EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

2-Methylresorcinol

COLIPA N° A44

Adopted by the SCCP
during the 9th plenary meeting of 10 October 2006

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1. BACKGROUND

Submission I of 2-Methylresorcinol was submitted in June 1985 by COLIPA[1,2].

The Scientific Committee on Cosmetology (SCC) has at its meeting on 10 October 1988 expressed its opinion and revised it in its 54th meeting on 10 December 1993 as follows:

"*The SCC requires a teratogenicity study*."

The substance is currently regulated by the Cosmetic Directive (76/768/EC), Annex III, Part 2 under entry 37 on the List of substances, provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission II for this substance was submitted in July 2005 by COLIPA. According to this submission 2-Methylresorcinol is used as a precursor for oxidative hair colours. The substance is proposed to be used as an ingredient in oxidation dye formulations as well as in dyeing formulations without a hydrogen peroxide based developer mix with a final on head concentration of 1.8%.

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

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 2-Methylresorcinol safe for use in oxidative and non-oxidative hair dye formulations up to an on-head concentration of 1.8% taken into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of 2-Methylresorcinol in any oxidative and non-oxidative hair dye formulations?*

[1] COLIPA – The European Cosmetic Toiletry and Perfumery Association

[2] According to the records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2-Methylresorcinol (INCI)

3.1.1.2. Chemical names

1,3-Benzenediol, 2-methyl- (CA INDEX NAME, 9CI)
1,3-Dihydroxy-2-methylbenzene
2-Methylbenzene-1,3-diol
2-Methyl-1,3-dihydroxybenzene
2,6-Dihydroxytoluene

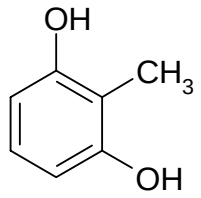
3.1.1.3. Trade names and abbreviations

Ro 261

3.1.1.4. CAS / EINECS number

CAS : 608-25-3
EINECS : 210-155-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_7H_8O_2$

3.1.2. Physical form

Colourless to light brown crystals

3.1.3. Molecular weight

Molecular weight: 124.17 g/mol

3.1.4. Purity, composition and substance codes

Batch 221201 = SAT 030385 = SAT 030630 = SAT 040278
Identification by NMR, IR and UV spectrometry
Purity by NMR assay: > 97.7%
Purity by HPLC assay: > 98.3% (peak area)

Only data on batch 221201 is submitted

Applicant declares that "The batch of A44 used in the acute oral toxicity is not fully analytically described. However, information is available from the laboratories that have synthesised 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 characterised batch 221201".

3.1.5. Impurities / accompanying contaminants

Batch 221201 contains:

Water:	0.16% (w/w)
Acetate anions:	0.3% (w/w)
Sulfate ash:	<0.1% (w/w)
Toluene:	0.06% (w/w)
Resorcinol:	0.37% (w/w)
5-*tert*.-butylbenzene-1,3-diol:	0.7% (HPLC peak area)
4-*tert*.-butylbenzene-1,2-diol:	<200 ppm (detection limit)
2,5-dimethylbenzene-1,3-diol:	<200 ppm (detection limit)
4-ethylbenzene-1,3-diol:	<200 ppm (detection limit)
4-methylbenzene-1,2-diol:	<200 ppm (detection limit)

3.1.6. Solubility

Readily soluble in water and ethanol

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 1.22 ± 0.21, calculated

3.1.8. Additional physical and chemical specifications

Organoleptic properties:	colourless to light brown crystals
Melting point *:	116-123°C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/

* Melting point reported here is taken from Submission II. Melting point reported in the report R9800896 of June 1985 is 65°C. *A large variation in the melting point of the substance may indicate variable amounts of impurities in the test substance.*

3.1.9. Stability

No data

General Comments on Physico-chemical characterisation

- Stability of 2-methylresorcinol in test solutions and in marketed products is not reported.
- Log P_{ow}: calculated values cannot be accepted as estimates of the true physical constants without justification indicating that the reported values are realistic.
- No documentation is provided for the similarity of physico-chemical properties of batches SAT 030385, SAT 030630, SAT 040278 of 2-methylresorcinol with those of batch 22120.
- Two different melting points (65°C and 116-123°C) of 2-methylresorcinol are reported

