EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

1,2,4-Trihydroxybenzene

COLIPA N° A33

Adopted by the SCCP
during the 7th plenary meeting of 18 March 2006

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1. BACKGROUND

Submission I on 1,2,4-Trihydroxybenzene was submitted by COLIPA (European Cosmetics Toiletry and Perfumery Association) in August 1981. On 4 November 1991, the Scientific Committee on Cosmetics (SCC) deferred classification of that substance due to inadequate data. Submission II was made by COLIPA in September 1994 and Submission III in August 2001.

Submission IV presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes ((http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Is 1,2,4-Trihydroxybenzene safe for use in hair dye formulations taken into account the data provided ?*

2. *Does the SCCP recommend any restrictions with regard to the use of 1,2,4-Trihydroxybenzene in hair dye formulations?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1.	Chemical identity

3.1.1.1.	Primary name and/or INCI name

1,2,4-Trihydroxybenzene (INCI name)

3.1.1.2.	Chemical names

1,2,4-Trihydroxybenzene,
Benzene-1,2,4-triol,
Hydroxyhydroquinone

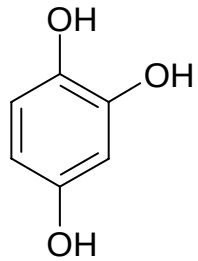
3.1.1.3.	Trade names and abbreviations

Trade name: IMEXINE OAM
COLIPA n°: A33

3.1.1.4. CAS / EINECS number

CAS: 533-73-3
EINECS: 208-575-1

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_6H_6O_3$

3.1.2. Physical form

Beige granular powder

3.1.3. Molecular weight

Molecular weight: 126.11

3.1.4. Purity, composition and substance codes

Batch/Lot Identification	**0506382**	**Op.29**	**0502124**
Titre (g/100g)	98.1 (HPLC)	99.5 (potentiometry)	HPTLC one main spot
Water content (g/100g)	0.2	0.2	-
Total impurity content (%)	< 2	< 0.2	-
Melting point (^{o}C)	144.5	139.6	139
IR-spectrum	In accordance with the proposed structure	-	-
Mass spectrum	In accordance with the proposed structure	-	-
^{1}H and ^{13}C NMR spectra	In accordance with the proposed structure	-	-

The identity of 1,2,4-trihydroxybenzene was established by infra-red spectrophotometry and UV spectrophotometry.
The quantification was performed by HPLC and potentiometry as described in the table above.

3.1.5. Impurities / accompanying contaminants

Total impurities content: Not more than 2%
Heavy metals: < 10 µg/g

Four impurities were detected by HPLC with a relative UV purity above 0.1%. The identity was determined by HPLC/MS and tandem mass spectrometry:

Impurity A: exact mass = 142.03,
molecular formula = $C_6H_6O_4$
proposed structure = tetrahydroxybenzene.
Impurity B: exact mass = 250.05
molecular formula = $C_{12}H_{10}O_6$
proposed structure = 1,1'-biphenyl-2,2',4,4',5,5'-hexol.
Impurity C: exact mass = 124.02
molecular formula = $C_6H_4O_3$
proposed structure = 2-hydroxybenzo-1,4-quinone
Impurity D: exact mass = 203.97
molecular formula = $C_6H_4O_6S$
proposed structure = 1,3,2-benzodioxathiole-5,6-diol 2,2-dioxide

Residual solvents

Isopropanol: not detected (GC detection limit < 500 µg/g)
Dichloromethane: 300 µg/g
n-Propanol: 1500 µg/g
Ethyl acetate: not detected (GC detection limit < 250 µg/g)

3.1.6. Solubility

Water: 10% (w/w)

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.2

Comment
The value was not experimentally derived.

3.1.8. Additional physical and chemical specifications

organoleptic properties

- melting point: 145 – 150 °C
- flash point: /
- vapour pressure: /
- boiling point: /
- density at 20 °C: /
- viscosity: /

- pKa: /
- UV absorption spectrum: /
- Refractive index at 20 °C: /

3.1.9. Stability

Solutions of 1,2,4-trihydroxybenzene were stable after 2 and 4-hour storage at room temperature protected from light and under inert gas atmosphere at the following concentrations:

- 50 mg/ml in purified water,
- 2.5 mg/ml in DMF,
- 10 mg/ml in DMF,
- 250 mg/ml in DMF.

