100, and 1000 µg/ml Plated hepatocytes: 0.889, 8.89, and 88.9 µg/ml
Reference substance:	7-Ethoxycoumarin
Incubation time:	Suspended hepatocytes: 3 hours Plated hepatocytes: 24 hours
GLP statement:	No

In vitro metabolism of 2-amino-5-methylphenol was determined in suspended or plated cryopreserved human hepatocytes. In both test systems, 7-ethoxycoumarin, a known substrate for multiple cytochrome P450 enzymes, was used to assess the metabolic activity of the hepatocytes by measuring 7-hydroxycoumarin formation. Chemical stability of the test substance during incubation was evaluated by incubating each dose solution in a test well that contained no cells. The loss of parent compound and formation of metabolites were quantified via HPLC/RAD/Q-ToF/MS. Accurate mass identifications were made with mass spectrometry while quantification was performed with radioanalytical detection. In the suspended hepatocyte study quantification was done using a calibration curve prepared from the dose solution standards. In the plated hepatocyte study rapid dimerization of the dose solutions occurred, making it impossible to obtain an accurate standard curve. Therefore the quantitative results were expressed as % of the total radioactivity integrated for all peaks in the HPLC radiochromatograms.

Suspended hepatocytes: Each concentration of the test substance, 1, 10, 100, and 1000 µg/ml was tested in triplicate. At times 0, 1, 2, and 3 hours the wells were quenched with acetonitrile to stop cellular activity. At the end of the study, the plates were centrifuged at 4000 rpm for 10 minutes and a 200 µl aliquot of the supernatant was taken and stored at -80ºC until analysis.
Plated hepatocytes: Approximately 350,000 cells in hepatocyte plating media were added to each well and the plates were incubated for 4 to 6 hours to allow attachment. After the attachment period, cells were washed with growth media. The growth media was removed and growth media containing Geltrex was added. After incubation overnight, cells were dosed with growth media containing 0.889, 8.89, or 88.9 µg/ml 2-amino-5-methylphenol. Each concentration of the test substance was tested in triplicate. After a 24 hour incubation period the supernatant was drawn off the cell layer and centrifuged at 10,000 rpm for 10 minutes. Supernatants were stored at -80ºC until analysis.

Results
2-Amino-5-methylphenol was readily metabolized and the profile of metabolites formed was concentration dependent. Two metabolites of 2-amino-5-methylphenol, O-glucuronide- 2-amino-5-methylphenol and O-sulfate- 2-amino-5-methylphenol, were detected in studies with both suspended and plated hepatocytes. The substrate concentration dependency of formation of these metabolites was similar in both studies. However, a marked increase in sulfonation was observed at 1000 µg/ml in suspended hepatocytes which may be attributed to a shift from SULT1A1 to SULT1B1 as the responsible catalytic entity (ref 7, submission V). A third metabolite, N-acetyl-O-glucuronide- 2-amino-5-methylphenol, was detected only

with plated hepatocytes, a test system that may more closely reflect the *in vivo* metabolic capability. This metabolite was only found at the low and mid concentration of the test substance in amounts of up to 15%. Major amounts of a dimer of 2-amino-5-methylphenol were found at high substrate concentrations.
In plated hepatocytes, at lower concentrations (~0.9 µg/ml) O-sulfonation was the major route of metabolism (75% of total), and O-glucuronidated metabolites (N-acetyl-O-glucurono-2-amino-5-methylphenol and O-glucurono-2-amino-5-methylphenol) were less predominant. At an intermediate substrate concentration (~9 µg/ml), O-sulfonation and O-glucuronidation were equally important as biotransformation pathways, while at a high substrate concentration (~90 µg/ml) only O-glucurono-2-amino-5-methylphenol was detected. These changes may be due to the fact that sulfonation was completely inhibited. At the lower concentrations (up to ~9 µg/ml) metabolism was essentially complete. No parent compound was found in samples after 24 h at any of the three concentrations tested. However, metabolism was incomplete at the high concentration (~90 µg/ml). 2-amino-5-methylphenol equivalents that were unmetabolized were recovered as a dimer of 2-amino-5-methylphenol representing 42% of total radioactivity. The dimer of 2-amino-5-methylphenol was formed via a non-enzymatic chemical reaction, since it was also present in dose solutions and chemical stability samples.