3.2. Function and uses

2-Methylresorcinol is used in oxidative hair dye formulations at a maximum concentration of 1.8%, after mixing with peroxide developer. The hair dye formulation mixed with peroxide developer is applied for 30 min on head.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: mouse, CF1
Test substance: Ro 261
Batch: not specified
Purity: not specified
Vehicle: water
GLP: not in compliance

Male mice, 10 per dose group, were administered the test substance via gavage in dose levels of 251, 316, 398, 425, 448, 501 mg/kg bw. No clinical effects were noted after administration of the lowest dose. The LD_{50} was calculated to be 390 mg/kg bw.

Ref.: 3

Comment
Although this is an old study (1976), the results are useful for the purpose of this assessment (but the LD_{50} is very low compared to the dose levels used in the 90 day study. This might be explained by the (unknown) impurities in the batch used for the LD_{50} 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: New Zealand White rabbits
Group: 3 males
Test substance: A044/SAT030630
Batch: 221201
Purity: 98.6%
Dose: 0.5g
GLP: in compliance

An aliquot of 0.5 g of the moistened test substance was exposed to the intact shaved back skin of each animal for 4 hours under semi-occlusive conditions. Animals were examined approximately 1 hour, 24, 48 and 72 hours after termination of the exposure.

Results
The test substance caused orange staining of the skin but the investigators said that this did not affect observations.
Under the conditions of the study, application of the undiluted test substance resulted in very slight erythema in the treated skin-area of one rabbit one hour after instillation.

Conclusion
The test substance applied undiluted to rabbit skin showed mild and transient skin irritation.

Ref.: 4

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405 (2002)
Species:	New Zealand White rabbit
Group:	1 male
Test substance:	A044/SAT030630
Batch:	221201
Purity:	98.6%
Dose:	50.6mg (approx 0.1ml)
GLP:	in compliance

The equivalent of 0.1 ml of the test substance was instilled into the conjunctival sac of one eye of the test animal. The substance remained in permanent contact with the eye until rinsing with warm tap water, 24 hours after instillation. The other eye served as control.
The eye irritation reactions were scored approx. 1 hour, 24, 48 and 72 hours and 7 days after instillation of the test solution.

Results
The instillation of the undiluted test substance into the eye of one rabbit resulted in effects on the cornea, iris and conjunctiva.
The cornea injury consisted of opacity (maximum grade 3) 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 from days 1 to 7 after instillation. The irritation of the conjunctiva consisted of redness and chemosis. Redness persisted up to termination. In addition, the eyelids and/or nictitating membrane showed grey-white discoloration (sign of necrosis) between days 1 and 7 after instillation. Reduced elasticity of the eyelids was noted between 72 hours and 21 days after exposure.

Conclusion
Based on the degree and persistence of the eye injury, it was concluded that ocular corrosion had occurred by instillation of undiluted test substance into the rabbit eye. Undiluted A044/SAT030630 is severely irritant to the rabbit eye.

Ref.: 5

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species:	CBA mice
Group:	5 females per group
Test substances:	A044/SAT030630
Batch:	221201
Purity:	98.6%
Dose:	vehicle control (acetone/olive oil 4:1); volume 25 µl
	1%
	10%
	25%
	50%
GLP	in compliance

Four dose groups and a control group (receiving the vehicle only) of 5 female mice each, were chosen.

A homogenous dilution of the test item in a mixture of acetone:olive oil (4:1 v/v) was made shortly before each dosing. The highest non-irritating test item concentration was found in a pre-test with two female mice. Based on these results 1, 10, 25 and 50% solutions were chosen for the main study. The stability in the chosen vehicle was confirmed in separate tests.
Each test group of mice was treated by topical (epidermal) application to the dorsal surface of each ear lobe (left and right) with different test item concentrations. The application volume, 25 µl, was spread over the entire dorsal surface of each ear lobe once daily for three consecutive days. The negative control group was treated with the vehicle exclusively. Five days after the first topical application, all mice were administered with radio-labelled thymidine (^{3}HTdR) by intravenous injection via a tail vein.

