EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

2,7-Naphthalenediol

COLIPA n° A19

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:10.2772/27368

ISBN 978-92-79-12752-6
ND-AQ-09-024-EN-N

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, hair dye, A19, 2,7-naphthalenediol, directive 76/768/ECC, CAS 582-17-2, EC 209-478-7

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 2,7-naphthalenediol, 21 September 2010

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1. BACKGROUND

The first opinion on 2,7-Naphthalenediol was adopted by the Scientific Committee on Cosmetology (SCC) the 19 February 1991 with the conclusion, that .."*requires a cytogenetic and a mouse lymphoma gene mutation in vitro study with full specifications of the compound tested and the nature and quantity of impurities eventually present, including mono-, di-, and trioxide naphthalene.*"
This opinion was revised June 29, 1993 and approved December 10, 1993.

The second opinion (SCCNFP/0232/99) on 2,7-Naphthalenediol was adopted by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) the 17th February 2000 with the conclusion, that "*2,7-Naphthalenediol can be used safely in permanent hair dye formulations at a maximum concentration of 1.0%. However, as permanent hair dyes are mixed with hydrogen peroxide before application, the maximum in-use concentration should not exceed 0.5 %"*.

The substance is currently regulated in Annex III, part 2 under entry 4 on the preliminary list of substances, which cosmetic products must not contain except subject to restrictions and conditions laid down on the basis of the above opinion.

After the adaptation of the hair dye strategy all hair dyes substances should be evaluated or re-evaluated according to the Notes of Guidance.

The third opinion (SCCP/1061/06) on 2,7-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.
2,7-naphthalenediol is a moderate sensitizer.
2,7-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".

According to the submission III, 2,7-Naphthalenediol is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye-stuff. The reaction can be accelerated by addition of an oxidising agent (e.g. hydrogen peroxide), but it can also be achieved by air oxidation. The final concentration on the scalp is of 1.0% in oxidative hair dye formulations.

The current submission contains a new dermal absorption study.

2. TERMS OF REFERENCE

1. *Does SCCS consider 2,7-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 2,7-Naphthalenediol in oxidative hair dye formulations?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1.	Chemical identity

3.1.1.1.	Primary name and/or INCI name

2,7-Naphthalenediol (INCI)

3.1.1.2.	Chemical names

Naphthalene-2,7-diol
2,7-Naphthalenediol
2,7-Dihydroxynaphthalene

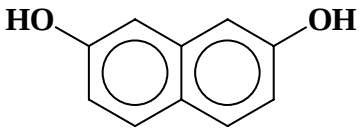
3.1.1.3.	Trade names and abbreviations

C.I. 76645
Ro 575
COLIPA A 019

3.1.1.4.	CAS / EC number

CAS: 582-17-2
EC: 209-478-7

3.1.1.5.	Structural formula



3.1.1.6.	Empirical formula

Formula: $C_{10}H_8O_2$

3.1.2.	Physical form

Light grey, slightly yellow, amorphous powder

3.1.3.	Molecular weight

Molecular weight: 160.17

3.1.4.	Purity, composition and substance codes

Material used on the market

Purity by NMR assay: > 98% (w/w)
Purity by HPLC assay: > 99% (area)

Water (Karl-Fischer):	< 1.0% (w/w)
Impurities:	
1-Naphthol:	< 500 ppm
2-Naphthol:	< 1000 ppm
2,6-Dihydroxynaphthalene:	< 250 ppm
Sulphated Ash:	< 0.5% (w/w)
Heavy Metal Content	
Pb:	< 20
Sb and Ni:	< 10
As and Cd:	< 5
Hg:	< 1 ppm

Ref.: 1

Batch 20020517 = SAT 030628 = SAT 030387 = SAT 040232

The structural identity of the test sample Naphthalene-2,7-diol, batch 20020517 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.

Identity verified by NMR-spectroscopy, IR-spectrometry and UV-spectrometry.

Purity:		
NMR assay:	101% (w/w)	
HPLC assay:	99.9% (area)	
Water (Karl-Fischer):	< 0.1% (w/w)	
Impurities:		
1-Naphthol:	< 50 ppm	
2-Naphthol:	763 ppm	(banned according to directive 768/76/EEC: annex II, entry n° 241)
2,6-Dihydroxynaphthalene:	< 10 ppm	
1-Naphthylamine:	< 20 ppm	(banned according to directive 768/76/EEC: annex II, entry n°242)
2-Naphthylamine:	< 20 ppm	(banned according to directive 768/76/EEC: annex II, entry n°242; EC CMR carcinogenic category 1)
Sulphated ash:	< 0.1% (w/w)	

Ref.: 2

Other batches
The batch of A19 used in the acute oral toxicity test is not fully analytically described. However, information is available from the laboratories that have synthesized this batch concerning the identity and purity of the material produced at that time. From this information it can be concluded that the former not fully described batch is representative and its specification is quite similar to the fully characterized batch 20020517.