Conclusions
Two metabolites of 2-amino-5-methylphenol, O-glucurono-2-amino-5-methylphenol and 2-amino-5-methylphenol-O-sulfate, were detected in studies with both suspended and plated hepatocytes. The substrate concentration dependency of formation of these metabolites was similar in both studies. A third metabolite, N-acetyl-O-glucurono-2-amino-5-methylphenol, was detected in minor amounts only with plated hepatocytes, a test system that may more closely reflect the *in vivo* metabolic capability. In plated hepatocytes at the low test concentration, the major metabolite was 2-amino-5-methylphenol-O-sulfate, while at higher substrate concentrations, O-glucuronidation became the predominant metabolic pathway. Metabolism was incomplete at the high concentrations both in suspended and plated human hepatocytes, suggesting saturation of phase II metabolism or enzyme inhibition.

Ref.: 5, Submission V

Comment
The human donors were not phenotyped regarding their acetylator status as slow or rapid metabolizers.
Under the conditions of the study, the dimer (trade name Oxygelb Dimer, $C_{14}H_{14}N_2O_2$, formula weight 242) is probably a product of an autoxidation reaction of 2-amino-5-methylphenol formed at increased concentrations in presence of oxygen. It cannot be excluded that the formation of the dimer of 2-amino-5-methylphenol interfered with metabolism under the test conditions, e.g., by substrate consumption (by dimer formation) or mild cell toxicity or enzyme inhibition by the dimer itself, as 40-80% of the radioactivity accounted for the dimer at high substrate concentrations in both suspended and plated hepatocytes.

N-acetylation of 4-amino-3-hydroxytoluene in the human keratinocytes cell line (HaCaT)

Guideline:	/
Species/strain:	human keratinocytes cell line (HaCaT)
Test substance:	4-amino-3-hydroxytoluene (WR 23080)
Batch:	95290 T0001 Fass 9
Purity:	99.6 area% (GC/HPLC)
Concentration range:	0.25 to 25 µg/ml
Incubation time:	24 h
Method of analysis:	HPLC

GLP statement: not in compliance
Study period: 8 September – 3 October 2005

To determine the capacity of human skin to acetylate 4-amino-3-hydroxytoluene, the i*n vitro* acetylation by HaCaT cells, a cell line derived from human keratinocytes was investigated. The test substance was incubated for 24 h in Dulbecco's Modified Eagle Medium (DMEM) buffer. After termination the supernatant was extracted with ethylacetate, concentrated and analysed by means of HPLC-DAD. The detection limit for 4-acetylamino-3-hydroxytoluene was 0.5 µg/ml.

Results
With concentrations up to 1.5 µg/ml N-acetylation reached more than 80% and acetylation rates were linear to substrate concentrations. With increasing substrate concentration no further increase of acetylated metabolite occurred. Impaired cell viability was observed at conc. of 10 and 25 µg/ml. Cytotoxicity of the substrate may explain the reduced acetylation rates at these concentrations.

Conclusion
The results demonstrate the capacity of the human keratinocytes-derived cell line HaCaT to efficiently N-acetylate 4-amino-3-hydroxytoluene.

Ref.: 21

Comment: For the cytotoxicity observed at conc. of 10 and 25 µg/ml. see also the recent study in HaCaT cells from 2010 below (Ref. 2, Submission V).

N-acetylation of 4-amino-3-hydroxytoluene in the human keratinocytes cell line HaCaT

Guideline:	/
Test system:	human keratinocytes cell line HaCaT Clone B (Lot #: 300493-75, passage 33)
Cell density:	Approx. 500,000 cells/well
Test substance:	1) WR23080 (Oxygelb), (termed 2-amino-5-methylphenol (A075) in the study), free base
Batch:	99290T0002, Dotticon Exclusive Synthesis AG
Purity:	98.4-99.7 area% (HPLC), 98.1 weight% (NMR); 0.55% dimeric A075 as an impurity 2) ^{14}C-2-amino-5-methylphenol (A075) Amersham Code CFQ40755, Batch B1, specific activity 473 mCi/mg
Purity:	98.9 (retested by the method combination below: >96%)
Concentration range:	0.625, 1.25, 2.5, 5, 10, 25, 80, 200 µg/ml
Reference substance:	1) p-aminobenzoic acid (PABA) (Sigma #A9878, batch #039K0124)
Purity:	99% 2) 4-acetamidobenzoic acid (Sigma #133337, batch #1395915)
Purity:	98%
Incubation time:	24 h
Method of analysis:	HPLC-UV/RAD/Q-ToF/MS
GLP statement:	No, but with some GLP elements such as archival
Study period:	2010