Approximately five hours after ^{3}HTdR application all mice were killed. The draining lymph nodes were excised and pooled for each experimental group. After preparation of the lymph nodes, disaggregation and overnight precipitation of macromolecules, these precipitations were re-suspended and transferred to scintillation vials.

The level of ^{3}HTdR incorporation was then measured by scintillation counting. The proliferative response of lymph node cells is expressed as the ratio of ^{3}HTdR incorporation into lymph node cells of treated animals relative to that recorded in control mice (stimulation index).

An appropriate reference (α-hexylcinnamic aldehyde) was used as positive control to demonstrate the sensitivity of the test system. There was no contemporaneous positive control done.
The proliferative capacity of pooled lymph node cells was determined by the measurement of the incorporation of ^{3}H-methyl thymidine. A test item is regarded as a sensitizer if the exposure to at least one concentration resulted in an at least 3-fold increase in incorporation of ^{3}HTdR compared with concurrent controls, as indicated by the stimulation index (S.I.).

Results
Slight skin irritation was noted on the ear dorsum of the treated mice at concentrations above 10% (w/v).
The Stimulation Index (S.I.) was below 3 in the dose groups 1, 10 and 25%. In the group treated with 50% a S.I. of 3 was found.

The individual findings are summarized in the following table:

Test Item Concentration	S.I.
1% (w/v)	0.7
10% (w/v)	0.6
25% (w/v)	1.1
50% (w/v)	3.0

The EC 3 value was determined to be 50%.

The positive control undertaken in August 2003 induced an increase of the stimulation index demonstrating the validity of the test system.

Conclusion
Based on the criteria of the test system A044/SAT030630 was found to be a sensitizer when tested at 50% (w/v) in acetone:olive oil (4:1) in mice. No concurrent positive control experiment had been performed.

Ref.: 6

3.3.4. Dermal / percutaneous absorption

In vitro absorption/percutaneous penetration out of a basic hair dyeing formulation with and without a developer (excised pig skin)

Guideline:	OECD draft 428
Species:	pig
Group:	0.38 mm dermatomed skin from 2 animals; total 8 chambers per each of 3 experiments
Test substances	A044; (radio-labelled [^{14}C]-A044)
Batch:	SAT030630; (radio-labelled SAT 030759)
Purity:	/ (radio-labelled: 99.65%)
Dose:	1.8% A044 in formulations with coupler and/or hydrogen peroxide; 1.8% in water
Receptor fluid:	Physiological buffered saline
GLP:	in compliance

The dermal absorption/percutaneous penetration of A044 out of a basic cream (mixed with a developer with and without hydrogen peroxide) and from a solution in water was studied on the clipped excised skin of suckling pigs (aged 6-8 weeks). The pig skin was dermatomed to a mean thickness of 0.38 mm.
The integrity of the skin discs was checked by measuring the trans-dermal electrical resistance. The intact, clipped excised pig skin of the flanks area was exposed for 30 minutes to the test substance in the basic hair dyeing formulation without occlusion (10 mg/cm²).

The basic cream consisted of:

Ingredient	**Concentration in %**
COLIPA A044 (traced with [^{14}C] radio-labelled material)	3.6
tetraaminopyrimidine sulfate x H_2O (COLIPA A 053)	6.8 (4% free base)
sodium laureth sulfate	4.05
cocoamidopropyl betaine	3.75
cetearyl alcohol	8.5
fatty alcohol C12-18 (Lorol)	2.0
ceteareth-20	0.75
ascorbic acid	0.2
sodium sulphite	0.2
ammonium sulfate	0.4
etidronic acid	0.12
sodium silicate [SiO_2:Na_2O = 3.3], 38.2% active matter	0.5
ammonia, 25% (as solvent and for pH-adjustment)	15.5
water	ad 100

The developer mix with and without hydrogen peroxide, respectively, consisted of:

Ingredient	**with H_2O_2 in %**	**without H_2O_2 in %**
ammonia, 25%	0.65	0.65
dipicolinic acid	0.1	0.1
sodium diphosphate, acidic	0.03	0.03
etidronic acid	0.9	0.9
sodium laureth sulfate	0.56	0.56
silicon emulsion, 10% active substance	0.067	0.067
acryl polymer	4.2	4.2
hydrogen peroxide	6	-
L(+)-tartaric acid (pH adjustment)	0.125	-
water	ad 100	ad 100