3.1.5. Impurities / accompanying contaminants

Solvent content (water):	< 1.0% (w/w)
Impurities:	
1-Naphthol:	< 500 ppm

2-Naphthol:	< 1000 ppm
2,6-Dihydroxynaphthalene:	250 ppm
Sulphated Ash:	< 0.5% (w/w)

Ref.: 1

3.1.6.	Solubility

Water:	1 – 10 g/l	room temperature
Ethanol:	> 100 g/l	room temperature
DMSO:	> 100 g/l	room temperature

Ref.: 1

3.1.7.	Partition coefficient (Log P_{ow})

$P_{o/w}$	189.7	
Log $P_{o/w}$	2.278	(EU method A.8)

Ref.: 18

3.1.8.	Additional physical and chemical specifications

Melting point:	184 - 185 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_VIS spectrum:	229 nm (minor peaks at 283 and 325 nm)

3.1.9.	Homogeneity and Stability

No data submitted

General Comments to physico-chemical characterisation

- The stability of the test substance in typical hair dye formulations was not reported.
- The impurity '2-naphthol' is banned according to Directive 768/76/EEC on cosmetic products (Annex 2, entry n° 241).

3.2. Function and uses

2,7-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

Taken from SCCNFP/0232/99

LD_{50} mice CD1, oral: 720 (655-792) mg/kg bw
rat, oral: > 5000 mg/kg bw
(1% of formulation containing 2,7-dihydroxynaphthalene)

Ref.: 1, 2 (opinion SCCNFP/0232/99)

Guideline: /
Species/strain: Rat, CFY
Group size: 10 (5male, 5 female)
Test substance: 2,7-Dihydroxynaphthalene
Batch: /
Purity: /
Doses: 1600, 2000, 3200 and 4000 mg/kg bw
20% suspension in aqueous carboxymethylcellulose by gavage
Observation: 2 weeks
GLP: /

2,7-Dihydroxynaphthalene was given by gavage as a 20% suspension in aqueous carboxymethylcellulose and mortalities and clinical-toxicological observations were recorded for 2 weeks.

Results
At all doses, animals showed lethargy and pale extremities. Hunched posture and rough fur were temporarily observed. With increasing doses, the rats collapsed or died immediately. Post-mortem revealed hemorrhagic oedema and hyperaemia of the lung and the liver, hemorrhagic erosion in the stomach mucosa and partial hyperaemia of the duodenum with bloody-mucous content.

Sex	***Dosage (mg/kg bw)***	***Mortality (no. deaths/ no. dosed)***
male	1600	2 / 5
	2000	5 / 5
	3200	5 / 5
	4000	5 / 5
female	1600	1 / 5
	2000	1 / 5
	3200	4 / 5
	4000	5 / 5

The LD_{50}-value was calculated to be 2160 mg/kg bw.

Ref.: 3

Comment
This is an old study. However, despite deficiencies (predating OECD guidelines and GLP, lack of data of the test substance), it is not necessary to repeat the study.

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/strain:	male New Zealand White rabbits
Group size:	3
Test substance:	2,7-naphthalenediol
Batch:	20020517
Purity:	99.9%
Vehicle:	neat (powder moistened with water) 2,7-naphthalenediol
GLP:	in compliance

0.5 g of 2,7-naphthalenediol was moistened with water (0.4 ml) to ensure a close contact to the skin. Test material was applied to gauze patch (semi-occlusive dressing), then to shaved intact skin on one flank of each rabbit for 4 hours. Patch was removed and the site washed with water. The animals were examined 1, 24, 48 and 72 hours after patch removal. Adjacent areas of the treated skin of each animal served as controls.

Results
None of the treated animals showed any response to treatment, no skin irritation was observed after 4 hours exposure to 2,7-naphthalenediol.

Conclusion
Under the conditions of the experiment, 2,7-naphthalenediol was not irritant to the rabbit skin.

