EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

1,5-Naphthalenediol

COLIPA n° A18

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 8th plenary meeting

of 21 September 2010

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 Evaluation 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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ISSN 1831-4767
doi:10.2772/27149

ISBN 978-92-79-12751-9
ND-AQ-09-023-EN-N

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

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, hair dye, A18, 1,5-naphthalenediol, directive 76/768/ECC, CAS 83-56-7, EC 201-487-4

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 1,5-naphthalenediol, 21 September 2010

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1. BACKGROUND

Submission I for 1,5-Naphthalenediol was submitted in June 1985 by COLIPA[1].

The first opinion (SCCNFP/XXIV/1285/97) on 1,5-Naphthalenediol was adopted by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) the 20 Mai 1998 with the conclusion, that "*Based on the data presented in the two submissions it can be concluded that 1,5-naphthalenediol does not pose a hazard to human health for the intended use and at the dose level of 1% in hair dye formulations, which corresponds to 0.5% upon application*".

Submission II for 1,5-Naphthalenediol was submitted in Dec 2005 by COLIPA.

The second opinion (SCCP/1060/06) on 1,5-Naphthalenediol was adopted by the Scientific Committee on Cosmetic Products the 21 March 2007 with the conclusion "*that the information submitted is insufficient to allow a final risk assessment to be carried out.*
Before any further consideration, an in vitro percutaneous absorption study should be performed following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.
The results from a LLNA study indicate that 1,5-naphthalenediol is a skin sensitiser.
1,5-Naphthalenediol itself has no mutagenic potential in vivo.
However, studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance."

The substance is currently regulated in Annex III, part 2 under entry 32 on the preliminary list of substances, which cosmetic products must not contain except subject to restrictions and conditions laid down.

According to submission III 1,5-Naphthalenediol is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye-stuff. The reaction is accelerated by addition of an oxidising agent (e.g. hydrogen peroxide). The final concentration on the scalp is proposed to be 1.0%.

The current submission contains a new dermal absorption study.

2. TERMS OF REFERENCE

1. Does SCCS consider 1,5-Naphthalenediol safe for use in oxidative and non-oxidative hair dyes with a maximum concentration up to 1.0% taken into account the scientific data provided?

2. And/or does the SCCS has any further scientific concerns with regard to the use of 1,5-Naphthalenediol in oxidative hair dye formulations?

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

1,5-Naphthalenediol (INCI)

3.1.1.2. Chemical names

Naphthalene-1,5-diol
1,5-Naphthalenediol
1,5-Dihydroxynaphthalene

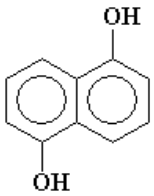
3.1.1.3. Trade names and abbreviations

COLIPA A 018
C.I. 76625
Durafur Developer E
Rhodol 15 N
Ro 576

3.1.1.4. CAS / EC number

CAS: 83-56-7
EC: 201-487-4

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{10}H_8O_2$

3.1.2. Physical form

Grey to light brown powder

3.1.3. Molecular weight

Molecular weight: 160.17

3.1.4. Purity, composition and substance codes

Material used in the market

Purity by NMR assay:	> 97.5% (w/w)
Purity by HPLC assay:	> 99% (area)
Water (Karl-Fischer):	< 1% (w/w)
Sulphated ash:	< 0.3% (w/w)
Impurities:	1-Naphthol < 0.3% (w/w)
	2-Naphthol < 100 ppm
Heavy metals	
	Hg: < 1 ppm
	Pb: < 20 ppm
	Sb, Ni: < 10 ppm
	Cd, As: < 5 ppm

Ref.: 1

Batch 820211/01 = SAT 030627 = SAT 040279

The structural identity of the test sample Naphthalene-1,5-diol, batch 820211/01 has been confirmed by ^{1}H, ^{13}C- and DEPT NMR-spectra and is additionally supported through the IR- and UV-spectra.

Quantitative ^{1}H-NMR-spectroscopy of the test sample was carried out using an internal standard for quantification. The purity was determined by HPLC with UV-detection. The quantification of impurities was made through calibration with 1-naphthylamine as external standard. Experiments with 2-naphthol, naphthalene-2,6-diol and 1-naphthylamine as external standard showed that the concentration of each of these substances was below the detection limit.