At low concentration levels (0.0625, 0.1 and 0.156 mg/ml in water and 0.1 and 1 mg/ml in DMF), the compound deteriorated rapidly up to 27-64 % within 2 hours.

Ref. (14)

General Comments on Physico-chemical characterisation

- The characterisation of batch 0502124 was limited to HPTLC only. A quantification by HPLC would be desirable for batch 0502124.
- Batch OP29 was investigated using HPLC for quantification in several studies, resulting in yields from 99.4 to 99.8%.
- Stability in market formulation is not reported.

3.2. Function and uses

1,2,4-Trihydroxybenzene is an ingredient used in direct hair colouring products, *i.e.* without mixing with an oxidative agent, at a maximum use concentration of 3.0%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	/
Species/strain:	Rats, OFA (Sprague-Dawley derived)
Group size:	5 male and 5 female
Test substance:	1,2,4-Trihydroxybenzene (1% in carboxymethylcellulose/water)
Batch:	/
Purity:	/
Dose:	100, 250, 350, 500 and 1000 mg/kg bw
Observation:	14 days

GLP: not in compliance

The test substance was administered orally by gavage to the animals. The animals were observed during the first hours after intubation and then for the following 14 days. Mortality was checked during the 14-day observation period. Body weights were presented for the beginning of the study only.

Results
Clinical symptoms were presented in French only. Death occurred within 24 hours after substance administration.
Based on the observed mortality rates, the LD_{50} figure was calculated using three methods (Probit method, Litchfield & Wilcoxon´s method, Arcsinus method).
The LD_{50} is between 350 and 500 mg/kg bw. This value was calculated for both sexes.

Ref.: 1

Comment
The description of the procedure was very short. It was not possible to compare the method used with OECD 401. There was no information on the batch that was tested, the study was not carried out according to GLP, and no data were presented on the development of the body weights. Additionally, the study protocol did not include a necropsy. However, the outcome of this study adds some information on the toxicity of the compound.

3.3.1.2. Acute dermal toxicity

Guideline:	OECD 402
Species/strain:	Rats, Sprague-Dawley
Group size:	5 male and 5 female
Test substance:	1,2,4-Trihydroxybenzene (1% in carboxymethylcellulose/water)
Batch:	0506382
Purity:	98.1%
Dose:	2000 mg/kg bw
Observation:	14 days
GLP:	in compliance

Trihydroxybenzene was applied to the skin of a group of 10 rats (5 males and 5 females) at the dose level of 2000 mg/kg bw. The test site was then covered by a semi-occlusive dressing for 24 hours. Mortality, clinical signs and body weight gain were observed for a period of 14 days following the single administration.

Results
No mortality occurred during the study. From day 2 after removal of the dressings, hypoactivity, piloerection and dyspnea were observed in all females until day 8. 1 of 5 females showed tremors. The overall body weight gain of 9/10 animals was similar to the historical control animals, one female showed a slightly reduced body weight gain during the second week of the study.
A black coloration of the skin was noted in all animals from day 2 until day 15 (end of study). An erythema was observed in 2/5 males on day 2 and persisted in one of them on day 3.
An oedema was recorded between day 2 and day 5 in 2/5 males and all females between day 2 and day 6. No apparent abnormalities were noted at necropsy in any animal.

Conclusion
The maximal non-lethal dose of 1,2,4-trihydroxybenzene was 2000 mg/kg bw by dermal route in rats.

Ref.: 2

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404
Species/strain:	New Zealand White rabbit
Group size:	3 males
Test substance:	1,2,4-Trihydroxybenzene (3% in water)
Batch:	0506382
Purity:	98.1%
Application:	0.5 ml, for 3 minutes, 1 hour and 4 hours in one rabbit. 1 hour and 4 hours in two rabbits, each.
GLP:	in compliance

Doses of 0.5 ml of a 3% 1,2,4-trihydroxybenzene solution were placed on a dry gauze pad, which was then applied to the flanks of the animals. The flanks were clipped before treatment and the clipping was repeated thereafter on several days up to day 9. The gauze pad was held in contact with the skin by means of an adhesive hypoallergenic aerated semi-occlusive dressing and a restraining bandage. The untreated skin served as control.

Results
After a 3-minute exposure (one animal) a very slight or well defined erythema (grade 1 or 2) was noted from day 2 up to day 6.
After a 1-hour exposure (three animals) a very slight or well defined erythema (grade 1 or 2) was noted from day 1 up to day 8 in the first treated animal. In the two other animals, a discrete erythema was noted on day 1 and 2 in one of them; no erythema was observed in the other one.
After a 4-hour exposure (three animals) a brown coloration of the skin was noted in all animals from day 1 up to day 2, 6 or 9. This could have masked a very slight or well-defined erythema (grade 1 or 2). No other cutaneous reactions were recorded during the study.