Ref.: 4

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species/strain:	male New Zealand White rabbits
Group size:	3
Test substance:	2,7-naphthalenediol
Batch:	20020517
Purity:	99.9%
Vehicle:	neat (powder) 2,7-naphthalenediol
GLP:	in compliance

Test preparation (neat material) was instilled into one eye of one of the rabbits; 55.1 mg of powder corresponding to a volume of 0.1 ml. Observations were made 1, 24, 48 and 72 hours and 7, 14 and 21 days after instillation. Based on the severity of the ocular lesions observed during the study, the two further rabbits assigned to the study were not treated.

Results
Instillation of the test substance resulted in effects on the cornea, iris and conjunctivae. The corneal injury consisted of opacity (maximum grade 2) and epithelial damage (maximum 100% of the corneal area). As a result of the corneal injury, pannus (neovascularisation of the cornea) was apparent 7, 14 and 21 days after instillation. Iridial irritation grade 1 was observed between 24 and 72 hours after treatment. The irritation of the conjunctivae consisted of redness, chemosis and discharge. Redness remained present up to termination.

Reduced elasticity of the eyelids was noted after 7 days. In addition, grey-white discolouration of the nictating membrane (sign of necrosis) was observed 48 and 72 hours after instillation.

Conclusion
Under the conditions of the experiment, 2,7-naphthalenediol, instilled as the neat powder, is extremely irritant and corrosive to rabbit eyes.

Ref.: 5

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species/strain:	mouse – CBA/CaOlaHsd
Group size:	5 females (10-11 weeks) per dose group
Test substance:	2,7-naphthalenediol in acetone:olive oil 4:1 (v/v)
Batch:	20020517
Purity:	99.9%
Doses:	0.5%, 1.0%, 2.5%, 5.0%, 25% and 50% (w/v)
Positive control	alpha-hexylcinnamaldehyde (5, 10 and 25%)
Vehicle control	Acetone/Olive oil (4:1, v/v)
GLP:	in compliance

25 µl of test material or vehicle control and positive control was applied to the dorsal surface of both ear lobes once daily for 3 consecutive days. 5 days after the first application animals were injected iv with 250µl ^{3}H-methyl thymidine in the tail vein. Mice were sacrificed 5 hours later. Draining lymph nodes were excised and pooled to prepare a single cell suspension for each group. Thymidine incorporation was measured by β-scintillation counting. The disintegrations per minute per lymph node (DPM/node) were measured and expressed as the ratio of the control group (stimulation index, S.I.).

Results

Treatment	***induction***	***mean DPM ± SD***	***SI ± SD***
vehicle control	acetone : olive oil	213 ± 170	1.0
experimental	5% hexylcinnamaldehyde (HCA)	250 ± 171	1.2 ± 1.0
experimental	10 % HCA	580 ± 365	2.7 ± 1.0
experimental	25% HCA	3575 ± 1207	16.8 ± 0.9
experimental	0.5% test substance	218 ± 139	1.6 ± 0.7
experimental	1.0% test substance	241 ± 147	1.8 ± 0.7
experimental	2.5% test substance	191 ± 34	1.4 ± 0.3
experimental	5.0% test substance	684 ± 440	4.5 ± 0.9
experimental	25% test substance	1878 ± 416	12.4 ± 0.7
experimental	50% test substance	632 ± 151	4.2 ± 0.7
vehicle control	acetone : olive oil (4:1 v/v)	151 ± 94	1.0
vehicle control	acetone : olive oil (4:1 v/v)	134 ± 40	1.0

The EC3 value calculated from these data was 2.8%.

Conclusion
The results indicate that 2,7-naphthalenediol is a moderate skin sensitiser.

Ref.: 6

3.3.4. Dermal / percutaneous absorption

In Vitro Percutaneous Absorption (pig skin)

Guideline:	OECD 428
Species:	pig
Tissue:	Pig skin from 4 pigs (3 males, 1 female). 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 <4KΩ
Substance:	A019
Batch:	SAT 090021; Batch 20020517
Purity:	99.9% HPLC
Radiolabelled	A019 [^{14}C]; SAT10001B, Batch CFQ40748B1; 99% HPLC
Test formulation:	Hair dye formulation containing A019 at 2% mixed 50:50 with sham developer (no peroxide); Hair dye formulation containing A019 at 2% mixed 50:50 with developer containing 6% hydrogen peroxide.
Dose:	1% A019 in both formulations, applied at 20mg/cm^2
Receptor fluid:	PBS; 0.9% NaCl
Solubility in receptor	1-10g/L in water
Detection	liquid scintillation counting
GLP:	in compliance
Date	February 2010