Shortly before topical application to skin the basic cream was mixed (1:1) with the developer mix with and without hydrogen peroxide as study B and A, respectively. Additionally, a third formulation C was produced by dissolving A044 (traced with [^{14}C] radio-labelled material) in water. The content of A044 in the final application formulations was 1.8%.
The dermal absorption/percutaneous penetration of the test substance was investigated for the open application of about 10 mg formulation per cm² pig skin. Therefore the resulting dose of the test substance was approx. 0.25 mg/cm² skin. Skin discs of 0.79 cm² were exposed to the formulations for 30 minutes, terminated by gently rinsing with a 3% Teepol® solution.
Each of the two formulations and the solution were analysed with eight replicates for adsorbed, absorbed and penetrated amount of the test substance. The receptor fluid used was physiological buffered saline.

In the static system, samples of receptor fluid were taken manually using a positive displacement pipette at recorded intervals (0, 0.5, 2, 4, 6, 12, 24, 30, 36 and 48 hours). The volume of fluid in the receptor chamber was maintained by the addition of 0.1 ml fresh receptor fluid to the chamber immediately after the removal of each sample.

Results
The quantities that had penetrated during the 30 minute exposure to A44 containing formulations and within the 48 hours after application are shown in the following table. Both the absorbed and penetrated amounts were taken as systemically available.

Analysed Sample	**Formulation A without H_2O_2**		**Formulation B with H_2O_2**		**Solution C**	
	[% of dose]	**[µg/cm²]**	**[% of dose]**	**[µg/cm²]**	**[% of dose]**	**[µg/cm²]**
Skin rinsings	99.3	-	93.7	-	86.5	-
Adsorption (stratum corneum)	0.712	1.330	0.510	0.964	3.86	7.25
Considered NOT available	**100.01**	-	**94.21**	-	**90.36**	-
Absorption (epidermis/dermis)	2.79	5.22	0.238	0.450	8.75	16.4
Penetration (receptor fluid)	1.69	3.16	0.211	0.400	8.43	15.8
Considered available	**4.48**	**8.38**	**0.449**	**0.850**	**17.18**	**32.2**
Total recovery / mass balance	**105**	-	**94.5**	-	**108**	-

In this *in vitro* dermal penetration study in pig skin the amount of A44 systemically available from a standard cream formulation with or without hydrogen peroxide was found to be 0.85 ± 0.78 µg/cm² (0.45%) and 8.38 ± 5.41 µg/cm² (4.48%), respectively and 32.2 ± 11.91 µg/cm² for the aqueous solution.
The maximum value observed in the experiment using hydrogen peroxide was 2.07 µg/cm² and this is the value which will be used for calculating the MOS.

Ref.: 14

Comment:
Skin penetration of the formulation with and without H_2O_2 differs greatly.

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:	Wistar rats Crl (WI) BR
Group size:	17 animals per sex at control and high dose (each 5 for recovery) 12 animals per sex at low and mid dose
Test substance:	A44
Batch number:	221201
Purity:	>98.6 % (HPLC)
Vehicle:	propylene glycol
Dose levels:	0, 100, 200 and 450 mg/kg/day
Route:	Oral (gavage)
Exposure period:	90 days
Recovery period:	28 days
GLP:	in compliance

The subchronic toxicity was investigated in a 90-day study using 12 rats per group and sex. The test substance was administered as an aqueous suspension in propylene glycol at dose levels of 0, 100, 200 and 450 mg/kg bw by daily gavage. The application volume was 10 ml/kg bw. In addition, 5 males and 5 females were included in the control and high dose group for a 4-week recovery period. The test substance solution was analyzed for homogeneity and accuracy. The stability over 8 days in the refrigerator was also determined. Clinical examinations covered clinical signs, mortality, body weight and food consumption and functional observations. Haematological, clinico-chemical and urinalysis were performed in all animals. Ophthalmology was carried out prior to treatment and at the end of the treatment in control and high dose animals. At termination of treatment and after recovery had elapsed, all animals were sacrificed and macroscopically examined, organs were weighed and histopathology was performed.