Results	
Identity verified by:	^{1}H-, ^{13}C-NMR-spectroscopy, DEPT-spectrum in D_2O / NaOD, IR-spectrometry and UV-spectrometry
Purity:	99.9% (area) determined by HPLC
	97.7% (w/w) as determined by NMR
Water (Karl-Fischer):	0.2% (w/w)
Sulphated Ash:	0.4% (w/w)
Impurities:	
1-Naphthol:	940 ppm
2-Naphthol:	< detection limit (50 ppm)
Naphthalene-2,6-diol:	< detection limit (20 ppm)
1-Naphthylamine:	< detection limit (20 ppm) (banned according to Directive 76/768/EEC: Annex II, entry n° 242)

Ref.: 2

Batch Ro 576 (internal material code 774)

Identity:	verified by IR-spectrometry and UV-spectrometry
Purity:	96.8% (area) determined by HPLC
Impurities:	
1-Naphthol:	0.57%
2-Naphthol:	39 ppm
Hg:	< 0.5 ppm
Ni, Cr, Co, Pb, Cu, Mn:	< 5 ppm

Cd, As:	< 1 ppm
Fe:	< 10 ppm

Ref.: 3

Batch used in the in vitro chromosomal aberration test:
Only limited information is available on this batch. The purity (HPLC) is given as > 98% [area], which is lower than that of currently used material (> 99%).

3.1.5. Impurities / accompanying contaminants

Solvent content (water): < 1% (w/w)

Impurities:

1-Naphthol	< 1000 ppm
2-Naphthol	< 100 ppm

Ref.: 1

3.1.6. Solubility

In water:	< 1 g/l	room temperature
In ethanol:	10-100 g/l	room temperature
In DMSO:	> 100 g/l	room temperature

Ref.: 1

3.1.7. Partition coefficient (Log P_{ow})

$P_{o/w}$	50.07
Log $P_{o/w}$	1.70 (method EU A.8)

Ref.: 17

3.1.8. Additional physical and chemical specifications

Melting point:	259-261 °C
Boiling point:	decomposes at > 152 °C
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum:	peaks at 330, 316, 299 and 225 nm

Ref. 1 and Summary of submission I

3.1.9. Homogeneity and Stability

Stability of the chromatographic System and End Solutions
The chromatographic system and end solutions containing the test substance (1.01 and 8.02 mg/l) were stable for at least 4.6 hours when stored at room temperature in the dark.

Stability of Standard Solutions
Standard solutions (584 mg/l and 586 mg/l) of the test substance in Tetrahydrofuran were stable for at least 46 hours when stored at room temperature in the dark.

Stability of the Chromatographic System and End Solutions

A 1.01 mg/l and a 8.02 mg/l solution of the test substance in end solution were injected five times throughout the validation sequence (including the beginning and end). The results are summarised in Table 3.

Table 3: Stability of the chromatographic system and end solutions

Elapsed time (hours)	Concentration [mg/l]	Coefficient of variation of the response (n=5) [%]
4.6	1.01	0.8
4.6	8.02	0.6

Since the coefficient of variation at both concentration levels was less than 20%, it was concluded that the chromatographic system was stable over at least a 4.6-hour time interval at the concentration levels tested.

Stability of Standard Solutions

Two standard solutions of 584 and 586 mg/l in Tetrahydrofuran were measured 46 hours after preparation together with two freshly prepared standard solutions of 602 and 652 mg/l. As the coefficient of variation of the response factor was 0.6%, it was concluded that the standard solutions were stable during storage (at room temperature and in the dark).

Ref.: 13

General Comments to physico-chemical characterisation

- The stability of the test substance in typical hair dye formulations was not reported.

3.2. Function and uses

1,5-Naphthalenediol is used in oxidative and non-oxidative hair dye formulations with a maximum on-head concentration of 1%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 423 (2001), acute toxic class method
Species/strain:	Rat, Wistar strain Crl:(WI) BR (outbred, SPF-Quality)
Group size:	3 of each sex
Test substance:	1,5-naphthalenediol
Batch:	820211/01
Purity:	> 99.9 area % (HPLC)
Dose:	2000 mg/kg bw in propylene glycol
GLP:	In compliance

The test substance was administered by oral gavage to three Wistar rats of each sex at 2000 mg/kg bw. Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice (day 15).

Results
No mortality occurred. Hunched posture and piloerection were noted in all animals on day 1. Two males showed piloerection on day 2. The body weight gain shown by the animals over the study period was considered to be normal. No abnormalities were found at macroscopic post mortem examination of the animals.