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 019 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)	97.17	196.05	97.67	203.73
Adsorption (*stratum corneum*)	0.81	1.63	0.80	1.67
Not Bioavailable	97.98	197.68	98.47	205.40
Absorption (epidermis/dermis)	1.43	2.88	1.85	3.86
Penetration (receptor fluid)	0.16	0.31	0.28	0.58
Bioavailable	1.59±0.55	3.19±1.12	2.13±0.82	4.45±1.71
Total recovery / mass balance	99.57	200.87±6.03	100.62	209.85±3.39

The amount of A 019 considered as being systemically available from a standard hair dyeing formulation containing 1% A019 (on head) was 3.19±1.12 µg/cm² (1.59 ± 0.55% of the applied amount) in the presence of H_2O_2 and 4.45±1.71 µg/cm² (2.13 ± 0.82% of the applied amount) in the absence of H_2O_2 in this *in vitro* dermal penetration study.

Ref.: 17

Comment
This was a well conducted study.

For calculating the MOS the amounts of A019 considered to be available from a hair dye formulation containing it at 1% (on head) are (mean +1SD) 4.31 µg/cm² in the presence of H_2O_2 and 6.16 µg/cm² in the absence of H_2O_2.

An in vitro dermal absorption study (Ref. 13) 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 shortcomings 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

Range finding study

Guideline:	OECD 408 (1998)
Species/strain:	Rat, Wistar (HsdBrlHan:WIST)
Group size:	5 per sex and per dose and control group
Test substance:	A 019 (2,7-naphthalenediol)
Batch:	20020517
Purity:	99.9% (HPLC)
Doses:	0, 80, 250 and 750 mg/kg bw
Vehicle:	0.5% aqueous carboxymethylcellulose (CMC)
GLP:	in compliance

A 28-day oral range finding study to determine the doses for the 90-day study was conducted. The doses were 0, 80, 250, 750 mg/kg bw/day. The control animals received the vehicle alone (0.5% CMC).

Results
One female rat of the 750 mg/kg bw dose group was found dead on day of the treatment 11. It exhibited symptoms viz., salivation, lacrimation, nasal discharge, tremor, hyperaesthesia, excessive grooming of snout and hopping gait on day 10 and 11.

Animals in the low dose group did not exhibit treatment related clinical signs. The animals treated with 250 mg/kg bw and above revealed excessive grooming of snout immediately post oral gavage throughout the experiment. However, this clinical sign was observed only for approximately 5 minutes post-dosing.
The majority of the animals of the mid and high dose group revealed treatment related signs such as salivation, lacrimation and nasal discharge post dosing throughout the experiment. The severity of clinical signs in the rats of the mid dose group was mild as compared to those of the high dose group.

No significant alteration in body weight was observed in the mid and high dose groups as compared to the low dose group. However, in the high dose group males, mean body weight was found to be 7.4 and 9.3% less during the 3rd and 4th week of treatment, respectively, as compared to the control group. No significant variation in food consumption was observed in rats belonging to 3 treatment groups as compared to the control group.
Absolute and relative spleen weight was significantly higher in male rats from the high dose group as compared to the control group. In female rats, relative spleen weight was slightly increased in the high dose group.

External examination of carcass of either sex of the control and dose groups did not reveal any lesion of pathological significance. Some animals from control and dose groups revealed varying degree of gross lesions viz, lungs-consolidation and spleen-whitish deposits. The observed gross lesions were considered as spontaneous/incidental. The visceral examination

of found dead animal revealed various gross lesions such as lung-congestion, spleen-whitish deposition, thymus-congestion, liver-mottling and adrenal-congestion.

The histopathology of gross lesions encountered in different groups did not show any correlation with treatments. Hence these findings could be considered as spontaneous/incidental. Histopathology of kidney from control and high dose group did not reveal any significant pathological changes.