Conclusion
Due to the skin colouration by 1,2,4-trihydroxybenzene after a 4-hour exposure, it was not possible to definitely conclude on the irritant potential. Based on the results obtained with the 1-hour exposure, trihydroxybenzene at 3% in water was slightly irritant for the rabbit skin.

Ref.: 3

3.3.2.2. Mucous membrane irritation

Guideline: OECD 405

Species/strain:	New Zealand White rabbit
Group size:	3 males
Test substance:	1,2,4-Trihydroxybenzene
Batch:	0506382
Purity:	98.1%
Dose:	0.1 ml
GLP:	in compliance

0.1 ml of a 3% dilution of 1,2,4-trihydroxybenzene in water was applied into the conjunctival sac of the left eye of 3 male rabbit, the right eye served as control. The eyes were not rinsed after administration of the test item. Ocular reactions were observed 1 hour, 24, 48 and 72 hours after the administration.

Results
A very slight chemosis and a very slight redness of the conjunctiva were observed in all animals on day and persisted in 2 of 3 animals up to day 3. No other ocular reactions were observed during the study.

Conclusion
1,2,4-Trihydroxybenzene at 3% in water is slightly irritant when administered by ocular route to rabbits.

Ref.: 4

3.3.3. Skin sensitisation

Local Lymph Node Assay

Guideline:	OECD 429
Species/strain:	Mice CBA/J
Group size:	4 animals per group
Test substance:	1,2,4-Trihydroxybenzene
Batch:	0506382
Purity:	98.1%
Concentrations:	Experiment 1: 0.25, 0.5, 1, 2.5 or 5% (w/v) in DMF Experiment 2: 0.01, 0.05, 0.1, 0.25 or 0.5%.
Negative control:	DMF only
Positive control:	alpha-hexylcinnamaldehyde at the concentration of 25% (v/v)
GLP:	in compliance

The skin sensitising potential of 1,2,4-Trihydroxybenzene was investigated in CBA/J mice by measuring the cell proliferation in the draining lymph nodes after topical application on the ear. In each experiment, the test solution, vehicle or reference solution was applied over the ears (25 µl per ear) for three consecutive days. After 2 days of resting, the proliferation of the lymph node cells was measured by incorporation of tritiated methyl thymidine. The obtained values were used to calculate stimulation indices.
The irritant potential of 1,2,4-Trihydroxybenzene was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6.

Results

No clinical signs and no mortality related to treatment were observed during the study.
In the first experiment, dryness of the skin was noted on day 6 in 2/4 and 4/4 animals given 1,2,4-Trihydroxybenzene at the concentrations of 1 and 2.5%, respectively. In addition, a moderate increase in ear thickness (up to 45%) was observed at the concentrations of 2.5 and 5%, showing the irritant potential of 1,2,4-Trihydroxybenzene at these concentrations.
No cutaneous reactions and no noteworthy increases in ear thickness were observed in the second experiment.
In the first experiment, positive lymphoproliferative responses were observed at all tested concentrations but without clear evidence of a dose-response relationship.
In the absence of local irritation, the positive responses observed at the concentrations of 0.25 and 0.5% were attributed to delayed contact hypersensitivity.
In the first experiment, the stimulation indices ranged from 12.68 to 26.41 using concentrations in the range from 0.25 to 5%.
In the second experiment, a dose related increase in the stimulation indices (except at 0.1%) was noted and the threshold positive value of 3 was exceeded at the concentration of 0.25%. The EC_3 value for the 1,2,4-Trihydroxybenzene calculated on the basis of the results obtained in the second experiment is equal to 0.08%.

Conclusion
1,2,4-Trihydroxybenzene induced delayed contact hypersensitivity in the murine Local Lymph Node Assay.
According to the EC_3 value obtained in this experiment, 1,2,4-Trihydroxybenzene should be categorised as an extreme sensitizer.