Conclusion
The oral LD50 value of 1,5-naphthalenediol in Wistar rats was established to exceed 2000 mg/kg bw.

Ref.: 4

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404
Species:	Rabbit, New Zealand White
Group:	3 male
Substance:	A018 / SAT 030627 (1,5-naphthalenediol)
Batch:	820211/01
Purity:	99.9%
Dose:	0.5 g of the test substance moistened with 1 ml water on a semi-occlusive patch
GLP:	in compliance

An aliquot of 0.5 g of the moistened test substance was applied to the clipped back skin of each animal. The patch was removed 4 hours after semi-occlusive contact and the skin was

cleaned, using water and ethanol. The skin reactions were assessed at approximately 1, 24, 48 and 72 hours (all animals) and 7 days (2/3 animals) after removal of the dressings and test substance. Adjacent areas of untreated skin served as control in each animal.

Results
In one animal, slight (24 hours) to well-defined (48, 72 hours) erythema, slight (1, 24 hours) to very slight (48 hours) oedema, and scaling (7 days) was observed after dressing removal. No erythema, oedema or scaling were recorded in 2/3 animals. Yellow staining of the treated skin area was noted 1 to 72 hours after removal of the test substance.

Conclusion
Under the conditions of the study, the test substance caused some irritation to rabbit skin, but did not fulfil the EU criteria for classification as skin irritant.

Ref.: 5

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species:	Rabbit, New Zealand White
Group:	3 male
Substance:	A018 / SAT 030627 (1,5-naphthalenediol)
Batch:	820211/01
Purity:	99.9%
Dose:	39.7 mg (approximately 0.1 ml)
GLP:	in compliance

The equivalent of 0.1 ml of the undiluted test substance was instilled into the conjunctival sac of one eye of each test animal. The eye lids were held together for about one second to avoid loss of test substance. Eye irritant reactions were assessed approximately 1, 24, 48 and 72 hours and 7 and 14 days after instillation. On day 2, after fluorescein examination, the treated eye was rinsed with warm tap water. The untreated eye served as control.

Results
Conjunctival redness was observed in 3 animals at 1 hour to 7 days. Chemosis was observed in 3 animals at 1, 24 and 48 hours and in 1 animal also at 72 hours. Mean value of eye irritation scores (24-72 hours) for each animal were for redness 3.0, 2.7, 2.7; and for chemosis 1.7, 1.0, 1.0. No iridial irritation or corneal opacity was observed.

Conclusion
Under the conditions of the study, A018 was irritant to the rabbit eye.

Ref.: 6

3.3.3. Skin sensitisation

Local Lymph Node Assay

Guideline:	OECD 429
Species:	mice, CBA/J
Group:	females, 3 groups of 5 animals for each of 2 experiments (30 animals)
Substance:	A018 / SAT 030627 (1,5-naphthalenediol)
Batch:	820211/01
Purity:	99.9%
Dose:	Experiment I: 25 µl of A018 at 5, 25 and 50% in acetone/olive oil (4:1 v/v) Experiment II: 25 µl of A018 at 0.25, 1.0 and 2.5% in acetone/olive oil (4:1 v/v)
GLP:	in compliance

A Local Lymph Node Assay was performed to investigate the sensitisation potential of 1,5-naphthalenediol. Initially, 3 groups of animals were treated with 3 test substance concentrations respectively. Based on the results, 3 additional groups were treated with 3 lower concentrations. In each experiment, during the induction phase, the test item was applied over the dorsal surface of each ear (25 µl per ear) for 3 consecutive days (days 1, 2, 3). On day 6, each animal was administered with radio-labelled thymidine (^{3}HTdR) by intravenous injection. After 5 hours, the animals were euthanized and the draining lymph nodes were excised and pooled to prepare single cell suspension for each animal. ^{3}HTdR incorporation was measured by scintillation counting. The proliferative response of lymph node cells was expressed as the ratio of ^{3}HTdR incorporation into lymph node cells of treated animals relative to that recorded in control animals.

Reference was made to separate experiments with vehicle control animals, and for reliability check with alpha-hexylcinnamaldehyde as positive control, respectively.

Results
Experiment I:

Concentration (%)	***Stimulation index (SI)***
5	18.4
25	16.7
50	6.1

Experiment II:

Concentration (%)	***Stimulation index (SI)***
0.25	1.4
1	2.5
2.5	2.8

Stimulation Index values (SI) were calculated for the groups of the Experiments I and II. The data showed a dose-response and an EC3 value of 3.4% was calculated, based on data from the two experiments.

