Health and Consumers
Scientific Committees

Scientific Committee on Consumer Safety

SCCS

OPINION ON

1,2,4-Trihydroxybenzene

COLIPA n° A33

The SCCS adopted this opinion at its 17th plenary meeting

of 11 December 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 on 1,2,4-trihydroxybenzene was submitted by COLIPA[1] in August 1981. On 4 November 1991, the 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 contains full and updated scientific data on the above substance which is in line with the second step of the strategy on the evaluation of hair dyes.

The SCCP adopted during the 7th plenary meeting of 18 March 2006 the opinion (SCCP/0962/05) on 1,2,4-trihydroxybenzene with the conclusion 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."

The current submission is the Industry reply to the request from the SCCP in opinion SCCP/0962/05

2. TERMS OF REFERENCE

1. *Is 1,2,4-trihydroxybenzene safe for use in direct hair dye formulations at a maximum use concentration of 3% taken into account the data provided?*

2. *And/or does the SCCS recommend any other restrictions with regard to the use of 1,2,4-trihydroxybenzene in hair dye formulations?*

[1] The European Cosmetic 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

1,2,4-Trihydroxybenzene (INCI name)

3.1.1.2. Chemical names

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

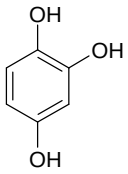
3.1.1.3. Trade names and abbreviations

Trade name: IMEXINE OAM
COLIPA n°: A33

3.1.1.4. CAS / EC number

CAS: 533-73-3
EC: 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 powder

3.1.3. Molecular weight

Molecular weight: 126.11 g/mol

3.1.4. Purity, composition and substance codes

Identification of 1,2,4-trihydroxybenzene of batch 0506328 was performed by NMR, MS and IR spectrophotometry.

Batch/Lot Identification	0506382	Op.29	0502124
Titre (g/100g)	98.1 (HPLC)*	99.5 (potentiometry, NaOH)	HPTLC one main spot
Water content, g/100g	0.2	0.2	-
Loss on drying, g/100g	<0.5	-	-
Melting point (°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	-	-

* quantification was performed by HPLC using Op.29 as reference standard. Op.29 was considered to be 100% pure.

Comments

- The identification of 1,2,4-trihydroxybenzene in batches Op.29 and 0502124 is not convincing: identification of 1,2,4-trihydroxybenzene in batch Op.29 is based only on HPLC retention time and melting point, and the identification of 1,2,4-trihydroxybenzene in batch 0502124 is based only on HPTLC and melting point. In addition, the melting points of batches Op.29 (139.6°C) and 0502124 (139°C) were significantly different from melting point 145°C of properly chemically characterised batch 0506382.
- The content of trihydroxybenzene in batch 0506382 should be considered semi-quantitative, because the quantification was performed by HPLC using Op.29 as reference standard, which was not adequately characterised but was considered to be 100% pure.
- There was no information on purity of 1,2,4-trihydroxybenzene in batch 0502124.

3.1.5. Impurities / accompanying contaminants

Total impurities content:

Batch 0506382: Total impurities <2% (HPLC area%)

Identification and quantification of impurities in batch 0506382: Four impurities were detected by HPLC with a relative HPLC area% > 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)

Heavy metals

Al:	3 mg/kg
Cr:	3 mg/kg
Fe:	11 mg/kg
Ni:	2 mg/kg
Zn:	2 mg/kg
Ag, As, Ba, Bi, Cd, Co. Cu, Mn, Mo, Pb, Pd, Pt, Sb, Se, Sn, Ti, V:	each < 1 mg/kg
Hg:	<0.1 mg/kg

Batch Op.29: total impurities <0.2% (HPLC area%). No other information was provided.
Batch 0502124: no information on impurities in this batch was given

3.1.6. Solubility

Water solubility according to OECD method A6: 486 g/L at 20°C

Solubility (g/100 ml, 22°C, 24 h)
Ethanol: 1<S<10
DMSO: 10<S<20

3.1.7. Partition coefficient (Log P_{ow})

Log Pow: 0.2 (calculated*)

* As the test item was not stable in water, the shake-flask method (EC A.6) was not applicable.