To determine the capacity of human skin to acetylate 4-amino-3-hydroxytoluene, the HaCaT cells were plated in 6 well culture plates and incubated for 24 hours with the test compound at concentrations ranging from 0.625 to 200 µg/ml. After incubation samples of the culture media were taken for metabolite identification and quantification. PABA (10 µg/ml) as a specific substrate of human NAT1 and formation of the metabolite 4-acetamidobenzoic acid were used to demonstrate that the cells were metabolically viable throughout the 24 h incubation. Quantification was performed by HPLC/RAD/ToF/MS with radio-analytical detection while mass spectrometry was used for accurate mass determination to confirm metabolite identification.

Results
The N-acetylated test substance was the only metabolite formed, apart from traces of some other reaction products or impurities which could not be identified. Dimerization of the test substance non-enzymatically occurred at higher concentrations but did not disturb the N-acetylation up to 10 µg/ml. With concentrations up to 5 µg/ml, the N-acetylation of the test substance was rapid and almost linear between substrate concentration and the amount of metabolite formed per million cells. At 25 µg/ml and above, cytotoxicity occurred. Metabolic viability of the cells throughout the 24 h incubation was demonstrated by addition of PABA to separate plates for the last 3 hrs of the incubation.

Conclusion
The results demonstrate the capacity of the human keratinocytes-derived cell line HaCaT to efficiently N-acetylate 4-amino-3-hydroxytoluene up to a concentration of 5 µg/ml. Above this concentration, increasingly inhibition of N-acetylation occurred. N-acetyl-4-amino-3-hydroxytoluene was the only metabolite identified under the conditions of the study, thus confirming the findings of the earlier study in HaCaT cells above.

Ref.: 2, Submission V

***In vitro* metabolism study in viable human skin**

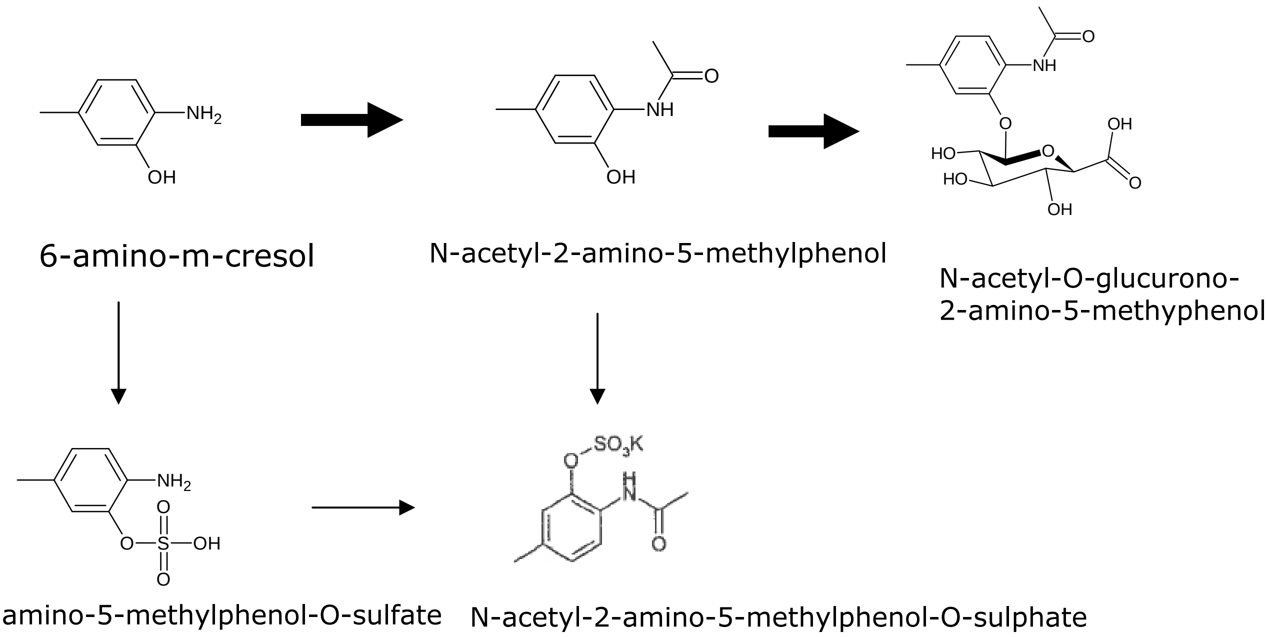
Guideline:	/
Tissue:	human skin (1 breast, 2 abdomen from 3 females) thickness 580-650 µm
Viability of skin samples:	MTT assay
Diffusion cell:	6-well plate on a Netwell insert, static system (Internal area 0.64 cm^2)
No. of chambers:	12 from 3 donors
Test substance:	[^{14}C]-2-amino-5-methylphenol
Batch:	992990T0002 (Oxygelb, non-radio-labelled) CFQ40717 (radio-labelled)
Purity:	99.5% HPLC, 98.1% by NMR (non-radio-labelled) 99% by HPLC (radiochemical)
Reference substances:	a) N-sulfate Oxygelb, purity >99 % b) N-acetyl-O-sulfate Oxygelb, purity >99% (HPLC), 90.3% (^{1}H-NMR) c) N-acetyl- Oxygelb, purity >99% (HPLC), 98.4% (^{1}H-NMR) d) O-sulfate-Oxygelb, purity ca. 99% (HPLC, ^{1}H-NMR) e) Oxygelb dimer, purity >99% (HPLC), 91.5% (^{1}H-NMR)
Test item:	Oxidative hair dye formulation containing 1.5% 6-amino-m-cresol
Area dose:	100 mg/cm^2)
Time period:	60 min (3 and 24 hours)
Receptor fluid:	Dulbecco's minimum eagle medium (DMEM) pH 7.21-7.37
Solubility in receptor:	/
Stability:	/
Method of Analysis:	LC-MS
GLP:	in compliance
Study period:	December 2009