Conclusion
Based on the results of this range finding study, the following doses were selected for the 90-day study: 0, 70, 210 and 630 mg/kg bw/day

Ref.: 11

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

Taken from SCCNFP/0232/99

2,7-Dihydroxynaphthalene was administered daily by oral gavage, over a period of 12 weeks to 15 male and 15 female Wistar rats (Mu Ra Han 67 SPF) for each group, at dose levels of 0-20-60-180 (5.5 weeks) /360 (6.5 weeks) mg/kg bw/day (10 ml/kg bw in aqueous suspension). The highest test dose produced weight increase in liver, spleen and kidney, liver pigmentation, increased haematopoiesis in the spleen, and hyaline deposition in the kidney. The other doses (20 and 60 mg/kg bw/day) did not show clinical, biochemical and pathological-anatomical signs of a systemic cumulative toxicity. The dose of 60 mg/kg bw/day represents the dose with the NOAEL.

Ref.: 11 (opinion SCCNFP/0232/99)

New study

Guideline:	OECD 408 (1998)
Species/strain:	Rat, Wistar (HsdBrlHan:WIST)
Group size:	20 (10 per sex), except high dose 24 (12 per sex)
Recovery group:	High dose 24 (12 per sex), control 20 (10 per sex)
Test substance:	A 019 (2,7-naphthalenediol)
Batch:	20020517
Purity:	99.9% (HPLC)
Doses:	0, 70, 210 and 630 mg/kg bw; 10 ml/kg bw by gavage
Vehicle:	0.5% aqueous carboxymethylcellulose
GLP:	in compliance

Twenty rats (10 per sex) were used per dose and the control group, except for the high dose group with 24 rats (12 per sex). A recovery group was kept for a further four weeks after the last dosing, to check for treatment-related effects.
The doses of test substance in 0.5% CMC were made freshly daily prior to gavage since the stability decreased by 8-10% after 4h.

During the study the mortality, signs of intoxication, the body weight and the food consumption were recorded. The animals of the recovery groups were additionally examined during the 4-week treatment-free period. At the end of the study, the animals were sacrificed and subjected to pathological investigations.

Results
Twelve treatment-related mortalities were observed during the experiment in the male and female high dose and high dose recovery groups.

In the male low dose group there was a marginal reduction in weight gain. Significant reduction in weight gain and food intake were seen in the high dose and recovery group (from week 2) and in the mid dose (from week 7). These were considered treatment related. Food intake and weight gain in all females were similar to the controls.

No clinical or behavioural signs were observed in the low dose group. In the mid and high dose groups, there were no treatment related neurobehavioral observations. However, the majority showed excessive grooming of snout immediately post-gavage throughout the treatment period. This was transient, lasting approximately five minutes post-dosing. Other transitory clinical signs (salivation, lacrimation, nasal discharge, gasping; approx. 30 minutes post-dosing) were observed in animals from the mid and two high dose groups. Tremors were observed in few animals belonging to the two high dose groups. No ophthalmoscopic changes were attributable to the treatment.
During open field observations, in high dose females, the mean rearing count was significantly lower during week 4 and in the high dose recovery group during week 3, 5, 7, 9 and 10 of exposure. The males were comparable to the control.
Sensory reactivity tests did not reveal any treatment related abnormality.
Significantly reduced haemoglobin and haematocrit values in the mid and high dose males were seen compared with the controls. In high dose males, RBC was also significantly lower. In high dose females, significantly higher MCV and MCH than in the control group were noted. After the recovery period, complete haematological recovery/regeneration was observed with a significant increase in haematocrit in males and haemoglobin in females.
A significant increase was observed in the serum GGT in males and ALT levels in females of the high dose group. Total bilirubin was significantly increased in the mid and high dose males and in high dose females. Total protein levels were significantly increased in the mid and high dose females.

In the high dose males, there was a significantly lower urinary pH. The urine of all high dose animals (male and female) showed a brownish yellow and cloudy appearance, probably due to urinary excretion of the test substance or its metabolites. In some high dose males, blood was seen in urine, but not in urine at the end of recovery period. All dosed females showed significant increase in urinary volume.
Absolute liver and spleen weights were significantly increased in high dose females. Relative weights of liver, spleen and kidneys were significantly higher in the high dose males and females. In the mid dose group, relative weight of spleen of males was increased while in females both liver and spleen weights were increased.
After the recovery period, the absolute and relative spleen weights remained significantly higher in the high dose group. The relative kidney weight in both males and females and relative liver weight in females were also significantly higher.
There were no gross pathological changes at *post-mortem* attributable to the treatment. However, the dead animals of the two high dose groups showed nasal discharge, salivation and lacrimation.
Histopathology of the high dose group showed some treatment related changes: degeneration and necrosis of hepatocytes, bile duct hyperplasia and foci of erythropoiesis in liver; increased extramedullary haematopoiesis with connective tissue proliferation in spleen and degeneration and necrosis of tubular epithelial cells in outer medulla and cortex of kidney. These histopathological changes in liver and kidneys were reversible.