3.1.8. Additional physical and chemical specifications

Melting point:	139°C, 139.6°C and 144.5°C for 3 different batches
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm):	λmax 291 nm

3.1.9. Homogeneity and Stability

Solutions of 1,2,4-trihydroxybenzene were stable (variation <2%) 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, when stored at room temperature protected from light and under inert gas atmosphere.

Ref.: 14

In the dermal absorption study, a degradation of circa 8% within one week of 1,2,4-trihydroxybenzene (content 3%) in the test formulation was indicated, even though the test item was stored under an inert atmosphere.

Ref.: 12

Oxidisability of 1,2,4-trihydroxybenzene

The half wave potential oxidation of 1, 2, 4-trihydroxybenzene by determined by polarography at pH=10 in a carbonate buffer:
El/2 = -0,375 V/SCE.
This means that 1, 2, 4-trihydroxybenzene is much more oxidisable than ascorbic acid for which El/2 = -0,175 V/SCE. Ascorbic acid is very easily oxidised.

Ref.: 0, subm IV

Comment
1, 2, 4-trihydroxybenzene in solutions at low concentration was not stable even though these solutions were stored in dark and under inert atmosphere. Although 1,2,4-trihydroxybenzene in the test solutions of high concentrations was shown to be stable, it should be noted that the stability testing of these was performed after storage in dark and under inert atmosphere.

General Comments to physico-chemical characterisation

- The identification and quantification of 1,2,4-trihydroxybenzene in the batches Op.29 and 0502124 was not sufficiently performed. A complete identification and quantification of 1,2,4-trihydroxybenzene in these batches, using state of art methods, is required. Identification and determination of impurities in these batches should also be performed.-
- The content of 1,2,4-trihydroxybenzene, determined using Op.29 as reference standard, can only be considered as semi-quantitative determination
- The stability testing of 1,2,4-trihydroxybenzene in solutions is inadequate, because it is performed after storage of test solutions in dark and under inert atmosphere. The consumer is exposed to 1,2,4-trihydroxybenzene in ambient air.
- Stability of 1,2,4-trihydroxybenzene in typical hair dye formulations was 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% on head.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCP/0962/05

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:	/

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
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

Taken from SCCP/0962/05

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

Taken from SCCP/0962/05

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 the other two rabbits,
GLP:	in compliance
Date:	January-February 2004

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 was noted from day 2 up to day 6.
After a 1-hour exposure (three animals) a very slight or well defined erythema 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. 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, 1,2,4-trihydroxybenzene at 3% in water was slightly irritant for the rabbit skin.

Ref.: 3

3.3.2.2. Mucous membrane irritation

Taken from SCCP/0962/05

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 rabbits, the right eyes 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 1 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 the ocular route to rabbits.

Ref.: 4

3.3.3. Skin sensitisation

Taken from SCCP/0962/05

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 lympho-proliferative 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

Taken from SCCP/0962/05

Guideline:	OECD 428 (draft guideline)
Tissue:	human skin from three female donors; 400µm
No. of chambers:	8
Membrane integrity:	tritiated water
Method:	Flow-through diffusion cells; 9mm
Test substance:	1,2,4-trihydroxybenzene
Batch:	0506382
Purity:	98.1%
Radiolabel:	1,2,4-trihydroxy[U-^{14}C]-benzene
Batch:	CFQ13623 (labelled trihydroxybenzene)
Purity:	radiochemical purity 93.5%
Dose:	20 mg formulation per cm^2, containing 2.78% active dye corresponding to 556 µg/cm^2 applied for 30 minutes
Receptor:	PBS containing 0.01% sodium azide
Solubility:	100g/L in water
Stability:	"Trace of oxygen may cause oxidation"
Detection:	Liquid scintillation counting
GLP:	in compliance
Date:	February 2004

The skin absorption of 1,2,4-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.
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, 1,2,4-trihydroxybenzene was removed by washing the skin with water (10x), 2% sodium dodecylsulfate solution 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

	Percentage of dose									
Replicate no.	A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4		
Donor no.	1	4	2	4	1	2	2	4	**Mean**	**SD**
Skin wash	106.1	105.4	104.7	106.7	105.6	108.8	105.6	98.8	105.2	2.9
Cotton swabs	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Donor compartment	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.01	0.01
Tape strips	0.02	0.02	0.02	0.03	0.02	0.04***	0.02	0.03	0.02	0.01
Dislodgeable dose*	106.1	105.4	104.8	106.7	105.6	108.8	105.6	98.8	105.2	2.9
Skin	0.01	0.01	0.00	0.01	0.02	0.03	0.02	0.01	0.01	0.01
Receptor fluid + receptor wash	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total recovery	106.2	105.4	104.8	106.7	105.6	108.9	105.6	98.9	105.3	2.9
Total absorption**	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.01	0.01	0.01