The metabolism of 2-amino-5-methylphenol from a typical oxidative hair dye formulation was investigated in viable human skin obtained from 3 female donors.

At 24 h post dose, the experiment was terminated by removing the skin sample. Samples of the receptor fluid and skin extract were analysed for metabolite profiling and identification. The reference standards include N-acetyl-2-amino-5-methylphenol, 2-amino-5-methylphenol-O-sulfate and N-acetyl-2-amino-5-methylphenol-O-sulfate.

Results
The capacity for viable human skin to metabolize 2-amino-5-methylphenol was evident. Three metabolites were identified: N-acetyl-2-amino-5-methylphenol, 2-methyl-5-aminophenol-O-sulfate and N-acetyl-2-amino-5-methylphenol-O-sulfate. In addition, a fourth metabolite was detected in both receptor fluid and exposed skin samples, and this metabolite was postulated to be N-acetyl-O-glucurono-2-amino-5-methylphenol on the basis of the mass spectrometry analysis.
The metabolite profiling results indicate that N-acetylation is the major route of metabolism of 2-amino-5-methylphenol and enters through the viable human skin. The amount of N-acetylated metabolites was calculated to be 0.93 µg/cm^2, i.e at least 34% of the total amount of 2-amino-5-methylphenol that was found in the receptor fluid or in the skin (2.77 µg/cm^2) was present in the form of these N-acetylated metabolites.

Proposed biotransformation pathway of 2-amino-5-methylphenol in human skin (Major pathway is indicated with bold arrows)



A dimer of 2-amino-5-methylphenol (trade name Oxygelb Dimer, $C_{14}H_{14}N_2O_2$, formula weight 242) and a related substance (Unknown 3, formula weight 245) were also identified in the receptor fluid and skin extract samples but not quantified. The oxygelb dimer is probably formed under the oxidative conditions of the hair dye formulation.