Conclusion
Based on the effects on the spleen and liver, the No Observed Adverse Effect Level (NOAEL) of 2,7-Naphthalenediol in Wistar rats exposed over a period of 90 days is considered to be 70 mg/kg bw/day.

Ref.: 11

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.2 Mutagenicity/Genotoxicity *in vitro*

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 019 (2,7-naphthalenediol)	
Solvent:	DMSO	
Batch:	Lot 20020517	
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	

2,7-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 and TA100 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
In experiment I toxic effects were observed at 2500 µg/plate for TA102 and at 5000 µg/plate for TA98, TA1535 and TA1537; in experiment II at 2500 µg/plate for TA102 and at 5000 µg/plate for TA98, TA100, TA1535 (with S9-mix only) and TA1537 (without S9-mix only). Reduction in background growth was reported in experiment I at 5000 µg/plate for TA 1535, TA1537, TA100 (with S9-mix only) and TA102 (without S9-mix only); in experiment II at 2500 µg/plate for TA102 and at 5000 µg/plate for TA 100, TA1535 and TA98 (with S9-mix only).
In both experiments 2,7-naphthalenediol treatment did not result in a biologically relevant increase in revertant colonies in any of the five tester strains neither in the absence nor in the presence of S9-mix.

Conclusion
Under the experimental conditions used 2,7-naphthalenediol was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 7

***In Vitro* Mammalian Cell Gene Mutation Test**

Guideline:	OECD 476	
Cells:	L5178Y Mouse lymphoma cells	
Replicates:	two parallel cultures in 3 independent experiments	
Test substance:	A 019 (2,7-naphthalenediol)	
Solvent:	DMSO	
Batch:	20020517	
Purity:	99.9%	
Concentrations:	Experiment I:	25.0 - 300.0 µg/ml (without S9-mix) 1.4 – 22.5 µg/ml (with S9-mix)
	Experiment II:	12.5 - 200.0 µg/ml (without S9-mix) 2.0 - 10 µg/ml (with S9-mix)
	Experiment IIA:	8.0 - 16.0 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.
	Experiment IIA:	4 h treatment with S9-mix; expression period 72 h and selection period of 10-15 days.
GLP:	In compliance	

2,7-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 (without S9-mix experiment II) 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 nor in osmolarity even at the maximal concentration of 2,7-naphthalenediol (1600 µg/ml ≈ 10 mM) measured in the pre-test without S9-mix.
In experiment II and experiment I culture I in the absence of S9-mix a data point with appropriate toxicity (10-20% survival after the highest dose) was not present pointing to insufficient exposure of the cells.
Exclusively, in experiment I culture II in the presence of S9-mix a more or less dose dependent increase in the total number of mutant colonies was found which was at the highest not too toxic (<10% survival) and outside the historical control range. Since this result was not reproducible it is considered not biologically relevant.

Conclusion
Under the experimental conditions used, 2,7-naphthalenediol was not genotoxic (mutagenic and /or clastogenic) in this gene mutation tests in mammalian cells.

Ref.: 8

In Vitro Mammalian Chromosome Aberration Test

Guideline:	OECD 473
Replicates:	duplicate cultures
Cells:	V79
Test substance:	A 019 (2,7-naphthalenediol)
Solvent:	culture medium (Minimum essential medium, MEM)
Batch:	Lot 20020517
Purity:	> 99.9%
Concentrations:	50.0 - 200.0 µg/ml without S9-mix 0.5 - 1.5 µg/ml with S9-mix
Treatment:	4 h treatment and harvest time 18 after start of treatment both in the absence and presence of S9-mix
GLP:	In compliance

2,7-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 range finding pre-test on cell number and cell morphology with 4 h and 24 h treatment. The highest dose in the pre-test was the prescribed maximum concentration (1610 µg/ml ≈ 10 mM). Cells were treated for 4 h and harvested 18 h after the start of treatment. 2.5 h before harvest, each culture was treated with colcemid (final concentration 0.2) to block cells at metaphase of mitosis. Liver S9 fraction from phenobarbital/β-naphthoflavone-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 draft guideline.

Results
Neither precipitation nor relevant influence of test item on the pH value or osmolarity was observed.