Results
From week 4 of treatment in animals of the high dose group (and to a lesser extent also in animals of the 200 mg/kg bw group), clonic spasms, salivation and scratching movements were noted. These signs resolved during the recovery phase

Body weight gain of high dose males was slightly reduced from week 4 of treatment onwards. This reduction was not noted anymore during the recovery phase.
Slight increase in body weight in males of the 450 and 200 mg/kg/day group was observed. In these groups, also the relative liver weight was increased (although not statistically significant).
There was an increased glucose level in males at 450 mg/kg/day.
Increased ASAT and/or ALAT levels were noted in 1 male of the 100 mg/kg bw group, 2 males from the 200 mg/kg group and 2 males from the 450 mg/kg group. All these effects were not observed after the recovery phase.

Based on the effects on the body weight, the NOAEL in this study is 100 mg/kg bw. The NOEL is lower than 100 mg/kg bw.

Ref.: 11

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline	OECD 471
Species/strain	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537
Replicates	Two independent tests with and without S9 mix (plate incorporation test and a pre-incubation test)
Test substance:	A 044 in ethanol
Batch:	221201
Purity:	98.6% (area%, HPLC)
Concentrations:	33-5000 µg/plate (6 concentrations)
GLP:	in compliance

The Ames-test was performed with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with and without fortified rat liver postmitochondrial fraction (S9 mix) induced with phenobarbital and β-naphthoflavone. A 044 was tested in ethanol (>99%) at six concentrations in the range of 33 to 5,000 µg/plate. This range was based upon the results of a pre-experiment. The main assay was performed in two independent experiments both with and without liver microsomal activation. A direct plate incorporation assay as well as a pre-incubation experiment as the second run of the main test were performed. Sodium azide (10 µg/plate) served as a positive control for TA100 and TA1535, 4-nitro-o-phenylene-diamine (10 µg/plate) for TA1537 and TA98 and methyl methane sulfonate (4 µg/plate) for TA102 without S9 mix. The enzyme activity of S9 mix was separately controlled by testing with 2-aminoanthracene (2.5 µg/plate) in all tester strains. The solvent ethanol and the untreated fresh cell suspension served as negative controls.

Results

Irregular background growth was observed at 5000 µg/plate in strain TA1537 without S9 mix and in strain TA102 with S9 mix in the second experiment. Toxic effects, evident as a reduction in the number of revertants, were observed in strain TA1537 without S9 mix in experiment I, and in strains TA1537, TA98, and TA100 with S9 mix and strains TA98 and TA102 without S9 mix in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with A 044 at any dose level, either in the presence or absence of metabolic activation. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. The positive controls used showed a distinct increase of induced revertant colonies.

Conclusion

A 044 was not considered to be mutagenic in the *in vitro* bacterial mutagenicity test in the presence or absence of S9 mix.

Ref.: 7

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

Guideline:	OECD 476	
Species/strain:	L5178Y mouse lymphoma cells *($tk^{+/-}$ locus)*	
Replicates:	Two independent tests, each with two parallel cultures	
Test substance:	A 044 in DMSO	
Batch:	221201	
Purity:	98.6% (area%, HPLC)	
Concentrations:	Experiment I:	100, 200, 400, 800, 1000 µg/ml without S9 mix,
		25, 50, 100, 200, 400, 600, 800 µg/ml with S9 mix.
	Experiment II:	25, 50, 100, 200 µg/ml without S9 mix
GLP:	in compliance	

The mouse lymphoma cell line L5178Y was used to examine the potential of A 044 to induce mutations at the thymidine kinase locus. The assay was performed in the presence and absence of phenobarbital- and β-naphthoflavone-stimulated rat liver microsomes (S9 mix). The test procedure followed the OECD guideline and was conducted in compliance with the principles of GLP. The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with a treatment period of 4 h with and without metabolic activation. In the second experiment, the cells were treated for 24 h without metabolic activation. The test article was dissolved in DMSO and, according to the results of the pre-test, at least four adequate concentrations were chosen for the experiment. In experiment I (72 h expression phase), five concentrations (100-1000 µg/ml) were analyzed. In experiment II (24 h without S9 mix), four concentrations were analyzed (25-200 µg/ml; cultures treated with 400, 600, and 800 µg/ml were not continued due to exceedingly severe toxic effects). Methyl methane sulfonate (13 µg/ml) without S9 mix and cyclophosphamide (6 µg/ml) with S9 mix were used as positive controls.