	$\mu g_{eq}.cm^{-2}$									
Replicate no.	A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4		
Donor no.	1	4	2	4	1	2	2	4	**Mean**	**SD**
Skin wash	565.2	586.8	583.3	504.9	612.5	633.6	616.5	580.4	585.4	39.6
Cotton swabs	0.02	0.01	0.02	0.02	0.02	0.06	0.01	0.02	0.02	0.01
Donor compartment	0.01	0.02	0.00	0.08	0.00	0.01	0.00	0.11	0.03	0.04
Tape strips	0.09	0.11	0.12	0.12	0.14	0.22	0.10*	0.15	0.13	0.04
Dislodgeable dose*	106.1	105.4	104.8	106.7	105.6	108.8	105.6	98.8	105.2	2.9
Skin	0.05	0.03	0.03	0.03	0.09	0.16	0.09	0.04	0.07	0.05
Receptor fluid + receptor wash	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00
Total recovery	565.4	586.9	583.5	505.1	612.8	634.1	616.7	580.7	585.7	39.6
Total absorption**	0.06	0.04	0.03	0.03	0.10	0.17	0.09	0.05	0.07	0.05

* Amount in skin wash, the cotton swabs and the donor compartment wash

** Amount in receptor fluid, the receptor compartment wash and the skin (excluding tape strips)

*** The tape stripping procedure was stopped after 9 tape strips, because the epidermis was disrupted. The tape strips containing (pieces of) the epidermis was pooled with the skin membrane. Stopping of the tape stripping procedure can cause a slight overestimation of the total absorption because not all of the stratum corneum may have been removed from the skin membrane.

	% of dose applied	$\mu g_{eq}.cm^{-2}$
Dislodgeable dose	105.2 ± 2.9	585.4 ± 39.6
Stratum corneum	0.02 ± 0.01	0.13 ± 0.04
Receptor fluid	0.0003 ± 0.0003	0.0019 ± 0.0017
Skin (epidermis + dermis)	0.01 ± 0.01	0.07 ± 0.05
Unabsorbed dose*	105.2 ± 2.9	585.5 ± 39.6
Absorbed dose**	0.01 ± 0.01	0.07 ± 0.05
Total recovery	105.3 ± 2.9	585.7 ± 39.6

* Unabsorbed dose is defined as the amount recovered from the wash and in the stratum corneum

** Absorbed dose (dermal delivery) is defined as the amount in the receptor fluid, the receptor compartment wash and skin membrane, excluding tape strips

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 0.01 ±0.01% of the applied dose or 0.07 ±0.05µg/cm^2.

Ref.: 12

Comment
The experiment was conducted with a direct dye formulation containing 2.78% 1,2,4-trihydroxybenzene and not 3%. Only 0.0003% of the applied dose was found in the receptor fluid. The dose was slightly below that requested for use and stability in the receptor is not quantified. Therefore, the amount considered as being absorbed is the mean + 2SD. This is 0.03% of the applied dose or 0.17 µg/cm^2.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral toxicity

No data submitted

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

Taken from SCCP/0962/05

Guideline: OECD 408
Species/strain: Rat, Han Wistar
Group size: 15 males and 15 females per group, four additional satellite groups of 6 males and 6 females 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 1,2,4-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*

Taken from SCCP/0962/05 (but modified)