Conclusion
The results indicate that 1,5-naphthalenediol is a moderate skin sensitiser.

Ref.: 7

3.3.4. Dermal / percutaneous absorption

***In vitro* percutaneous absorption (pig skin)**

Guideline:	OECD 428
Species:	pig
Tissue:	Pig skin from 4 pigs (1 male, 3 females). Dermatomed 0.80mm
Group:	12 membranes (3 from each pig) per experiment
Method:	Static diffusion cells, exposed membrane area 3.14 cm^2
Integrity	electrical resistance < 4 kΩ
Substance:	A018
Batch:	SAT 090020; Batch 820211/01
Purity:	99.9% HPLC
Radiolabelled	A018 [^{14}C]; SAT090072B, Batch CFQ40709B1; 98.4% HPLC
Test formulation:	Hair dye formulation containing A018 at 2% mixed 50:50 with sham developer (no peroxide); Hair dye formulation containing A018 at 2% mixed 50:50 with developer containing 6% hydrogen peroxide.
Dose:	1% A018 in both formulations, applied at 20mg/cm^2

Receptor fluid: PBS; 0.9% NaCl
Solubility in receptor: 1g/L.
Detection liquid scintillation counting
GLP: in compliance
Date December 2009

Skin discs of 3.14 cm² were exposed to the formulations for 30 minutes, and exposure was terminated by gently washing with a mild shampoo solution diluted with water (2% v/v).
Both formulations were analyzed with twelve replicates each for adsorbed, absorbed and penetrated amount of the test substance.
In the static system, samples of the receptor fluid were drawn 0.5, 1, 2, 4, 6 and 24 hours after application. The volume of each sample was replaced by fresh receptor fluid.

Results
The quantities penetrated during the 30 minute exposure of A 018 containing formulations and within the 24 hours after application are tabulated as follows:

Analysed Sample	Standard dye formulation in the presence of H_2O_2		Standard dye formulation in the absence of H_2O_2	
	[% of dose]	[µg/cm²]	[% of dose]	[µg/cm²]
Rinsings (total dislodgeable amount)	96.13	196.94	97.01	206.64
Adsorption (stratum corneum)	0.92	1.88	1.11	2.37
Not Bioavailable	97.05	198.82	98.12	209.01
Absorption (epidermis/dermis)	0.86	1.76	0.68	1.45
Penetration (receptor fluid)	0.02	0.05	0.06	0.12
Bioavailable	0.88 ± 0.17	1.81 ± 0.34	0.74 ± 0.17	1.57±0.35
Total recovery / mass balance	97.93	200.63 ± 5.53	98.87	210.58±3.16

The amount of A 018 considered as being systemically available from a standard hair dyeing formulation containing 1% A018 (on head) was 1.81±0.34 µg/cm² (0.88 ± 0.17% of the applied amount) in the presence of H_2O_2 and 1.57±0.35 µg/cm² (0.74 ± 0.17% of the applied amount) in the absence of H_2O_2 in this in vitro dermal penetration study.

Ref.: 16

Comment
This was a well conducted study.
For calculating the MOS the amounts of A018 considered to be available from a hair dye formulation containing it at 1% (on head) are (mean +1SD) 2.15 µg/cm² in the presence of hydrogen peroxide and 1.92 µg/cm² in the absence of hydrogen peroxide.

An *in vitro* dermal absorption study (Ref. 15) using pig skin under non-oxidative conditions only has been described in opinion SCCP/1060/06. This study was considered not acceptable due to methodological shortcoming and the lack of testing under oxidative conditions and thus is not included here.

3.3.5. Repeated dose toxicity

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

No data submitted

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

Guideline: OECD 408 (1998)
Species/strain: Rat, Wistar strain Crl:(WI) BR (outbred, SPF-Quality)
Group size: 12 animals per sex and dose, 5 per sex in recovery groups
Test Substance: 1,5-naphthalenediol

Batch:	820211/01
Purity:	>99.9 area % (HPLC)
Dose levels:	0, 50, 100, 300 mg/kg bw (in propylene glycol)
Route:	Oral, gavage
Exposure period:	13 weeks, 4 weeks recovery
GLP:	in compliance

Wistar rats were treated with 1,5-naphthalenediol for at least 90 consecutive days by oral gavage at dose levels up to 300 mg/kg/day, followed by a 28-day treatment-free recovery period. Mortality and viability were checked at least twice daily. Clinical observations were made in all animals once daily. During week 12-13 of treatment, functional observations tests were performed including hearing ability, pupillary, static righting and grip strength reflex and a motor activity test. Ophthalmoscopic examinations were conducted at pre-test with all animals and at week 13 with controls and the 300 mg/kg bw dose group. Body weights and food consumption were monitored weekly. Blood samples were collected for clinical laboratory investigations immediately prior to scheduled *post mortem* examination and the common parameters were determined.

