SCCS/1435/11
Revision of 27 March 2012

Health and Consumers
Risk Assessment

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene sulfonic acid (1:1)

4-Formyl-1-methylquinolinium-p-toluenesulfonate (INCI name)

COLIPA n° A157

The SCCS adopted this opinion at its 13th plenary meeting

of 13-14 December 2011

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene sulfonic acid (1:1), 13-14 December 2011

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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1. BACKGROUND

Submission I for 4-Formyl-1-methyl quinolinium, salt with 4-methylbenzenesulfonic acid salt (1:1) was submitted in January 2005 by COLIPA[1] according to COLIPA.

The first scientific opinion (SCCP/0923/05) was adopted 13 December 2005 by the Scientific Committee on Consumer Products (SCCP) with the conclusion:

"Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) has been evaluated as a hair dye. The SCCP is of the opinion that the information submitted is insufficient to assess the safe use of the substance.

Before any further consideration, the following information is required:

- *data on the stability of Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) in typical hair dye formulations should be provided, and*

- *studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP opinions and in accordance with its Notes of Guidance.*

Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) has no EINECS or ELINCS number."

The current submission II is a response to the above mentioned opinion from 2005.

2. TERMS OF REFERENCE

1. *Does SCCS consider Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) safe for use as an oxidative hair dye (used in the absence or presence of hydrogen peroxide) with a maximum on-head concentration up to 2.5% taken into account the scientific data provided?*

2. *And/or does the SCCS have any scientific concern with regard to the use of Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) in oxidative hair dye formulations?*

[1] COLIPA – The European Cosmetics Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

4-Formyl-1-methylquinolinium-p-toluenesulfonate (INCI)

3.1.1.2. Chemical names

CAS name: Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1)

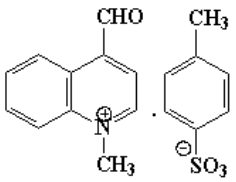
3.1.1.3. Trade names and abbreviations

Moe-HM-6116-190

3.1.1.4. CAS / EC number

CAS: 223398-02-5
EC: 453-790-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{11}H_{10}NO$. $C_7H_7O_3S$

3.1.2. Physical form

Yellow / beige powder

3.1.3. Molecular weight

Molecular weight: 343.41 g/mol

3.1.4. Purity, composition and substance codes

Chemical characterisation of Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene sulfonic acid (Batch Gro-HM-7733-092 and Moe-HM-6116-190) was performed by NMR and IR. UV spectrum in the range 200 nm - 400 nm shows λmax at 237 nm and significant absorbance also at 319 nm.

Purity and impurities

Chemical	Content	
	Batch No.Gro-HM-7733-092	**Batch No Moe-HM-6116-190**
4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid	By NMR > 97.0% (w/w) By HPLC 99.9 % (area %) * By HPLC 86.7% (area %) ***	By NMR > 99.0 %(w/w) By HPLC 99.3% area %) ** By HPLC 99.4% (area %) ***
4-methylquinoline	<detection limit, 75 ppm	<detection limit, 75 ppm
N,N´-dimethyl-4-nitrosoaniline	<detection limit, 40 ppm	<detection limit, 40 ppm
p-Toluenesulfonic acid methyl ester	80 ppm	109 ppm
Unknown impurity [a]	12.8 %	Not detectable
Solvent (water) content	<6.0% (w/w)	<6.0% (w/w)
Sulphate ash	<0.1% (w/w)	<0.1% (w/w)

[a] 12.8 % impurity of an unknown substance in one of the samples was observed when HPLC was performed using a diode array detector (detection wavelength of 468 nm)

* Ref 4, ** Ref. 3, *** Ref.2

Declaration by the Applicant

The specification of the material used in products on the market excludes the presence of the unknown impurity detected at 468 nm.