In the main test without S9-mix no toxic effects indicated by a reduced mitotic index compared to the negative controls were observed; with S9-mix a toxic effect at the highest dose (1.5 µg/ml, 31% reduction compared to the negative control) was found.
In both the absence and the presence of S9-mix, 2,7-naphthalenediol did not cause an increase in polyploidy.

In the absence of S9-mix, 2,7-naphthalenediol induced a dose dependent biologically relevant increase in cells with chromosomal aberrations. In the presence of S9-mix a dose dependent increase was found but the values were close to the values of the negative controls and within the range of the historical data. These observations with S9-mix can be regarded as not biologically relevant.

Conclusion
Under the experimental conditions used, the increase in cells with structural chromosomal aberrations shows genotoxic (clastogenic) activity of 2,7-naphthalenediol in V79 cells *in vitro*.

Ref.: 9

Comments
Since 2,7-naphthalenediol was considered clastogenic after experiment I with 4 h treatment, a second experiment with longer treatment was not performed.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474
Species/strain:	NMRI
Group size:	5 mice/sex/group
Test substance:	A 019 (2,7-Naphthalenediol)
Batch:	20020517
Purity:	99.9%
Dose level:	18.75, 37.5 and 75.0 mg/kg bw
Route:	*i.p.*
Vehicle:	aqueous DMSO
Sacrifice times:	24 and 48 h after the treatment.
GLP:	In compliance

2,7-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 with 2 animals per sex/group, 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, 18.75, 37.5 and 75.0 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 (1 h and 4 h after start of treatment) treated with 75 mg/kg bw were included for determination of blood concentrations of 2,7-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 6th 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 2,7-naphthalenediol did not result in substantially decreased PCE/TE ratios compared to the untreated controls indicating that 2,7-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 in almost all treated animals up to 24 h (highest dose) or 6 h (lower doses) after start of the treatment. 2,7-naphthalenediol could be quantified in the blood of the treated males confirming the bioavailability of 2,7-naphthalenediol.
Biologically relevant increases in the number of micronucleated PCEs compared to the concurrent vehicle controls were not found following treatment with 2,7-naphthalenediol at any time point or dose level tested.

Conclusion
Under the experimental conditions used 2,7-naphthalenediol did not induce an increase in bone marrow cells with micronuclei in treated mice and, consequently, 2,7-naphthalenediol is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 10

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

Embryotoxicity

Taken from SCCNFP/0232/99

The compound tested in the Hen's Egg Test was moderately toxic:

LD_{50}: 5.1 mg/egg (1day) and 2.05 mg/egg (5 days). The compound did not show evidence of teratogenic potential in this system.

Ref.: 15 (opinion SCCNFP/0232/99)

Comment
The test is not considered relevant for safety assessment.

3.3.8.2. Teratogenicity

Taken from SCCNFP/0232/99

2,7-Dihydroxynaphthalene administered daily by oral gavage to groups of 30 pregnant CD Sprague Dawley rats from day 5 to 15 of gestation at doses of 0-20-60-360 mg/kg bw showed at the highest test dose a slight retardation of average body weight during the treatment. No other difference was observed for other teratogenicity and embryotoxicity parameters. The dose of 60 mg/kg bw was the NOAEL.

Ref.: 16 (opinion SCCNFP/0232/99)

New study

Guideline:	OECD 414 (2001)
Species/strain:	Rat, Wistar
Group size:	25 (female 11 week old)
Test substance:	A 019/ SAT 040232
Batch:	20020517
Purity:	99.9%
Doses:	0, 65, 195 and 585 mg/kg bw/day; 10 ml/kg bw by gavage
Vehicle:	0.5% aqueous carboxymethylcellulose (CMC)
GLP:	In compliance

The females were paired with male rats of the same strain one to one with the day of mating determined by the vaginal plugs or sperm in the vaginal smear.
The doses of test substance in 0.5% CMC were made freshly daily prior to gavage.
Aliquots of 10 ml/kg bw of the test substance, at the 4 dose levels, were administered daily (GD 5-19) by gavage. Dosages were based on the results of the previously performed dose range-finding study (75, 225, 675 mg/kg bw/day). The mortality and the body weight gain were observed daily.
The dams were sacrificed on GD 20. The number of live and dead foetuses, their distribution and site in the uterus, early and late resorption, implantations and number of *corpora lutea* were determined. The weight of the foetuses, gravid uteri, uteri without foetuses, placentas and the sex of foetuses were recorded. Approximately one-half of the foetuses were selected at random and examined for visceral alterations. The remaining foetuses were examined for skeletal malformations, variations and retardation of the normal organogenesis after appropriate staining.