Ref.: 5

3.3.4.	Dermal / percutaneous absorption

3.3.4.1	Percutaneous penetration *in vitro*

Guideline:	OECD 428 (draft guideline)
Tissue:	human skin from three female donors
Method:	Flow-through diffusion cells
Test substance:	1,2,4-Trihydroxybenzene and 1,2,4-trihydroxy[U-^{14}C]-benzene
Batch:	0506382 and CFQ13623 (labelled trihydroxybenzene)
Purity:	98.1% and radiochemical purity 93.5%
Concentrations:	20 mg formulation per cm^2, containing 2.78 % active dye corresponding to 556 µg/cm^2 were applied for 30 minutes
No. of chambers:	8
GLP:	in compliance

Skin absorption of Trihydroxybenzene at a concentration of 2.78 % was investigated with human skin from three female donors. The formulation used contained 50% PEG-6 and approx. 47% water. The tissue was obtained directly after abdominal surgery. The transportation of the skin to the laboratory was carried out within approx. 1 h of dissection, while the skin was kept on ice. After arrival at the laboratory, subcutaneous fat was removed and skin was stored in aluminium foil at < -18 °C until use. After thawing of the skin, skin was dermatomed to a recorded thickness of approximately 400 µm. Subsequently, the dermatomed skin strip was cut into smaller pieces of approximately 2 cm. The exact thickness of all skin membranes was measured and recorded.

The integrity of the skin was monitored at the beginning of the experiment using tritiated water. The skin preparations were placed in 9 mm flow-through automated diffusion cells. The receptor fluid (PBS containing 0.01% sodium azide) was pumped at a speed of ca. 1.5 ml/h.
The experiments were performed with 8 samples. Thirty minutes after substance application, Trihydroxybenzene was removed by washing the skin with water (10x), 2% SDS-solution (Sodium dodecylsulfate) and water (10x) again. The washing solutions were combined and the amount of radioactivity was determined. The post exposure time was 23.5 hours.

Results
The recovery of radioactivity was 105%. Most of the substance was recovered in the skin wash after 30 min of exposure. Virtually no penetration of radioactivity into the receptor fluid after 24 hours was observed (0.0019 $\mu g_{eq}/cm^2$ or 0.0003 % of the dose applied).
The maximum absorption (dermal delivery), defined as the compound-related radioactivity present in the receptor fluid, the receptor compartment wash and skin membrane was 0.17 $\mu g/cm^2$.

Ref.: 12

Comments
A degradation of circa 8% within one week of the test substance (content 3%) in the test formulation was indicated, even though the test item was stored under an inert atmosphere.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data.

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

Guideline:	OECD 408
Species/strain:	Rat, Han Wistar
Group size:	15 males + 15 females, four additional satellite groups of 6 male and 6 female were included in the study to obtain blood samples for toxicokinetic analysis on day 1.
Observation:	90 days
Test substance:	Imexine OAM - 1,2,4-Trihydroxybenzene dissolved in water, daily freshly prepared in the dark under nitrogen
Batch:	0502124
Purity:	not stated
Dose:	50, 100 and 200 mg/kg bw (by oral gavage)
GLP:	in compliance

Three groups of 15 male and 15 female Han Wistar rats received the test substance, IMEXINE OAM, daily by oral gavage at dosages of 50, 100 and 200 mg/kg bw/day for 90 day. The control group of 15 rats/sex received the vehicle alone (sterile water for injections). Four additional satellite groups of 6 male and 6 female rats were included in the study to obtain blood samples for toxicokinetic analysis on day 1.
Animals were observed daily for mortality, clinical signs and water consumption. Examinations of individual animals for signs of reaction to treatment was carried out daily immediately after

dosing, and approximately 1 and 3 hours after dosing during the first 3 weeks of the study. Following evaluation of these observations were subsequently performed at approximately 15 minutes and 1 and 2 hours after dosing until the end of the study. Once before commencement of treatment and weekly thereafter, each animal was subjected to a detailed clinical examination including an evaluation of neurotoxicity. Body weight and food intake was recorded weekly. An ophthalmological investigation was performed before the start of the study and in week 12. The motor activity of the first 5 males and 5 females was measured once during week 12 of treatment. Haematology, blood clinical chemistry and urinalysis were performed in week 13 of treatment.
At the end of the treatment period, all animals were killed and subjected to macroscopic examination. Selected organs were weighed. Microscopic examination was performed for specified tissues and organs from all decedent rats, control and high dose rats killed at the end of the study, as well as for gross anomalies and lungs from all animals.