Ref: 3, Submission V

Comment
The acetylator status of the skin samples of the 3 donors regarding NAT1 (rapid or slow) is unknown. Thus, the evidence on the N-acetylation metabolic pathway in human skin is at best of semi-quantitative nature and does not allow drawing firm conclusions regarding the relevance of this metabolic pathway in the human population after dermal exposure to 2-amino-5-methylphenol.
The role of the oxygelb dimer and its derivative Unknown 3, both absorbed by human skin, need to be clarified.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

2-amino-5-methylphenol

Not applicable

3.3.14. Discussion

Physico-chemical properties
2-Amino-5-methylphenol is used as precursor of oxidative hair dye formulations, which are mixed with hydrogen peroxide developer in a ratio 1:1 or 1:3 before application. The maximum on-head concentration of 2-amino-5-methylphenol from the hair dye application is 1.5%.
Water solubility of 2-amino-5-methylphenol was described as 5.9 g/L and 4.2 g/L in two different references. The method of determination of water solubility was not described..
The Log P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.
Stability of 2-amino-5-methylphenol in typical hair dye formulations was not reported.
INCI name is scientifically not correct

Irritation, sensitisation
2-Amino-5-methylphenol in 1% aqueous solution is not irritant to the rabbit skin and eyes.
2-Amino-5-methylphenol is a strong skin sensitiser.

Dermal absorption
An *in vivo* study using rats not considered valid showed that 0.59% of the applied dose was bioavailable. Thereafter, an *in vitro* study with human skin was submitted.
Due to some shortcomings in the methodology employed, the Mean + 2SD (2.77 + 2 x 3.09 = 8.95 µg/cm²) should be considered as dermal delivery. As the concentration of 2-amino-5-methylphenol in the test formulation is half of the maximum use concentration, dermal absorption of 2 x 8.95 = 17.90 µg/cm² should be used to calculate MoS.

General toxicity
Acute oral toxicity was determined in male and female rats and mice. Clinical signs observed were sedation, tremor, accelerated respiration and exitus. No macroscopic organ changes were noted. The LD_{50} figures were calculated as being between 750 and 1375 mg/kg bw.
In a repeated dose (28 days) oral toxicity study in rats at 250 mg/kg bw/d alterations of haematology and clinical chemistry values were observed (reduction in erythrocytes and haemoglobin in males and females and iron in females; increase in reticulocytes and haematocrit in males and females). At autopsy increases in liver, kidney and spleen weights were found. The dose of 50 mg/kg bw/d represents the NOAEL.
The subchronic toxicity study in rats is considered inadequate since it does not conform to a guideline, batch and purity are unknown and only one dose group was used.
In a teratogenicity study with rats body weights and body weight gain of the dams were similar to controls. No embryotoxicity or teratogenicity was observed. The NOAEL of both embryo- and maternal toxicity was 200 mg/kg bw/d.
No study on reproductive toxicity was provided.

Toxicokinetics and metabolism

Toxicokinetics *in vivo*
Absorption, distribution, metabolism and excretion of ^{14}C- 2-amino-5-methylphenol were investigated in Sprague-Dawley rats after a single oral, intravenous or dermal dose. 2-Amino-5-methylphenol administered orally was well absorbed, readily distributed, extensively metabolised and excreted mainly via urine. There is weak analytical evidence that metabolism resulted in oxidised and N-acetylated derivatives.
After dermal application, absorption was 5.1% (0.019 mg/cm^2) from excretion, cage-wash, carcass and unexposed skin, and 6.8% (0.026 mg/cm^2) when adding the residue in the exposed skin. Excretion took place mainly via urine but elimination was slower compared to oral administration. The vehicle (acetone/water 1:1) used in this study was chosen to achieve a high bioavailability for comparison of the metabolite profiles.

Intestinal absorption
The bioavailability of 2-amino-5-methylphenol across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells *in vitro*. 2-amino-5-methylphenol (96 % recovery) revealed a P_{app} of 129.9 x 10^{-6} cm/sec and thus was classified to be of high permeability, indicating a complete absorption from the gastro-intestinal tract. As the absorption from the gastro-intestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption of 2-amino-5-methylphenol after oral administration.

Metabolism by primary hepatocytes
The metabolism of 2-amino-5-methylphenol was comparatively studied in primary hepatocytes of human, rat and mouse. The metabolism is similar for human, rat and mouse primary hepatocytes. The test substance was extensively metabolized by sulfate and glucuronide conjugation. Although the human donors were phenotyped as rapid acetylators, no N-acetyl-2-amino-5-methylphenol could be detected.