Results
In experiment I, relevant toxicity was detected in both parallel cultures at 400 µg/ml and above in the presence and absence of metabolic activation. In the second experiment, severe toxic effects occurred already at 100 µg/ml and above. No substantial and reproducible dose-dependent increase in mutant colony numbers was observed in either of the two main experiments. The positive controls showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large colonies.

Conclusion
It was stated that in the study described and under the experimental conditions reported, A 044 did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation. Therefore, A 044 was not considered to be mutagenic in this mouse lymphoma assay.

Ref.: 8

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

Guideline:	OECD 473
Species/strain:	Chinese hamster V79 lung cells
Replicates:	A single experiment with and without S9 mix
Test substance:	A 044 in MEM
Batch:	221201
Purity:	98.6% (area%, HPLC)
Concentrations:	50-500 µg/ml without S9 mix, 40-160 µg/ml with S9 mix
GLP:	in compliance

Chinese hamster V79 lung cells were used to examine the induction of chromosomal aberrations by the test substance. The chromosome aberration assay was performed in the presence and absence of β-naphthoflavone-stimulated rat liver microsomes (S9 mix). The test procedure followed the OECD guideline and was conducted in compliance with the principles of GLP. The cells were harvested 18 h after the start of treatment. The treatment interval was 4 hours with and without metabolic activation. 100 metaphases per culture were scored for structural chromosome aberrations. Considering the toxicity data and the occurrence of precipitation in a pre-test, A 044, dissolved in minimal essential medium (MEM), was tested in the range of 50-500 µg/ml in the absence and 40-160 µg/ml in the presence of S9 mix. Since the test item was considered to be clastogenic after 4 hours of treatment, a second experiment was not performed. Ethyl methane sulfonate (200 µg/ml without S9-mix) and cyclophosphamide (0.7 µg/ml with S9-mix) were used as positive controls.

Results

In the absence and the presence of S9 mix, no clear toxic effects, indicated by reduced cell numbers or mitotic indices, were observed up to the highest evaluated concentration. However, in the presence of S9 mix the highest concentration was not evaluable due to strong toxic effects. Statistically significant and biologically relevant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test item both in the absence of S9 mix (starting from 400 µg/ml) and the presence of S9 mix (starting from the lowest concentration tested, 80 µg/ml). No relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared with the frequencies of the controls. The positive controls induced statistically significant increases in cells with structural chromosome aberrations.

Conclusion

It could be stated that in the study described and under the experimental conditions reported, A 044 induced structural chromosome aberrations in the V79 cell line in the absence and presence of metabolic activation and was thus found to be clastogenic *in vitro*.

Ref.: 9

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	Mouse, NMRI
Group size:	5 males + 5 females
Test substance:	A 044 in water
Batch:	221201
Purity:	98.6%
Dose levels:	12.5, 25, 50 mg/kg bw (once intra-peritoneally)
Sacrifice time:	24 and 48 (highest dose group only) h after the treatment
GLP:	in compliance

This study was performed to investigate the potential of A 044 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The test item was formulated in deionised water, which was also used as vehicle control. The compound was administered by a single intraperitoneal injection, and bone marrow cells were collected 24 h and 48 h later. Ten animals (5 males, 5 females) per test group were included and at least 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. The ratio between polychromatic and total erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes. The following dose levels of the test item were investigated: 24-h preparation interval: 12.5, 25 and 50 mg/kg bw; 48-h preparation interval: 50 mg/kg bw. As estimated by pre-experiments, 50 mg A 044 per kg bw was the highest applicable dose without significant effects on the survival rates, but with clear signs of toxicity. At a higher dose (75 mg/kg) all treated animals had to be killed after 10 minutes due to the severity of the induced toxic effects.