Results
Twelve unscheduled deaths occurred during the course of the study. These included 1 male in each of the control, low- and intermediate-dose groups and 5 males and 4 females of the high-dose group. Microscopic examination indicated that the reason for death in animals of the first three groups was possibly due to mis-dosing. For the high dose animals, the main cause of death was considered to be due to stomach ulcerations.
Piloerection and salivation were observed in animals treated with 100 and 200 mg/kg bw/day.
An overall slight reduction in body weight gain was evident in treated males in comparison with controls from approximately one month of treatment. Furthermore, a 14% decrease in food consumption was found in week 13 in high dose males (200 mg/kg bw/day). These results were not observed in treated females.
A statistically significant increase in mean red blood cell volume, mean corpuscular haemoglobin and platelets and a statistically significant decrease in haematocrit, red blood cell count and haemoglobin were observed in animals treated with 100 and 200 mg/kg bw/day, when compared with controls of the study, although values remained within the normal range for this strain.
A statistically significant increase in bilirubin was observed in animals of the high dose group of both sexes. The authors attribute this to the colour of the test compound interfering with the methodology used. In addition, no toxicological significance was given to the statistically significant increase in urea seen in treated females only.
Statistically significant increases in the absolute weight and/or organ-to-body weight ratio were observed:

- in treated males at the following dose levels: spleen – all dose levels; liver and kidney – 100 and 200 mg/kg bw/day; testes and heart – 200 mg/kg bw/day;
- in females for the liver, spleen and kidneys at 200 mg/kg bw/day.

Ulcerations were observed in the non-glandular gastric region of 1/10 males and 1/11 females of the high dose group and in 1/14 males of the intermediate dose group at termination of the study. The histopathological evaluation of the stomach in the remaining animals of the intermediate dose group did not reveal any further treatment-related gastric lesions.
Dark brown, microgranular pigmentation was clearly evident in single cells or in the lumen of renal cortical tubes of 10/15 males and 10/15 females of the high dose group and in 2/15 males and 1/15 females of the intermediate dose group.

Conclusion

The investigators deduced a No Observed Adverse Effect Level of 50 mg/kg bw/day.

Ref.: 6

Comment
SCCP concluded that no NOAEL can be derived in this study. The content of the test solution has been analysed thrice for trihydroxybenzene (day 1, week 4, week 13). However, no data on purity were given in this report or in the analytical file. The analytical file stated "In accordance with the specification – one main spot" for batch 0502124.
The data on toxicokinetics were scheduled for day 1 of treatment and week 13 of treatment using satellite groups of animals. According to the investigators "analyses, carried out by the Analytical Chemistry Department at RTC, gave unreliable or negative results and are not reported". No further explanations were given.

3.3.5.3. Chronic (> 12 months) toxicity

See 3.3.7

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA102
Replicates:	Two independent test with and without S9 mix (with / without S9: plate incorporation test, with S9, second test: preincubation test)
Test substance:	1,2,4-trihydroxybenzene in purified water
Batch:	0506382
Purity:	98.1%
Concentrations:	Experiment 1: 15 – 2500 µg/plate (depending on the strain used) Experiment 2: 6 – 4000 µg/plate (depending on the strain used)
GLP:	in compliance

Trihydroxybenzene has been investigated for the induction of gene mutations in *Salmonella typhimurium.* Liver S9 fractions from rats treated with Arochlor 1254 were used as the exogenous metabolic activation system. Toxic effects (i.e. reduction in the number of revertant colonies and/or thinning of the bacterial lawn) was observed at higher concentrations with and without metabolic activation in nearly all strains used.

Results
Trihydroxybenzene induced gene mutations in *S. typhimurium* TA98 and TA100 strains in the absence of S9 mix.

Ref.: 7

***In vitro* mammalian cell gene mutation test**

Guideline:	OECD 476	
Cells:	L5178Y mouse lymphoma cells (HPRT)	
Replicates:	Two independent test with and without S9 mix	
Test substance:	Trihydroxybenzene	
Batch:	Op.29	
Purity:	99.4%	
Concentrations:	Experiment 1	(without S9 mix): 0.07813 – 40 µg/ml
		(with S9 mix): 1.25 – 640 µg/ml
	Experiment 2	(without S9 mix): 2.5 – 25 µg/ml
		(with S9 mix): 5 – 280 µg/ml
GLP:	in compliance	

Trihydroxybenzene was assayed for mutation at the *hprt* locus in mouse lymphoma cells using a fluctuation protocol. The study consisted of a cytotoxic range-finding experiment followed by two independent experiments, each conducted in the absence and presence of metabolic activation by an Arochlor 1254 induced rat liver S9 mix.