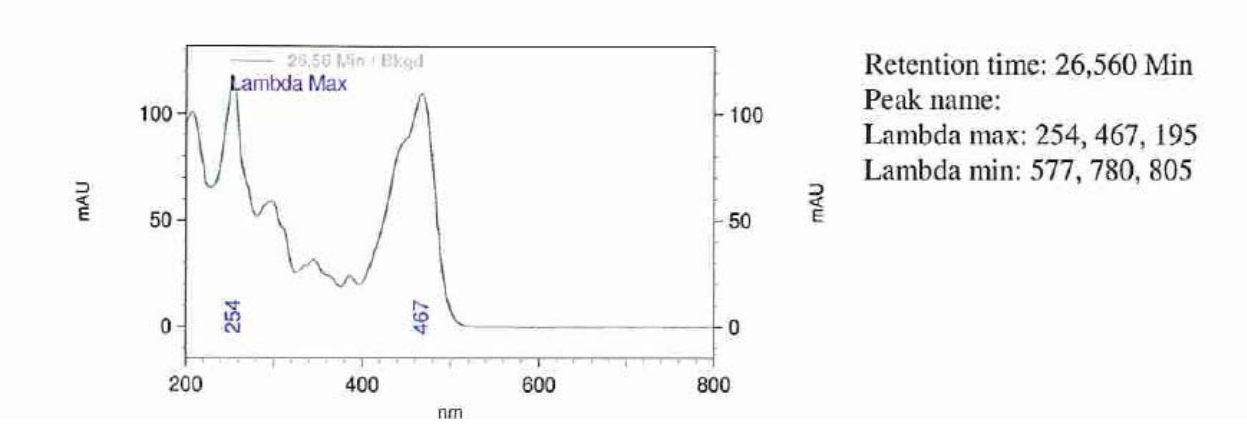
Additional Data submitted in November 2010

Re-analysis of Batch Gro-HM-7733-092 (dated 23 November 2010) by HPLC using 240 nm as detection and quantification wavelength revealed an unknown impurity to be 2.5%

Extraction of old HPLC chromatogram of Batch Gro-HM-7733-092 (analysis dated 30 November 2004) at 240 nm reveals the unidentified impurity to be 4.5%.

SCCS Comments to additional data

The UV-visible spectrum refers to the impurity with following UV-Vis spectrum



The absorption at 240 nm is lower than that at 467nm, and thus extracting the chromatogram at 240 nm will reveal a lower concentration (2.5% and 4.4% in two analyses) compared to 12.8% measured at its λmax 467 nm.
Having a significant absorption at 467 nm, this impurity should be quantified at 467 nm as it was done 2004 analysis, which showed impurity to be 12.8%.

Thus, the applicant's conclusion that "*the unknown impurity is neither a relevant nor a representative impurity of this chemical*" is not correct.

3.1.5.	Impurities / accompanying contaminants

See 3.1.4

3.1.6.	Solubility

Water: 10 g/l
Ethanol: < 1 g/l
DMSO: > 10 g/l

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow}: -1.42 (HPLC, 23 °C, pH 4.8)

3.1.8.	Additional physical and chemical specifications

Organoleptic properties: /
Melting point: 141-145 °C
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /

3.1.9	**Homogeneity and Stability**

The stability of 4-Formyl-1-methylquinolinium-p-toluenesulfonate at a concentration of 0.5% in deionized water, dimethylsulfoxide and acetone/olive oil (4:1) at room temperature was tested over a 48 h by quantification of the dye by HPLC. The recovery of the dye at 8, 24 and 48 hour in all cases was >96%.

General comments concerning physico-chemical specifications:

- The HPLC analysis of the sample with Batch No. Gro-HM-7733-092 showed that this sample contained 12.8% (HPLC peak area at 467 nm) of an unidentified impurity.
- Stability of 4-Formyl-1-methylquinolinium-p-toluenesulfonate in a typical hair dye formulations is not reported.

3.2. Function and uses

4-Formyl-1-methylquinolinium-p-toluenesulfonate is used in oxidative hair dye formulations *(used in the absence or presence of hydrogen peroxide)* up to a maximum concentration of 2.5% on the scalp.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCP/0923/05

Guideline:	OECD 423
Species/strain:	Wistar rats
Group size:	6 (3 per sex)
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch:	Moe-HM-6116-190
Purity:	97.9 %
Doses:	2000 mg/kg bw, single oral treatment
Observation period:	14 days
GLP:	in compliance

3 male and 3 female HanIbm:Wist (SPF) rats were treated with 2000 mg/kg bw of 4-formyl-1-methylquinolonium-p-toluenesulfonate in bi-distilled water. During the observation period of 14 days no mortality and clinical-toxicological findings were seen. The body weights were within the normal range. No macroscopic findings were recorded at necropsy. The LD_{50} is greater than 2000 mg/kg bw.