Bacterial gene mutation assay

Guideline:	OECD 471	
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA102	
Replicates:	triplicate cultures in two independent tests	
Test substance:	1,2,4-trihydroxybenzene	
Batch:	0506382	
Purity:	98.1%	
Solvent:	purified water	
Concentrations:	Experiment 1:	15.6, 31.3, 62.5, 125 and 250 µg/plate without S9-mix (for TA98, TA100, TA1535 and TA1537) 78.13, 156.3, 312.5, 625, 1250 and 2500 µg/plate without S9-mix (for TA102) 78.13, 156.3, 312.5, 625, 1250 and 2500 µg/plate with S9-mix (for all strains)
	Experiment 2:	6.25, 12.5, 25, 50 and 100 µg/plate without S9-mix (for TA98 and TA1537) 12.5, 25, 50, 100 and 200 µg/plate without S9-mix (for TA100) 25, 50, 100, 200 and 400 µg/plate without S9-mix (for TA1535) 100, 200, 400, 1000, 2000 and 4000 µg/plate without S9-mix (for TA102) 100, 200, 400, 1000 and 2000 µg/plate with S9-mix (for TA98, TA100, TA1535 and TA1537) 100, 200, 400, 1000, 2000 and 4000 µg/plate with S9-mix (for TA102)
	Experiment 3:	1000, 1250, 1500, 1750 and 2000 µg/plate with S9-mix (for TA98 and TA1537)
	Experiment 4:	1000, 1250, 1500, 1750 and 2000 µg/plate with S9-mix (for TA98 and TA1537)
Treatment:	Experiment 1 and 2 without S9-mix: direct plate incorporation with 48 - 72 h incubation without and with S9-mix	

	Experiment 2 with S9-mix, 3 and 4: pre-incubation method with 60 minutes pre-incubation and 48 - 72 h incubation without and with S9-mix
GLP:	in compliance
Study period:	30 January 2004 – 18 March 2004

1,2,4-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.
Test concentrations were based on the results of a preliminary toxicity test with strains TA98, TA100 and TA102. Toxicity was evaluated for 6 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or thinning of the bacterial background lawn. Negative and positive controls were in accordance with the OECD guidelines.

Results
No precipitation of 1,2,4-trihydroxybenzene was observed. Without S9-mix toxic effects (i.e. reduction in the number of revertant colonies and/or thinning of the bacterial lawn) was observed at a concentration of ≥ 100 µg/plate for TA98 and TA1537, of ≥ 200 µg/plate for TA100 and TA1535 and of ≥ 2000 µg/plate for TA102. With S9-mix toxic effects was observed at a concentration of ≥ 2000 µg/plate for TA1537 and of ≥ 2500 µg/plate for TA98, TA100 and TA1535.
1,2,4-Trihydroxybenzene induced an increase in the number of revertants in *S. typhimurium* TA98 and TA100 strains in the absence of S9 mix. In the presence S9-mix occasional increases in the number of revertants in TA1537 and TA98 were not reproducible and considered not biologically relevant.

Conclusion
Under the experimental conditions used 1,2,4-trihydroxybenzene was genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 7

In vitro mammalian cell gene mutation test (*hprt* locus)

Guideline:	OECD 476	
Cells:	L5178Y mouse lymphoma cells	
Replicates:	duplicate cultures in two independent tests	
Test substance:	1,2,4-Trihydroxybenzene	
Batch:	0506382	
Purity:	99.4%	
Vehicle:	degassed purified water	
Concentrations:	Experiment 1:	0.07813, 0.1563, 0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 µg/ml without S9-mix 1.25, 2.5, 5, 10, 20, 40, 80 and 160 µg/ml with S9-mix
	Experiment 2:	5, 7.5, 10, 12.5, 15, 17.5, 20 and 22.5 µg/ml without S9-mix 10, 20, 40, 80, 120, 160, 200 and 240 µg/ml, with S9-mix
Treatment	3 h treatment both without and with S9-mix; expression period 7 days and a selection period of 11-12 days.	
GLP:	in compliance	
Study date:	3 September 2004 – 8 November 2004	

1,2,4-Trihydroxybenzene was assayed for mutation at the *hprt* locus in mouse lymphoma cells using a fluctuation protocol with 8 cells/ml. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were

based on the results of a cytotoxic range-finding experiment with a wide range of test concentrations up to 1260 µg/ml, equivalent to 10 mM, both without and with S9-mix measuring raw plate counts and relative survival. In the main test, cells were treated for 3 h followed by an expression period of 7 days, to fix the DNA damage into a stable *hprt* mutation. Toxicity was measured as relative survival to the survival of the vehicle control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
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.
A biologically relevant increase in the mutant frequency was not observed following treatment with 1,2,4-trihydroxybenzene at any concentration level tested, in the absence or presence of S9-mix in both experiments.
Mutant frequencies in negative control cultures fell within normal ranges, and clear increases in mutant frequency were induced by the positive control.