Results
In experiment 1, the highest concentration tested in the absence of S9 (40 µg/ml) and the highest two concentrations tested in the presence of S9 (320 and 640 µg/ml) were later excluded from the final test statistics due to excessive toxicity.
In experiment 2, the lowest concentration tested in absence and presence of S9 (2.5 and 5 µg/ml, respectively) was non-toxic and was not plated to determine viability and 6-thioguanine resistance). Furthermore, the highest concentration level tested in the absence of S9 (25 µg/ml) was considered too toxic for selection.
The maximum concentrations tested in the absence of S9 mix were 20 µg/ml (experiment 1) and 22.5 µg/ml (experiment 2), resulting in 8 and 13% relative survival, respectively. The maximum concentrations tested in the presence of S9 mix were 160 µg/ml (experiment 1) and 240 µg/ml (experiment 2), resulting in 16 and 18% relative survival, respectively.
No statistically significant increase in mutant frequency was observed following treatment with Trihydroxybenzene at any dose level tested, in the absence or presence of S9.

Negative (solvent) and positive control treatments were included in each mutation experiment in the absence and presence of S9. Mutant frequencies in negative control cultures fell within normal ranges, and clear increases in mutation were induced by the positive control chemicals 4-nitroquinoline 1-oxide (without S9) and benzo(a)pyrene (with S9).

Conclusion
Trihydroxybenzene is not mutagenic at the HPRT locus in mouse lymphoma cells.

Ref.: 8

***In vitro* mammalian chromosome aberration test**

Guideline:	OECD 473	
Cells:	Human lymphocytes from 2 healthy donors	
Replicates:	Two independent test with and without S9 mix	
Test substance:	Trihydroxybenzene	
Batch:	Op.29	
Purity:	99.4%	
Treatment / preparation time:	Experiment 1	24 / 24 hours (- S9 mix) 2 / 24 h (+ S9 mix)
	Experiment 2	24 / 24 and 48 / 48 hours (- S9 mix) 2 / 24 and 2 / 48 hours (+ S9 mix)
Concentrations tested:	Experiment 1	(without S9 mix): 1.25 - 5 µg/ml (with S9 mix): 3.75 - 15 µg/ml
	Experiment 2	(without S9 mix): 2.5 – 7.5 µg/ml (with S9 mix): 10 – 20 µg/ml
GLP:	in compliance	

Trihydroxybenzene has been investigated for induction of chromosome aberrations in cultured human lymphocytes. Liver S9 mix from rats treated with Arochlor 1254 was used as the exogenous metabolic activation system. Negative and positive controls were included.

Results
The test substance did not induce any significant increase in the aberrant cell frequency, with or without S9.

Conclusion
Trihydroxybenzene did not show clastogenic activity in this chromosomal aberration test performed in cultured human lymphocytes under the test conditions described. However, the concentrations tested did not induce the required degree of cytotoxicity and an insufficient number of cells was evaluated in some cases. Altogether, this test is not acceptable.

Ref.: 9

Comment
Results published in the scientific literature were not supplied by the applicants. They are discussed at 3.3.14.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	Mouse, Swiss OF1
Group size:	5 males + 5 females
Test substance:	1,2,4-Trihydroxybenzene dissolved in water
Batch:	Op.29
Purity:	99.4%
Dose levels:	50 mg/kg bw (single i.p. injection)
Sacrifice time:	24 hours (all groups) and 48 hours (Trihydroxybenzene and vehicle only)

GLP: in compliance

Trihydroxybenzene has been investigated for the induction of micronuclei in the bone marrow cells of mice. Due to results of preliminary toxicity tests, 50 mg/kg bw was selected. Negative and positive controls were in accordance with the OECD guideline

Results
At the two sampling times, the number of micronucleated polychromatic erythrocytes in mice exposed to 1,2,4-Trihydroxybenzene did not differ statistically from the simultaneous vehicle control values. The ratio of polychromatic to normochromatic erythrocytes decreased significantly ($p < 0.05$) 24 hours after treatment and ($p < 0.001$) 48 hours after treatment, indicating a toxic effect of the test substance to bone marrow cells.

Conclusion
Trihydroxybenzene did not induce cytogenetic damage to the bone marrow cells of mice when treated by intraperitoneal route at 50 mg/kg bw in the micronucleus test. However, the protocol of this test is not in accordance with the current OECD guideline, because only one dose was tested.