Results
The number of PCEs was not substantially decreased in the treated animals as compared with the vehicle control group, indicating that A 044 did not exert any cytotoxic effects on the bone marrow. The bioavailability of the test item was, however, suggested by chemical analysis of the blood of the treated animals. In comparison with the corresponding vehicle controls, there was reported to be no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used.

It should be noted that in the 24-h sampling time all doses of A 044 resulted in about doubling of the micronucleus frequency of the vehicle controls. The difference to control was 2.4-, 2.1-, and 2.0-fold, at 12.5, 25, and 50 mg/kg, respectively and was of borderline significance at 12.5 mg/kg (P=0.0658) and at 25 mg/kg (P=0.0816) in the Mann-Whitney test. No dose-response was, however, evident, and the micronucleus frequencies were in general low and within historical control range.

Conclusion
Under the experimental conditions reported, the test item did not significantly induce micronuclei in mouse bone marrow polychromatic erythrocytes. Therefore, A44 was considered

to be negative in the micronucleus assay. Negative and positive controls were in accordance with the OECD guideline. Dose selection was based on a dose range-finding assay.

Ref.: 10

Comment
At the 24-h sampling time, there was a doubling of mean micronucleus frequencies at all doses used. The differences were not statistically significant, although borderline, the numbers of micronucleated cells were, in general, low, and there was no dose-response. Consequently, it is not biologically relevant.

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Study 1, taken from SPC/1267/93

Rats (20 pregnant females) received 2 ml/kg topical applications of a formulation containing 1% of 2-methylresorcinol on days 1, 4, 7, 10, 13, 16 and 19 of gestation. The study resulted inadequate because the treatment was performed every 3 days and only one dose was tested.

Study 2, taken from SPC/1267/93

Sprague-Dawley rats (25 females for each dose) were treated with 0, 0.1, 0.4 or 1.5% of 2-methylresorcinol in the diet during the period of major organogenesis. The analyses were carried out on day 20 of gestation. At the doses of 0.4 or 1.5%, a slight increase (not significant statistically) was observed in the mean post-implantation loss with corresponding decrease in the number of viable foetuses and implantation sites. This result was considered to represent biological variance. The compound was neither embryotoxic nor teratogenic. The dose of 1.5% (i.e. 900 mg/kg bw) represents the NOAEL.

Study 3

Guideline:	OECD 414
Species/strain:	Wistar rats Crl (WI) BR
Group size:	25 females
Test substance:	A44
Batch number:	221201
Purity:	>98.6 % (HPLC)
Vehicle:	propylene glycol

Dose levels: 0, 40, 200 and 400 mg/kg/day
Treatment period: day 6 - 20 post-coitum, inclusive
GLP: in compliance

The test substance was given in propylene glycol daily at dose volumes of 10 ml/kg bw by oral gavage. The doses were selected on the basis of the results of a preliminary study in rats. Maternal evaluations and measurements included daily clinical signs and body weight/food intake recorded at designated intervals.
The dams were sacrificed on day 21 post-coitum by carbon dioxide asphyxiation and subjected to necropsy. The number of alive and dead foetuses, their distribution and site in the uterus, early and late resorption, implantation and number of *corpora lutea* was determined. The weight of the foetuses, gravid uteri, uteri without foetuses, placenta and the sex of foetuses was recorded. The foetuses examined for visceral alterations. Alternate foetuses were examined for skeletal malformations, variations and retardation of the normal organogenesis after appropriate staining.

Results
No maternal toxicity was observed, as mortality, clinical appearance, body weights, food consumption, and macroscopic examination were unaffected by exposure to the test substance.
No reproductive toxicity was observed, as no effects were noted on pregnancy outcome, post-implantation loss, litter size, and sex distribution.
No developmental toxicity was observed, as foetal body weights, placental weights, and external, visceral and skeletal examination did not reveal any adverse effects of the test substance.
Based on the results in this embryotoxicity and teratogenicity study, the No Observed Adverse Effect Level (NOAEL) for teratogenicity and maternal toxicity was 400 mg/kg bw/day.