Conclusion
Under the experimental conditions used, 1,2,4-trihydroxybenzene was not considered genotoxic in this gene mutation assay 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:	duplicate cultures in two independent experiments	
Test substance:	Imexine OAM	
Batch:	Op.29	
Purity:	99.4%	
Vehicle:	DMSO	
Concentrations:	Experiment 1:	1.25, 2.5 and 5 µg/ml without S9-mix 3.75, 7.5 and 15 µg/ml with S9-mix
	Experiment 2:	2.5, 5 and 7.5 µg/ml without S9-mix 10, 15 and 20 µg/ml with S9-mix
Treatment:	Experiment 1:	24 h treatment without S9-mix; harvest time 24 h after the start of treatment 2 h treatment with S9-mix; harvest time 24 h after the start of treatment
	Experiment 2:	24 h and 48 h treatment without S9-mix; harvest time 24 h and 48 h after the start of treatment 2 h treatment with S9-mix; harvest time 24 h and 48 h after the start of treatment
GLP:	in compliance	
Study date:	13 July 1993 – 11 January 1994	

Imexine OAM has been investigated for induction of chromosome aberrations in cultured human lymphocytes in the absence and presence of metabolic activation. Liver S9 mix from rats treated with Arochlor 1254 was used as the exogenous metabolic activation system. After isolation the lymphocytes were stimulated to divide with 3.6% phytohaemagglutinin for 48 h. Test concentrations were based on the results of a preliminary toxicity assay with concentrations up to 2000 µg/ml measuring the mitotic index.
In the main test, cells were treated for 24 h or 48 h (without S9-mix) or 2 h (with S9-mix) and harvested 24 h or 48 h after the start of treatment. Approximately 2 h before harvest,

each culture was treated with colcemid (0.2 µg/ml culture medium) to block cells at metaphase of mitosis. Reduction in the mitotic index was taken as a measure for cytotoxicity. Negative and positive controls were in accordance with the OECD guideline.

Results
At the 48 h harvest times the mitotic indices found were reduced by approximately 40-50% compared to the untreated controls, for the other experiments the mitotic indices were similar or only slightly reduced compared to the controls.
For both tests the number of cells with chromosome aberrations in the untreated controls was within the range of the historical control data. Imexine OAM did not induce a biologically relevant increase in the number of cells with chromosome aberrations at any concentration level tested, in the absence or presence of S9-mix in both experiments.

Conclusion
Under the experimental conditions used, Imexine OAM was not genotoxic (clastogenic) in this chromosome aberration test in human lymphocytes both in the absence and the presence of S9 metabolic activation.

Ref.: 9

Comment
The concentrations tested did not induce the required degree of cytotoxicity and an insufficient number of cells was evaluated in some cases. Altogether, this value of this test is limited.

In vitro micronucleus test

1,2,4-Benzenetriol has been investigated for the induction of micronuclei in cultured human peripheral blood lymphocytes. The cytokinesis-block micronucleus test was performed in combination with *in situ* hybridisation with specific centromeric probes for chromosomes 7 and 8 to allow discrimination between a clastogenic and an aneugenic mode of action. Human lymphocytes were, after isolation, stimulated to divide with 1% phytohaemagglutinin for 24 h. Cultures of human peripheral blood lymphocytes were then treated with 10, 25, 50 and 100 µM 1,2,4-benzenetriol for 48 h. The final 28 h of incubation was in the presence of cytochalasin B (final concentration 6 µg/ml).
One thousand binuclear cells were scored for the presence of micronuclei.
A concentration-dependent and statistically significant increase in the number of lymphocytes with micronuclei was found. Also a concentration-dependent and statistically significant induction of aneuploidy of chromosomes 7 and 8 was observed. Next to loss of chromosomal material in the micronucleus, also non-disjunction of chromosomes 7 and 8 was seen.

Conclusion
Under the experimental conditions used 1,2,4-benzenetriol was clastogenic and aneugenic in cultured human peripheral blood lymphocytes.

Add. Ref.: 3

Comment
The data are from a publication in the open literature. The test was not conducted in compliance with GLP and OECD guidelines. The purity of 1,2,4-benzenetriol was not reported.