Ref.: 10

3.3.7. Carcinogenicity

Topical application, mice

Guideline:	/
Species/strain:	Swiss Webster mice
Group size:	50 animals per sex and dose
Test substance:	A semi-permanent hair dye formulations (P22) containing 0.5 % 1,2,4-trihydroxybenzene (The formulation used was not given in reference to the study, but was found in: E. Goldenthal. Formulae P-25 and P-26: Lifetime Chronic Toxicity/Carcinogenesis Study in Rats. IRDC Study No. 355-003 (c), 1979)
Batch:	/
Purity:	not stated
Dose level:	0.05 ml of a solution containing m-aminophenol and hydrogen peroxide
Route:	Topical, 1 application weekly
Exposure period:	23 months
GLP:	not in compliance

The experiment involved 12 treatment groups and 3 negative control groups.

Dye applied topically to a 1 cm^2 area on a clipped (24 hours prior to application) site in the interscapular region. Mice received a dose of 0.05 ml topically without occlusion once weekly from 8 – 10 weeks of age for 21 – 23 months. The animals were observed daily for mortality and signs of toxicity, and were weighed monthly. A continuous weekly record was maintained for any skin lesions noted. After 9 months of treatment, 10 males and 10 females per group were necropsied and the study was terminated after 23 months. Skin and internal organs were evaluated histologically.

Four males and 4 females survived to 23 months in the group receiving the semi-permanent formulation 1,2,4-trihydroxybenzene. At 23 months, there were 3 males and 8 females surviving in the control groups. There were no significant differences in absolute or relative liver or kidney weights in groups of 10 male and 10 female mice necropsied after 7 and 9 months. There were no statistically significant differences in the distribution of tumours among treated and control groups.

Ref.: 13

Comment
2,4-Diaminoanisole (EU, carc. Cat. 2) was tested in the same experiment and no response was found. While 1,2,4-trihydroxybenzene is used in hair colouring formulations at a maximum concentration of 3.0%, the substance was only present in a concentration of 0.5% in the carcinogenicity study.

No conclusion with regard to carcinogenicity can be made from the study.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD 414
Species/strain:	Rat, Sprague-Dawley (Crl CD (SD) BR)
Group size:	25 females / dose level
Observation:	20 days
Test substance:	1,2,4-Trihydroxybenzene dissolved in water
Batch:	Op.29
Purity:	99.8%
Dose:	0, 30, 100, 300 mg/kg bw/day (in water)
GLP:	in compliance

Three groups of 25 mated rats received 1,2,4-trihydroxybenzene by oral gavage at doses of 30, 100 and 300 mg/kg bw/day from day 6 to day 15 of gestation (24/25, 22/25, 22/25 pregnant females in each group). The control group received the vehicle alone (21/25 pregnant females). On day 20 of pregnancy, the females were sacrificed and the foetuses were delivered by caesarean section. The following litter parameter was recorded: number of corpora lutea, resorptions, live and dead foetuses and implantation sites. Live foetuses were weighed and examined externally. Half of the live foetuses per litter were submitted to skeletal examination; the remaining foetuses were submitted to soft tissue examination.

Results
No clinical signs and no deaths occurred in the control, 30 and 100 mg/kg bw/day groups.
In the 300 mg/kg bw/day group, 3 females (1 non-pregnant and 2 pregnant) died or were sacrificed in moribund conditions due to a misdosing as noted by the clinical signs (noisy respiration) and/or the macroscopic changes at necropsy (perforated oesophagus or foam in the lungs).

Another female died without any clinical signs preceding death.
At necropsy of these females, gaseous dilatation of the stomach and intestines and congested lungs were noted.
The mean body weight gain and food consumption of females with completed pregnancy were similar in the control, 30 and 100 mg/kg bw/day groups. In the 300 mg/kg bw/day group, the mean body weight gain was slightly lower than that of control animals between days 6 and 9 (3.8% vs 5.6%, not significant) and the food consumption was also slightly lower than that of control females by about 6.5% during the treatment period.
The litter parameters were comparable in the control and treated groups.
No foetal external malformations were observed in the control, 30 and 100 mg/kg bw/day groups.
In the 300 mg/kg bw/day group, 4 from the same litter out of 325 foetuses had an exencephaly associated with opened eyelids. Exencephaly has already been noted in foetuses coming from mothers treated with a non-teratogenic substance (mean incidence: 0.06% - range of incidence per study: 0.0% - 1.0%). The incidence observed in this study (1.2%) was slightly higher than that of the historical data. But, as these foetuses came from the same dam, and as no malformations were noted in foetuses from other litters, this exencephaly was considered as congenital malformation. The dam showed no sign of any toxicity.
No treatment-related foetal skeletal variations, anomalies and malformations and/or foetal soft tissues anomalies or malformations were observed.