Results
Two males and three females at 300 mg/kg bw died during the treatment phase. Mis-gavage was considered the cause of death for one high dose male based on microscopic assessment and the other high dose male died during blood sampling. Purple discolouration of the urine was noted in all treatment groups, which resolved immediately after discontinuation of treatment. This effect was probably caused by the test substance and/or a test substance metabolite and was not regarded as adverse.

Hearing ability, pupillary reflex, static righting reflex and grip strength were normal in all animals. Variations were noted in motor activity between treated and control animals but occurred in the absence of a dose-related response and supportive clinical signs. Therefore, they were considered to be of no toxicological relevance. There were no ophthalmology findings at pre-test and in week 13.

Body weights and body weight gain of treated animals remained in the same range as controls over the study period. Food consumption before or after allowance for body weight was similar between treated and control animals throughout the study period.

Haematological parameters of treated rats were considered not to have been affected by treatment. All statistically significant deviations from control mean showed no relationship to dose and were considered to be incidental in nature. The following statistically significant deviations in clinical biochemistry parameters that distinguished treated animals from control animals were considered to be related to treatment:

- increased bilirubin levels in females at 100 mg/kg bw/day and in males and females at 300 mg/kg bw/day
- reduced urea levels in males at 100 and 300 mg/kg bw/day
- increased glucose levels in males and females at 300 mg/kg bw/day
- increased potassium levels in males at 300 mg/kg bw/day
- increased total protein and albumin levels in females at 300 mg/kg bw/day
- reduced aspartate aminotransferase activity in females at 300 mg/kg bw/day

These changes had resolved at the end of the recovery period, whilst increased inorganic phosphate levels were recorded for high dose females.

At the end of treatment, increased absolute kidney weights and kidney to body weight ratios were measured for high dose males. In addition, absolute liver weights were increased in high dose females, while liver to body weight ratios were increased in high dose males and females. These deviations had resolved at the end of the recovery phase.

The following microscopic findings were noted at the end of treatment:

- brown/black tubular pigment in the kidneys (minimal to moderate degree) in 11/12 males and 8/11 females at 300 mg/kg bw/day and in 5/12 males at 100 mg/kg bw/day
- increased severity of hyaline casts was seen in 6/12 males and 2/11 females at 300 mg/kg bw/day and in 2/12 males and 1/12 females at 100 mg/kg bw/day
- increased severity (moderate) of corticomedullary basophilia in 5/12 males at 300 mg/kg/day
- hyperplasia of the squamous epithelium of the limiting ridge in the stomach (minimal to moderate degree) in 11/12 males and 6/11 females at 300 mg/kg bw/day and in 3/12 males and 3/12 females at 100 mg/kg bw/day.

Following the recovery period slight degree of brown/black tubular pigment in the kidneys was recorded in 2/5 males at 300 mg/kg bw/day. At 300 mg/kg bw/day one male had corticomedullary basophilia and slight squamous hyperplasia was recorded in 2/5 females after the recovery period.

Due to the effects on kidneys and the forestomach a No Observed Adverse Effect Level (NOAEL) for 1,5-naphthalenediol of 50 mg/kg bw/day was established.

Ref.: 12

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

Bacterial gene mutation assay

Guideline:	OECD 471	
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537.	
Replicates:	triplicates in 2 individual experiments both in the presence and absence of S9-mix	
Test substance:	A 018 (1,5-naphthalenediol)	
Solvent:	DMSO	
Batch:	820211/01	
Purity:	99.9 %	
Concentrations:	Experiment I:	33 - 5000 µg/plate without and with S9-mix
	Experiment II:	10 - 5000 µg/plate without and with S9-mix
Treatment:	Experiment I:	direct plate incorporation with at least 48 h incubation without and with S9-mix
	Experiment II:	pre-incubation method was used with 60 minutes pre-incubation and at least 48 h incubation without and with S9-mix
GLP:	In compliance	

1,5-Naphtalenediol was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a preliminary toxicity test with strains TA98, TA100, TA102, TA 1535 and TA 1537 both without and with S9-mix. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or thinning of the bacterial background lawn. Since in this pre-experiment evaluable plates were obtained for five concentrations or more in all strains used the pre-experiment is reported as experiment I.