In vitro sister chromatid exchange test

1,2,4-Benzenetriol has been investigated for the induction of sister chromatid exchanges in cultured human peripheral blood lymphocytes. The lymphocytes, isolated from one healthy

male, were stimulated to divide with 8µg concanavalin A/ml for 24 h. Cultures of human peripheral blood lymphocytes were then treated with 5, 50, 70, 100, 300 and 500 µM 1,2,4-benzenetriol for 48 h in the presence of 5-Bromo-2'-deoxyuridine (final concentration 5 µM). The final 4 h of incubation was in the presence of demecolcine (final concentration 1.35 µM).

The concentration-dependent decrease of the mitotic activity after treatment with 1,2,4-benzenetriol indicated to sufficient cellular exposure. A concentration-dependent increase in the sister chromatid exchanges frequency in human lymphocytes was observed.

Conclusion
Under the experimental conditions used 1,2,4-benzenetriol was genotoxic in this sister chromatid exchange test in cultured human peripheral blood lymphocytes.

Add. Ref.: 1

Comment
The data are from a publication in the open literature. The test was not conducted in compliance with GLP or OECD guidelines. The purity of 1,2,4-benzenetriol was not reported. The test has only limited value and can at most be used for confirmation purposes.

***In vitro* DNA strand break test**

1,2,4-Benzenetriol has been investigated for the induction of DNA strand breaks in cultured bone marrow cells of femurs of female ICR mice. Cultures of murine bone marrow cells were treated with 6, 12 and 24 µM 1,2,4-benzenetriol for 1 h. An alkaline DNA elution method (pH> 9.5) was used. Fractions were collected every 24 minutes over a total of 120 minutes. The amount of DNA eluted was determined by a microfluorometric assay using an emission of 525 nm and an excitation at 405 nm. Bone marrow cell viability was measured by trypan blue dye exclusion following a 1 or 2 h exposure.

A concentration-dependent increase in alkali-labile DNA single strand breaks in bone marrow cells was observed.

Conclusion
Under the experimental conditions used, 1,2,4-benzenetriol was genotoxic in bone marrow cells of the mouse.

Add. Ref.: 2

Comment
The data are from a publication in the open literature. The test was not conducted in compliance with GLP or OECD guidelines. The purity of 1,2,4-benzenetriol was not reported. The test has only limited value and can at most be used for confirmation purposes.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Taken from SCCP/0962/05 (modified)

Mouse bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	Mouse, Swiss OF1
Group size:	5 mice/sex/dose
Test substance:	Imexine OAM
Batch:	Op.29
Purity:	99.4%
Solvent:	Water

Dose levels: 50 mg/kg bw
Treatment: Single i.p. injection
Sacrifice time: 24 hours and 48 hours after treatment
GLP: in compliance
Study date: 21 September 1992 – 25 January 1993

Imexine OAM has been investigated for the induction of micronuclei in the bone marrow cells of mice. Test doses were based on the results of preliminary assays with i.p. doses between 50 and 2000 mg/kg bw using groups of 2 mice per dose. Mortality and clinical signs were recorded over a period of 48 h.
In the micronucleus test mice were treated by i.p. injection with 0 and 50 mg/kg bw Imexine OAM. Bone marrow cells were collected 24 h and 48 h 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 preliminary study the administration of 2000, 1000 and 500 mg/kg bw induced the death of all mice just after treatment. The administration of 100 mg/kg bw induced piloerection and hypokinesia 2 h after treatment, sedation after 6 h and the death of all mice 24 h after treatment. The administration of 50 mg/kg bw induced piloerection and hypokinesia 6 h after treatment in all mice and piloerection in most mice 24 and 48 h after treatment.
In the micronucleus test after treatment with Imexine OAM piloerection and hypokinesia were observed a few h after treatment. One mouse died and was replaced with a mouse from a supplementary treated group. The ratio of polychromatic to normochromatic erythrocytes decreased statistically 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. At the two sampling times, the number of micronucleated polychromatic erythrocytes in mice exposed to Imexine OAM did not differ from the simultaneous vehicle control values.

Conclusion
Under the experimental conditions used Imexine OAM did not induce a biologically relevant increase in the number of erythrocytes with micronuclei of treated mice and, consequently, Imexine OAM is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.
Ref.: 10

Comment
The protocol of this test is not in accordance with the current OECD guideline, because only one dose was tested.