In a recent study conducted both with suspended and plated hepatocytes, two metabolites of 2-amino-5-methylphenol, the O-glucuronide and the O-sulfate, were detected. The formation of these metabolites was similar in both studies. A third metabolite, N-acetyl-O-glucurono-2-amino-5-methylphenol, was detected in minor amounts only with plated hepatocytes, a test system that may more closely reflect the *in vivo* metabolic capability. In plated hepatocytes at the low test concentration (0.89 µg/ml), the major metabolite was the O-sulfate, while at higher substrate concentrations, O-glucuronidation was the predominant metabolic pathway. The metabolism of the test substance was incomplete at the high concentration (89 µg/ml). It is not clear whether saturation of phase II metabolism or enzyme inhibition occurred under the test conditions at high substrate concentration or (as an artefact) interference by the formation of a "dimer" of 2-amino-5-methylphenol (oxygelb dimer).

Skin metabolism

The metabolism of 2-amino-5-methylphenol from a typical oxidative hair dye formulation was investigated in viable human skin obtained from 3 female donors. The metabolite profiling results indicate that N-acetylation is the major route of metabolism of 2-amino-5-methylphenol in skin. N-acetyl-2-amino-5-methylphenol and two of its O-conjugates (sulphate ester and glucuronide) were found in receptor fluid and skin. The amount of N-acetylated metabolites was calculated to be 0.93 µg/cm^2 , i.e at least 34% of the total amount of 2-amino-5-methylphenol that was found in the receptor fluid or in the skin (2.77 µg/cm^2) was present in the form of these N-acetylated metabolites. However, the acetylator status of the skin samples of the 3 donors regarding NAT1 (rapid or slow) is unknown. Thus, apart from other methodological restrictions, the evidence on the N-acetylation metabolic pathway in human skin is at best of semi-quantitative nature.

To determine the capacity of human skin to acetylate 4-amino-3-hydroxytoluene, the *in vitro* acetylation by HaCaT cells, a cell line derived from human keratinocytes was investigated. With concentrations up to 1.5 µg/ml N-acetylation reached more than 80%. With increasing substrate concentration no further increase of acetylated metabolite occurred. The results demonstrate the capacity of the human keratinocytes-derived cell line HaCaT to efficiently N-acetylate 4-amino-3-hydroxytoluene.
In a recent study, the capacity of HaCaT cells to efficiently N-acetylate 4-amino-3-hydroxytoluene (same as 2-amino-5-methylphenol) up to a concentration of 5 µg/ml was demonstrated. Above that concentration, increasingly inhibition of N-acetylation occurred. N-acetyl-4-amino-3-hydroxytoluene (same as N-acetyl-2-amino-5-methylphenol) was the only metabolite identified under the conditions of the study, thus confirming the findings of the earlier study in HaCaT cells above.

Kinetic analyses with four human recombinant NAT1 and NAT2 isoenzymes representative for rapid and slow N-acetylation were performed. 2-amino-5-methylphenol was found to be an excellent substrate with similar affinities for both rapid and slow acetylating isoenzymes of NAT1 and NAT2. Both the NAT1 and NAT2 acetylation polymorphisms were clearly expressed by N-acetylation of 2-amino-5-methylphenol with approximately 10-fold higher catalytic efficiencies in rapid than in slow acetylators both with recombinant human NAT1 and NAT2.
From these results it can be expected that 2-amino-5-methylphenol would be metabolized in humans via N-acetylation by both NAT1 and NAT2. The relative importance of NAT1 versus NAT2 in the human metabolism of 2-amino-5-methylphenol will depend on the relative tissue expression of the two enzymes, the route of exposure to 2-amino-5-methylphenol and the presence of competing enzymes, such as UDP-glucuronosyltransferase and sulfotransferase enzymes, in the tissue. With respect to tissue distribution, NAT1 is important in human skin since it is highly expressed in that tissue whereas NAT2 is not expressed in skin but is expressed in liver. The high catalytic activity of NAT1 for 2-amino-5-methylphenol and its high expression in skin suggests that this compound can be expected to be readily metabolized via N-acetylation in human skin. However, the polymorphic differences between rapid and slow acetylators in catalytic activities of NAT1 for 2-amino-5-methylphenol and possible differences in the individual expression of NAT1 in human skin should be taken into account.