Experiment I was performed with the direct plate incorporation method, experiment II with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation of 1,5-naphthalenediol was observed in experiment I at 5000 µg/plate and in experiment II at 2500 and 5000 µg/plate both without and with S9-mix.
In experiment I without S9-mix toxic effects were observed at 2500 µg/plate for TA102 and TA1535 and at 5000 µg/plate for TA98, TA100 and TA1537; with S9-mix at 5000 µg/plate for TA100, TA102 and TA1535. In experiment II without S9-mix toxicity was reported at 1000 µg/plate for TA98 and TA100 and at 2500 µg/plate for TA102; with S9-mix at 1000 µg/plate for TA102 and at 2500 µg/plate TA98.
Both in experiment I and II no biological relevant increase in revertant colonies was seen in any of the five tester strains following treatment with 1,5-naphthalenediol neither in the absence nor in the presence of S9-mix.

Conclusion
Under the experimental conditions used 1,5-naphthalenediol was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 8

In Vitro **Mouse Lymphoma assay (*tk* locus*)***

Guideline:	OECD 476	
Cells:	L5178Y Mouse lymphoma cells	
Replicates:	two parallel cultures in 2 independent experiments	
Test substance:	A 018 (1,5-naphthalenediol)	
Solvent:	DMSO	
Batch:	820211/01	
Purity:	99.9 %	
Concentrations:	Experiment I:	15.0 - 60.0 µg/ml (without S9-mix) 0.25 - 2.0 µg/ml (with S9-mix)
	Experiment II:	6.25 - 50.0 µg/ml (without S9-mix) 0.125 - 1.25 µg/ml (with S9-mix)
Treatment	Experiment I:	4 h treatment without and with S9-mix; expression period 72 h and selection period of 10-15 days
	Experiment II:	24 h treatment without S9-mix; expression period 48 h and selection period of 10-15 days 4 h treatment with S9-mix; expression period 72 h and selection period of 10-15 days.
GLP:	In compliance	

1,5-Naphthalenediol was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9-mix metabolic activation. Test concentrations were based on the results of a pre-test on toxicity measuring relative suspension growth. In the main test, cells were treated for 4 h or 24 h (without S9-mix experiment II) followed by an expression period of 72 or 48 h to fix the DNA damage into a stable *tk* mutation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
There was no relevant shift in pH values even at the maximal concentration of 1,5-naphthalenediol in the pre-test nor in osmolarity measured at the highest concentration in experiment I without S9-mix.

In experiment I in culture I in the presence of S9-mix and in culture II in the absence of S9-mix the appropriate level of toxicity (10-20% survival after the highest dose) was not reached pointing to insufficient exposure of the cells. In experiment II both in the absence and presence of S9-mix the appropriate level of toxicity was reached.

Both in experiment I and II independent of the presence or absence of S9-mix a biological relevant, reproducible increase in the number of mutant colonies was not observed. The occasional increases in mutant frequency with an induction factor > 2 were found at high cytotoxic concentrations and predominantly within the range of the historical control data. Therefore these increases were considered biologically irrelevant

Conclusion
Under the experimental conditions used, 1,5-naphthalenediol was considered not mutagenic in the mouse lymphoma assay at the *tk*-locus.

Ref.: 9

In vitro chromosome aberration test

Guideline:	OECD 473		
Replicates:	duplicate cultures		
Cells:	V79		
Test substance:	naphthalin-1,5-diol (1,5-naphthalenediol)		
Solvent:	DMSO		
Batch:	not indicated by the sponsor		
Purity:	> 98%		
Concentrations:	Experiment I:	harvest time 18 h:	1.0 - 10.0 µg/ml (without S9-mix)
			0.1 - 1.0 µg/ml (with S9-mix)
	Experiment II:	harvest time 18 h:	1.0 - 5.0 µg/ml (without S9-mix)
			0.1 - 0.6 µg/ml (with S9-mix)
		harvest time 28 h:	5.0 µg/ml (without S9-mix)
			0.6 µg/ml (with S9-mix)
Treatment:	18 or 28 h followed immediately by harvest in the absence of S9-mix. 4 h treatment and harvest time 18 or 28 h after start of treatment in the presence of S9-mix		
GLP:	In compliance		

1,5-Naphthalenediol has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in V79 cells. Test concentrations were based on the results of a pre-test on cell number and cell morphology 4h and 18-20 h after start of treatment of high density cultures as well as on viability 18-20 h after treatment (XTT assay). The highest dose in the pre-test was the prescribed maximum concentration (1600 µg/ml ≈ 10 mM).
In the absence of S9-mix, cells were treated for 18 or 28 h and immediately harvested; in the presence of S9-mix, cells were treated for 4 h and harvested 18 or 28 h after the start of treatment. 2.5 h before harvest, each culture was treated with colcemid (final concentration 0.2 µg/ml) to block cells at metaphase of mitosis. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system.