Ref.: 5

3.3.1.2. Acute dermal toxicity

Taken from SCCP/0923/05

Guideline:	OECD 402
Species/strain:	Wistar rats
Group size:	5 males and 5 female animals
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch:	Gro-HM-7733-092
Purity:	95.3 % (NMR)
Dose level:	2000 mg/kg bw/day
Route:	dermal, on clipped skin
Exposure period:	24 h, semi-occluded
GLP:	in compliance

The test substance in deionised water was applied at a dose of 2000 mg/kg bw to the backs of the animals and covered with a semi-occlusive dressing. 24 h following application the dressing was removed. The animals were examined for mortality, clinical signs and body weight. On day 15 the study was terminated and necropsy was performed.

Results
No treatment-related findings were observed. The LD_{50} is greater than 2000 mg/kg bw in this study.

Ref.: 6

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCP/0923/05

Guideline:	92/69/EEC, B.4, Acute Toxicity – Skin Irritation
Species/strain:	New Zealand White Rabbits
Group size:	1 male, 2 females, age 15 weeks
Test substance:	Moe-HM-6116-190
Batch:	SAT 990705
Purity:	97.9% (HPLC peak area)
Application:	Semi-occlusive application of 0.5 g of the test substance to approximately 6 cm^2 of the intact skin of the shaved area on the left side of the animals
GLP:	in compliance

A 0.5 g of the test substance, moistened with bi-distilled water, was put to a surgical gauge patch (ca. 2.5 cm x 2.5 cm), and that was applied to approximately 6 cm^2 of the intact skin of the shaved area on the left side of the animals. The patch was covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape. Four hours after treatment, the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time. The skin reaction was assessed according to the numerical scoring system listed in the guideline at 1, 24, 48 and 72 h, as well as 7 and 15 days after the removal of the dressing, gauze patch and test substance.

Results
Well-defined erythema were noted in all animals at the 24 h examination and persisted through 15 days (1 animal), slightly decreased at the day examination (1 animal) or increased in severity at 48 h before diminishing to clear by day 15 (1 animal). Very slight to slight oedema was observed in all animals at the 1 h or 24 h examination to disappear at 24-48 hour examination in two animals and persisting through 48 h in one animal. A very slight oedema still appeared in one animal at the 72 h examination. Slight to moderate scaling was observed in all animals at the 7 day reading and persisted in one animal through 15 days.
Application of the test substance to healthy intact rabbit skin resulted in a primary irritation score of 2.78. Local signs (mean values from 24 to 72 hours) consisted of grade 2.22 erythema and grade 0.56 oedema.
No staining by the test substance of the treated skin was observed during the observation period. No irreversible alterations of the treated skin were observed in two animals nor were corrosive effects evident on the skin.

Conclusions
Moe-HM-6116-190 is considered to be an irritant to rabbit skin.

Ref.: 7

3.3.2.2. Mucous membrane irritation

Taken from SCCP/0923/05

Guideline:	92/69/EEC, B.5, Acute Toxicity – Eye Irritation
Species/strain:	New Zealand white rabbit
Group size:	1 male 2 females, 15-17 weeks old
Test substance:	Moe-HM-6116-190
Batch:	SAT 990705

Purity: 97.9% (HPLC peak area)
Application: 0.1g
GLP: in compliance

0.1 g of Moe-HM-6116-190 was placed in the conjunctival sac of the left eye of each animal after pulling the lower lid away from the eye ball. The lids were gently held together for about one second to prevent loss of test substance. The right eye remained untreated and served as reference control.
The ocular reaction was assessed according to the numerical scoring system listed in the guideline at approximately 1, 24, 48, 78 and 168 h after application

Results
Moderate to marked (watery) discharge was observed in all animals at the 1h examination disappearing at the 72 h examination in 2 animals at the 48 h in one animal, but being described as moderate mucous discharge at the 24 h. The sclera was not visible at the 1 h examination in two animals, being moderately reddened in one of the two animals at the 24 and 48 h reading, diminishing in severity at the 72 h or being slightly reddened at the 24 h examination in the second animal. All animals were observed at the 7-day examination. The eye reactions were cleared in all animals within 7 days.
The application of the test substance in healthy rabbit conjunctivae resulted in primary irritation score of 2.22