Ref.: 13

Comment
The dose levels were based on a range finding study. In this study severe clinical effects were noted at a dose level of 1000 and 500 mg/kg bw (after observation of the effects in the 1000 mg/kg dose group, the dose level was adjusted to 500 mg/kg bw on the second day of administration). It is remarkable that in the present study no maternal toxicity was observed up to a dose level of 400 mg/kg bw/day.

3.3.9. Toxicokinetics

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3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

/

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

/

3.3.11. Human data

/

3.3.12. Special investigations

/

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(Oxidative/permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**2.07 µg/cm^2**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.449 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.024 mg/kg**
No observed adverse effect level (mg/kg) (Sub-chronic toxicity)	**NOAEL**	**=**	**100 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**4167**

3.3.14. Discussion

Physico-chemical specifications
2-Methylresorcinol is used in oxidative hair dye formulations at a maximum concentration of 1.8%, after mixing with peroxide developer. Stability of 2-methylresorcinol in test solutions and in marketed products is not reported. Two different melting points (65°C and 116-123°C) of 2-methylresorcinol are reported in the submission documents. A large variation in the melting point of the substance may indicate variable amounts of impurities in the test substance.

General toxicity
LD50 from the only acute study available from 1976 was very low compared with the dose levels used in the 90-day study. This might be explained by (unknown) impurities in the batch used for the LD50 study.
Based on the effects on body weight, the NOAEL in a 90-day study was set at 100 mg/kg bw. The NOEL was lower than 100 mg/kg bw.
Based on the embryotoxicity and teratogenicity studies, the No Observed Adverse Effect Level (NOAEL) for teratogenicity and maternal toxicity was 400 mg/kg bw/day.

Irritation, sensitisation
The test substance applied undiluted to rabbit skin showed mild and transient skin irritation.
Undiluted test substance was severely irritant to the rabbit eye. The test substance was a sensitizer when tested at 50% (w/v) in acetone:olive oil (4:1) in mice; no concurrent positive control experiment was performed.

Dermal absorption
The amount of test substance systemically available from a standard cream formulation with or without hydrogen peroxide was found to be 0.85 ± 0.78 µg/cm² (0.45%) and 8.38 ± 5.41µg/cm² (4.48%), respectively, and 32.2 ± 11.91 µg/cm² for the aqueous solution.
The maximum value observed in the experiment using hydrogen peroxide was 2.07 µg/cm².

Mutagenicity
Several mutagenicity studies both *in vitro* and *in vivo* were performed with the test substance. *In vitro*, it was not mutagenic in the Ames test or in the mouse lymphoma cell line L5178Y, but induced chromosome aberrations in Chinese hamster V79 cells in the absence and presence of metabolic activation. *In vivo*, the test substance, administered intraperitoneally to mice, did not significantly increase micronuclei in bone marrow polychromatic erythrocytes. In the 24-h sampling time, the control level of micronuclei was, however, doubled at all of the 3 doses analysed, although no dose-effect was evident (the frequencies of micronucleated cells were generally low and within the historical control range). There was no decrease in the proportion of polychromatic erythrocytes, suggesting no bone marrow toxicity. However, the compound appeared to be bioavailable, as it was measured in blood of the animals. A dose that was 50% higher than the highest analyzed dose resulted in severe acute toxicity in the mice and could not be included in the micronucleus test.
In summary, although the test substance induced chromosome aberrations *in vitro* in the absence and presence of metabolic activation, an *in vivo* bone marrow micronucleus test in mice was negative, suggesting that the clastogenic potential of the test substance is not seen under *in vivo* conditions. However, several mutagenicity test reports, some of them quite recent, were not provided with the submission. These reports concerned the Ames test (Beevers, 2001; Wallat, 1980), the in vitro chromosomal aberration test (Whitwell, 2001; Darroudi, 1982), the micronucleus test in vivo (Gudi and Krsmanovic, 2002; Richold et al., 1980), and the dominant lethal test in vivo (Re et al., 1986; Rodwell, 1983).

Carcinogenicity
No data were submitted.

4. CONCLUSION

Based on the information provided, the SCCP is of the opinion that the use of 2-methylresorcinol itself as an oxidative hair dye ingredient at a maximum concentration of 1.8% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer.

Studies on the genotoxicity/mutagenicity of 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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7. ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

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