Toxicity was determined by measuring the decrease in the mitotic index. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations and the mitotic index. Negative and positive controls were in accordance with the OECD guideline.

Results

In the pre-test precipitation was observed at concentrations of 600 µg/ml and above. In the main test the mitotic indices after the highest doses were never reduced to about 50% compared to the negative controls both in the absence or presence of S9-mix.
In the absence of S9-mix, 1,5-naphthalenediol induced at both treatment times a more or less dose dependent biologically relevant increase in cells with chromosomal aberrations. In the presence of S9-mix an increase was found in experiment I (18 h harvest time, highest dose only) and experiment II at 28 h harvest time. A negative result was observed in experiment II at 18 h harvest time but the highest dose in this experiment was also negative in experiment I under identical conditions.
In both the absence and the presence of S9-mix, 1,5-naphthalenediol did not cause an increase in polyploidy.

Conclusion
Under the experimental conditions used, the increase in cells with structural chromosomal aberrations indicates a genotoxic (clastogenic) activity of 1,5-naphthalenediol in V79 cells *in vitro*.

Ref.: 10

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	NMRI
Group size:	5 mice/sex/group
Test substance:	A 018 (1,5-naphthalenediol)
Batch no:	820211/01
Purity:	99.9 %
Dose level:	12.5, 25.0 and 50.0 mg/kg bw
Route:	*i.p.*
Vehicle:	aqueous DMSO (30%)
Sacrifice times:	24 and 48 h after the treatment.
GLP:	In compliance

1,5-Naphthalenediol has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the acute toxicity in a pre-test, measured at various intervals around 1 to 48 h after treatment. In the main experiment mice were exposed to single *i.p.* doses of 0, 12.5, 25 and 50 mg/kg bw. 24 h or 48 h (highest dose only) after dosing bone marrow cells were collected. The animals of the highest dose group were examined for acute toxic symptoms 1, 2-4, 6 and 24 h after start of treatment.
Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Satellite groups of 3 male mice per sampling time (20 min, 40 min, 1 h and 4 h after start of treatment) treated with 50 mg/kg bw were included for determination of blood concentrations of 1,5-naphthalenediol.
Bone marrow preparations were stained with May-Grünwald and examined microscopically for the PCE/TE ratio and micronuclei. 5 mice/sex/group were analysed; the remaining 6^{th} animals of each group were only evaluated in case a mouse died spontaneously. Negative and positive controls were in accordance with the OECD draft guideline.

Results
Treatment with 1,5-naphthalenediol did not result in substantially decreased PCE/TE ratios compared to the untreated controls indicating that 1,5-naphthalenediol did not have cytotoxic properties in the bone marrow. In contrast, clinical signs like reduction in spontaneous activity, abdominal position and ruffled fur indicating systemic toxicity were observed at all doses in most treated animals up to 24 h after start of the treatment. 1,5-naphthalenediol could be quantified in the blood of the treated males 20 minutes after start

of the treatment but not at later time points confirming the bioavailability of 1,5-naphthalenediol.
Biological relevant increases in the number of micronucleated PCEs compared to the concurrent vehicle controls were not found following treatment with 1,5-naphthalenediol at any time point or dose level tested.

Conclusion
Under the experimental conditions used 1,5-naphthalenediol did not induce an increase in bone marrow cells with micronuclei in treated mice and, consequently, is 1,5-naphthalenediol not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.
Ref.: 11

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Prenatal development toxicity study

Guideline:	OECD 414
Species/strain:	Sprague-Dawley CD rats (outbred, SPF quality)
Group size:	30 pregnant females per dose group
Test Substance:	1,5-Naphthalenediol
Batch:	Ro 576
Purity:	/
Dose levels:	0, 20, 60, 360 mg/kg bw/day (in 1% carboxymethylcellulose with 0.5% cremophor in water)
Treatment period:	Days 6 - 15 of gestation, oral gavage
GLP:	In compliance

1,5-Dihydroxynaphthalene was administered to pregnant rats orally by gavage once daily from day 6 to day 15 post coitum to the four dose groups 0, 20, 60 and 360 mg/kg bw/day. Each group consisted of 30 rats. Control animals were dosed with the vehicle alone. Females were sacrificed on day 20 post coitum and the foetuses were removed by Caesarean section. The examination of the dams and foetuses was performed in accordance with international recommendations.