Mutagenicity
Overall, the genotoxicity of 2-amino-5-methylphenol is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. The same genotoxic endpoints were also sufficiently tested in *in vitro* genotoxicity tests for a metabolite, N-acetyl-2-amino-5-methylphenol. Under *in vitro* conditions 2-amino-5-methylphenol was genotoxic in all tests performed. It induced gene mutations in gene mutation tests in bacteria as well as in mammalian cells. In the latter one, performed with colony sizing, an increased in small colonies was found indicating a clastogenic rather than a mutagenic effect. Indications for a clastogenic and/or aneugenic potential of 2-amino-5-methylphenol were observed in an *in vitro* micronucleus test and for a clastogenic and/or mutagenic effect in an *in vitro* Comet assay.
The metabolite 2-acetylamino-5-methylphenol, which was found in human keratinocytes, did not induce gene mutations in bacteria nor clastogenic and/or mutagenic effects in an *in vitro* Comet assay of V79 cells. In an *in vitro* micronucleus test an increase in human peripheral blood lymphocytes with micronuclei was found.
Although the negative *in vivo* UDS test may indicate that 2-amino-5-methylphenol does not induce gene mutations *in vivo*, the positive *in vivo* micronucleus test points to a clastogenic and/or aneugenic potential of 2-amino-5-methylphenol. Unfortunately, the metabolite N-acetyl 2-amino-5-methylphenol was not tested in *in vivo* tests.
Based on the present reports 2-amino-5-methylphenol has to be considered as a compound with genotoxic potential.

Carcinogenicity
No data submitted

Weight of evidence approach proposed

The applicant proposed a weight-of-evidence approach to address the relevance of the in vivo genotoxicity hazard data 2-amino-5-methylphenol for human risk assessment. The hazard identification results from the in vivo micronucleus study in rats dosed by i.p. injection with the maximum tolerated dose of 2-amino-5-methylphenol are not considered to be relevant for human risk assessment under hair dye use conditions based on the following arguments:

1) The substantial capacity of human skin to N-acetylate 2-amino-5-methylphenol, a metabolic transformation that eliminates or reduces the genotoxic potential relative to the parent compound and reduces the amount of systemically available parent compound,
2) The large margin of exposure for the dose tested in the *in vivo* micronucleus assay compared to the human systemic exposure associated with hair dye use (i.e. factor 14800), and
3) The dose dependent saturation of phase II metabolism of 2-amino-5-methylphenol in human hepatocytes, which indicates that saturation of phase II metabolism is very likely to occur under maximum tolerated dose conditions, i.e. during hazard identification but not under human use conditions

The applicant investigated the metabolism of 2-amino-5-methylphenol in human skin and liver. The SCCS does not agree to the applicant's weight of evidence approach and conclusion.

Ad 1): There may be a reduction of the genotoxic potential by metabolism in human skin but a considerable part of the population belongs to the phenotype of slow acetylators so that these individuals are at higher internal exposure to 2-amino-5-methylphenol. This might be covered by conventional safety factors.

Ad 2): The *in vivo* micronucleus assay (TG 474) is a validated test only for hazard assessment and should not be used in quantitative risk assessment.

Ad 3): The dose dependent saturation of phase II metabolism of 2-amino-5-methylphenol in human hepatocytes is far from clear. For instance, a marked increase of sulfonation of 2-amino-5-methylphenol was observed in suspended human hepatocytes at the highest dose (1000 µg/ml). Furthermore, an artificial inhibitory or toxic effect of oxygelb dimer formed by autoxidation under the study conditions cannot be excluded, as 40-80% of substrate equivalents were found as of oxygelb dimer at high concentrations of the substrate. Glucuronidation was not markedly inhibited at high concentrations of 2-amino-5-methylphenol.

4. CONCLUSION

The present results indicate that 2-amino-5-methylphenol has genotoxic potential. In addition the metabolite, N-acetyl-2-amino-5-methylphenol, found in the skin was genotoxic in the in vitro micronucleus test.

There is no adequate experimental evidence that 2-amino-5-methylphenol is completely converted to non-toxic metabolites in the skin *in vivo*.

Therefore, the SCCS considers that 2-amino-5-methylphenol is not safe for consumers, when used in oxidative hair dye formulations with a concentration on the scalp of maximum 1.5% taking into account the scientific data provided.

The dimer of 2-amino-5-methylphenol is probably formed under the oxidative hair dye conditions and was found to be absorbed by human skin in vitro. The dimer was also found in high concentrations in a study with human hepatocytes when the concentration of the substrate 2-amino-5-methylphenol was high. The effects of the dimer require further elucidation.

5. MINORITY OPINION

Not applicable

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