Conclusion
Trihydroxybenzene administered by oral route to pregnant female rats was neither maternotoxic, neither embryotoxic nor teratogenic at 30 and 100 mg/kg bw/day dose levels.
The 300 mg/kg bw/day dose level was maternotoxic, but not embryotoxic or teratogenic.

Ref.: 11

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

Physico-chemical specifications
The characterisation of batch 0502124 was limited to HPTLC only. The quantification by HPLC would be desirable for batch 0502124. Batch OP29 was investigated using HPLC for quantification in several studies, resulting in yields from 99.4 to 99.8%. Stability in market formulation is not reported.

Toxicity
A No Observable Adverse Effect Level (NOAEL) of 50 mg/kg bw/day (90-day, oral, rat) was proposed by the applicant. The SCCP disagreed with this since the relative organ weight was increased significantly in the spleen of male rats treated with 50 mg/kg bw/day. This increase continued dose dependently in male rats treated with either 100 or 200 mg/kg bw/day. The absolute organ weight of the spleen increased also in male rats but this increase was not significant at the dose of 50 mg/kg bw/day. Therefore, the dose of 50 mg/kg bw/day was considered as Lowest Observed Adverse Effect Level (LOAEL).

No treatment related effects were seen in a prenatal developmental toxicity study on developmental toxicity parameters up to the highest tested dose of 300 mg/kg bw/day. At 300 mg/kg bw/day a slight maternal toxicity was noted.

Irritation
A 3% dilution of 1,2,4-trihydroxybenzene was found to slightly irritant to rabbit skin und to the rabbit eye.

Sensitisation
Local Lymph Node Assay (LLNA): 1,2,4-trihydroxybenzene was found to be an extreme skin sensitiser in mice.

Percutaneous absorption
The maximum absorption (dermal delivery), defined as the compound-related radioactivity present in the receptor fluid, the receptor compartment wash and skin membrane was 0.17 $\mu g/cm^2$.

Mutagenicity
1,2,4-trihydroxybenzene showed a slight mutagenic activity in the bacterial reverse mutation test with *S. typhimurium* TA98 and TA100 without metabolic activation. The compound was found not mutagenic in mouse lymphoma cells (*hprt* locus).
Additionally, several authors have reported on clastogenic or DNA-damaging properties of 1,2,4-trihydroxybenzene:

- Erexson described an increase of SCEs in human lymphocytes exposed to concentrations of 5 – 500 µM trihydroxybenzene (add. ref 1).
- Kawanishi et al. showed the DNA damaging effect of trihydroxybenzene by incubation of the compound with ^{32}P-labeled DNA fragments (add. ref 2).
- Lee et al. demonstrated that trihydroxybenzene induced alkali-labile DNA single-strand breaks of bone marrow cells (max. concentration 24 µM) (add. ref 3).
- Chung et al. showed micronucleus formation in human lymphocytes exposed to doses of 10 to 100 µM. Using FISH technique the authors showed a greater sensitivity of chromosome 7 and 8 to trihydroxybenzene (add. ref 4).

1,2,4-trihydroxybenzene was tested negative for clastogenicity in cultured human lymphocytes but the test conditions were considered as insufficient by the SCCP.

A negative in vivo micronucleus test indicates that the substance does not induce chromosome aberrations or damage to the mitotic apparatus in bone marrow cells under the conditions of the experiment. The test did not match the current OECD-guideline since only one dose was tested.

Carcinogenicity
No conclusion with regard to carcinogenicity can be made from the study.

4. CONCLUSION

The SCCP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required:

- Characterisation of the reaction product(s) to which the consumer is exposed, because of the reported instability of 1,2,4-trihydroxybenzene in aqueous systems.
- An *in vivo* micronucleus test to exclude the clastogenic potential.
- A thorough interpretation of the literature data should be done.
- Further testing on the potential to induce gene mutation is required.

This hair dye, like many other hair dyes, is a skin sensitiser.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References in italics are not submitted as full reports in the present dossier. They consist of reports for stability/homogeneity studies [14] and preliminary toxicity studies [15-17] or reports for studies considered inadequate [18-25], and can be provided upon request.

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7. ACKNOWLEDGEMENTS

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