Results
Deaths occurred in the high dose group and were considered to be treatment related.
Post-dosing clinical symptoms like lacrimation, nasal irritation, salivation and lethargy were observed in mid and high dose groups. Tremors and nasal discharge were seen in rats of the high dose group only. The symptoms were transitory, immediately post-dosing, recovery were within 1 to 2 hours.
The mean maternal weight and GD 20 corrected body weight in the dam were significantly decreased in the high dose group. Food consumption was significantly decreased in the mid and high dose groups.
At *post-mortem*, there were no gross pathological changes/lesions observed that were considered treatment-related. However, the lesions (lung- congestion/oedema; liver - pallor, mottling; brain – congestion) found in the 2 high dose females that died during the study were considered to be treatment-related.

Conclusion
The test substance did not exhibit any adverse effect on pregnancy rates. There were no significant differences in the incidences of malformation or birth defects between control and the groups dosed with the test substance.
In this study, the No Observed Adverse Effect Level (NOAEL) for maternal toxicity of 2,7-Naphthalenediol was determined to be 65 mg/kg bw/day, based on post-dosing symptoms. The NOAEL for foetal toxicity was 585 mg/kg bw/day.

Ref.: 12

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

2,7-naphthalenediol
(Non-oxidative conditions)

Absorption through the skin	**A (mean + 1SD)**	**=**	**6.10 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**3.54 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.06 mg/kg bw/d**
No observed adverse effect level (maternal toxicity in teratogenicity study, oral, rat)	**NOAEL**	**=**	**65 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**=**	**1083**

(Oxidative conditions)

Absorption through the skin	**A (mean + 1SD)**	**=**	**4.31 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**2.50 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.04 mg/kg bw/d**
No observed adverse effect level (maternal toxicity in teratogenicity study, oral, rat)	**NOAEL**	**=**	**65 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**=**	**1625**

3.3.14. Discussion

Physico-chemical specifications
The stability of the test substance in typical hair dye formulations was not reported. The impurity '2-naphthol' is banned according to Directive 768/76/EEC on cosmetic products (Annex 2, entry n° 241).

General toxicity
In all oral studies, 2,7-naphthalenediol caused decreased bodyweight, oedema in lungs, degeneration and necrosis of hepatocytes, bile duct hyperplasia and foci of erythropoiesis in liver; increased extra-medullary haematopoiesis with connective tissue proliferation in spleen and degeneration and necrosis of tubular epithelial cells in outer medulla and cortex of kidney in the mid and high dose groups. The histopathological changes in liver and kidneys were reversible. In a 90 day rat study, the No Observed Adverse Effect Level (NOAEL) is considered to be 70 mg/kg bw/day. The NOAEL for maternal toxicity of 2,7-naphthalenediol was determined to be 65 mg/kg bw/day and the NOAEL for foetal toxicity was 585 mg/kg bw/day.

Irritation / sensitisation
Under the conditions of the test, 2,7-naphthalenediol was not irritant to the rabbit skin. Instilled in the eyes as a neat powder, it is extremely irritant and corrosive to rabbit eyes.

The EC3 value calculated (2.8%) from the LLNA showed that 2,7-naphthalenediol was a moderate sensitizer.

Dermal absorption
An *in vitro* dermal absorption study with pig skin under oxidative and non-oxidative conditions has been performed. The amount of 2,7-Naphthalenediol considered as being systemically available from a standard hair dyeing formulation containing 1% 2,7-Naphthalenediol (on head) are 4.31 µg/cm² in the presence of hydrogen peroxide and 6.10 µg/cm² in the absence of hydrogen peroxide (representing mean +1SD).

Mutagenicity
2,7-Naphthalenediol did not produce gene mutations in bacteria nor in mammalian cells on the *tk* locus of mouse lymphoma cells. 2,7-Naphthalenediol induced an increase in the number of cells with chromosomal aberrations. An *in vivo* bone marrow micronucleus tests performed up to lethal doses in mice did not induce an increase in the number of micronucleated erythrocytes.
Overall, the genotoxicity program on 2,7-naphthalenediol is sufficient investigating three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. 2,7-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 2,7-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 2,7-naphthalenediol in combination with hydrogen peroxide have to be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

2,7-Naphthalenediol is a moderate sensitizer.

The SCCP is of the opinion that 2,7-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.

2,7-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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