3.3.7. Carcinogenicity

Taken from SCCP/0962/05

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 (GHS carc. Cat. 1B) 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

Taken from SCCP/0962/05

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
1,2,4-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)

Not applicable

3.3.14. Discussion

Physico-chemical specifications
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% on head. The identification and quantification of 1,2,4-trihydroxybenzene in the batches Op.29 and 0502124 was not sufficiently performed. A complete identification and quantification of 1,2,4-trihydroxybenzene in these batches, using state of art methods, is required.
Identification and determination of impurities in these batches should also be performed. The content of 1,2,4-trihydroxybenzene, determined using Op.29 as reference standard, can only be considered as semi-quantitative determination.
The stability testing of 1,2,4-trihydroxybenzene in solutions is inadequate, because it is performed after storage of test solutions in dark and under inert atmosphere. A degradation of circa 8% within one week of 1,2,4-trihydroxybenzene (content 3%) in the test formulation, used for dermal absorption study, was found even though the test item was stored under an inert atmosphere. It is known that 1,2,4-trihydroxybenzene is air-oxidised to form a dimer, which in the presence of ammonia forms a brown colour (Morrel and Christie 2011). The consumer is exposed to 1,2,4-trihydroxybenzene in ambient air. Therefore, for the safety assessment of 1,2,4-trihydroxybenzene, dermal absorption data not only for 1,2,4-trihydroxybenzene, but also for its dimer and the final colour by reaction with ammonia, under the use conditions of hair dye formulation, are required.

Stability of 1,2,4-trihydroxybenzene in typical hair dye formulations was not reported.

Toxicity
An acute dermal toxicity study in rats was performed, and the maximal non-lethal dose of 1,2,4-trihydroxybenzene was found to be 2000 mg/kg bw.
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 be slightly irritant to rabbit skin and to the rabbit eye.

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

Percutaneous absorption
The experiment was conducted with a direct dye formulation containing 2.78% 1,2,4-trihydroxybenzene and not 3%. The dose was slightly below that requested for use and stability in the receptor is not quantified. Therefore, the amount considered as being absorbed, is the mean + 2SD. This is 0.03% of the applied dose or 0.17 µg/cm^2.

Mutagenicity
Overall, the genotoxicity of 1,2,4-trihydroxybenzene is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. 1,2,4-trihydroxybenzene induced gene mutations in bacteria (a slight but reproducible mutagenic activity in *S. typhimurium* TA98 and TA100 without metabolic activation) but not in mammalian cells. 1,2,4-Trihydroxybenzene did not induce an increase in cells with chromosome aberrations but the relevance of this test is questionable since the test conditions were considered as insufficient by the SCCS. In an *in vitro* micronucleus test in combination with *in situ* hybridisation with specific centromeric probes for chromosomes 7 and 8, a concentration-dependent and statistically significant increase in the number of lymphocytes with micronuclei as well as in aneuploid cells was found. Moreover, 1,2,4-trihydroxybenzene induced an increase in sister chromatid exchanges in human peripheral blood lymphocytes and an induction of DNA single strand breaks in murine bone marrow cells.
The positive findings from the *in vitro* tests covering both chromosome aberrations and aneuplody were not confirmed in an *in vivo* test. In an *in vivo* micronucleus test, 1,2,4-trihydroxybenzene exposure of mice did not result in an increase in erythrocytes with micronuclei. However, the positive finding in the gene mutation test in bacteria was not confirmed nor overruled with an *in vivo* test measuring the same genotoxic endpoint.
Consequently, on the basis of these tests 1,2,4-trihydroxybenzene has to be considered as an *in vitro* genotoxin. *In vivo* testing would be required to explore the potential to induce gene mutations; such tests are no longer permitted.

Carcinogenicity
No conclusion with regard to carcinogenicity can be made from the mice topical application carcinogenicity study submitted.

4. CONCLUSION

The SCCS 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:

- Proper characterisation and quantification of 1,2,4-Trihydroxybenzene as well as identification and quantification of impurities in all test batches.

- Characterisation of the oxidation reaction product(s) of 1,2,4-trihydroxybenzene to which the consumer is exposed, because of the reported instability of 1,2,4-trihydroxybenzene in aqueous systems. In the case of relevant exposure to the reaction products, further toxicity data might be required.

- *In vivo* testing would be required to explore the potential to induce gene mutations; such tests are no longer permitted.

1,2,4-Trihydroxybenzene was found to be an extreme 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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