Results
The dams tolerated the applied dose of up to 360 mg/kg bw without lethality or symptoms of cumulative intoxication. But, the corrected mean body weight gain of the high dose group was significantly decreased compared with the control animals indicating maternal toxicity. It was argued that this was due to the concomitant increase in mean uterus weight. However, at 20 mg/kg bw, a similar increase in mean uterus weight was not associated with a change of the corrected mean body weight.
There were no treatment related effects in the gestation data. The examined foetuses showed no treatment related malformations. The skeletal ossification in all groups was considered to be within normal range. According to the authors, the test substance is not cumulative toxic to pregnant rats and does not reveal embryotoxic or teratogenic potential at dose levels up to 360 mg/kg bw/day.

Ref.: 14

Comment
The NOAEL of embryo/foetotoxicity and teratogenicity was 360 mg/kg bw/day. The NOAEL of maternal toxicity was considered 60 mg/kg bw/day.

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

1,5-naphthalenediol
(oxidative conditions)

Absorption through the skin	**A (mean + 1SD)**	**=**	**2.15 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.25 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.02 mg/kg bw/d**
No observed adverse effect level (90-day study, oral, rat)	**NOAEL**	**=**	**50 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**=**	**2500**

The value obtained for dermal absorption under non-oxidative conditions (1.92 µg/cm²) was comparable to the one used in the calculation above, resulting in a similar MoS. Therefore only the calculation with the higher value under oxidative conditions is given.

3.3.14. Discussion

Physico-chemical specifications
The stability of the test substance in typical hair dye formulations was not reported.

General toxicity
Due to the effects on kidneys and the forestomach a No Observed Adverse Effect Level (NOAEL) for 1,5-Naphthalenediol of 50 mg/kg bw/day was established in a subchronic oral toxicity study in rats.
In a teratogenicity study in rats the NOAEL of embryo/foeto-toxicity and teratogenicity was 360 mg/kg bw/day. The NOAEL of maternal toxicity was considered 60 mg/kg bw/day.

Irritation / sensitisation
Under the conditions of the study, the test substance caused irritation to rabbit skin and eye. The results from a LLNA study indicate that 1,5-naphthalenediol is a moderate skin sensitiser.

Dermal absorption
An *in vitro* dermal absorption study with pig skin under oxidative and non-oxidative conditions has been performed. The amount of 1,5-Naphthalenediol considered as being systemically available from a standard hair dyeing formulation containing 1% 1,5-Naphthalenediol (on head) are 2.15 µg/cm² in the presence of hydrogen peroxide and 1.92 µg/cm² in the absence of hydrogen peroxide (representing mean +1SD).

Mutagenicity
1,5-Naphthalenediol did not produce gene mutations in bacteria nor in mammalian cells on the *tk* locus of mouse lymphoma cells. Indications for clastogenicity were observed in an *in vitro* chromosome aberration test (V79 cells). In an *in vivo* bone marrow micronucleus tests in mice performed up to lethal doses, an increase in the number of micronucleated erythrocytes was not found.
Overall, the genotoxicity program on 1,5-naphthalenediol is sufficient investigating the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. 1,5-naphthalenediol did not induce gene mutations. The increase in cells with chromosomal aberrations *in vitro* was not confirmed in an adequate bone marrow micronucleus test in mice.
Consequently, 1,5-naphthalenediol itself can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.
To reach a definitive conclusion, appropriate tests with 1,5-naphthalenediol in combination with hydrogen peroxide have to be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

The results from a LLNA study indicate that 1,5-naphthalenediol is a moderate skin sensitiser.

The SCCP is of the opinion that 1,5-Naphthalenediol as an ingredient in oxidative and non-oxidative hair dye formulations at a maximum on-head concentration of 1% does not pose a risk to the health of the consumer, apart from its sensitising potential.

1,5-Naphthalenediol itself has no mutagenic potential *in vivo*.
However, studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

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