Conclusions
Moe-HM-6116-190 is considered to be irritating to rabbit eye under experimental conditions.
Ref.: 8

3.3.3. Skin sensitisation

Taken from SCCP/0923/05

Buehler test

Guideline: 98/54/EEC, B.6. Skin Sensitisation
Species/strain: Male guinea pigs, lbm: GOHI
Group size: 20 animals for treatment, 10 for control group and 4 animals for irritation screen, age 4-6 weeks
Test substance: Moe-HM-6116-190
Batch: SAT 990705
Purity: 97.9% (HPLC peak area)
Treatment: Induction: closed patches (Hill Top chambers) containing 50% test material in PEG 400, once a week for 3 week incubation period
Challenge: 50% test material in PEG 400 two weeks after the final induction application
GLP: In compliance

Range finding study
The skin irritation screen was performed by exposing the skin of each of the four animals with 10%, 15% 25% and 50% test material in PEG 400 for 6 hours, using 25 mm Hill Top chambers. The allocation of the different test concentrations to the sites on the four animals was altered in order to minimise site-to-site variation in response. The application sites were assessed for erythema and oedema 24 h and 48 h after removal of patches. No irritant effect was noted with the highest technically possible application concentration (50%).

Main study
Twenty male animals were treated topically with 50% test substance in PEG 400 for 6 hours, once a week for a 3 week incubation period. Two weeks after the final induction

application, the animals were challenged with the same test substance concentration of 50% in PEG 400 as used for induction.
The ten animals of the control group were not treated during the induction but were treated once at challenge with the test substance at 50% in PEG 400.

The exposed skin areas of the animals used for irritation screen and challenge were depilated, using depilatory VEET cream for 3-5 min, 21 h after the patches had been removed. It was then thoroughly washed with warm water. The visual skin responses were graded using the scoring system: 0 (no visible change), 1 (discrete or patchy erythema), 2 (moderate or confluent erythema) and 3 (intense erythema and swelling).

Results
None of the control and test animals were observed with skin reactions after challenge treatment performed with the highest technically applicable non-irritating concentration of Moe-HM-6116-190

Conclusions
Moe-HM-6116-190 was evaluated as non-sensitiser in Buehler Test. However, the vehicle used may not be suitable.

Ref.: 9

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species/strain:	Mice CBA/CaOlaHsd
Group size:	One control group and 3 test groups each with 4 females
Test substance:	Moe-HM-6116-190
Batch:	SAT 990705
Purity:	97.9% (HPLC peak area)
Concentrations:	5.0, 10.0 and 25.0 % (w/v) in acetone:olive oil (4:1, V/V)
GLP:	In compliance

The skin sensitising potential of Moe-HM-6116-190 was investigated in CBA/CaOlaHsd mice by measuring the cell proliferation in the draining lymph nodes after topical application on the ear.

Pre-test (excluded from GLP compliance)
To determine the highest non-irritant and technically applicable test item concentration, a non-GLP pre-test was performed in two mice with concentrations of 2.5%, 5%, 10% and 25% (w/v, in acetone:olive oil (4:1, v/v)). The top dose was the highest technically achievable concentration whilst avoiding systemic toxicity and excessive local irritation. No severe irritant effects were tolerated choosing the test concentrations. The test item in the main study was assayed in three consecutive concentrations.

Main Study
Each test group of mice was treated by topical application to the dorsal surface of each ear lobe with different test item concentrations of 5%, 10% and 25% (w/v) in acetone:olive oil (v/v). 25 µl of the test formulation (freshly prepared) was spread over the entire dorsal surface of each ear lobe once daily for three consecutive days. The mice in the control group were treated with the vehicle alone.
A hair dryer was used to dry the ears surface as quickly as possible after treatment.
Five days after the first topical application, all mice were administered with 250 µl of 78.68 µCi/ml ^{3}HTdR by intravenous injection via a tail vein. Five hours later, all mice were euthanized by intraperitoneal injection of VETANARCOL, and draining lymph nodes were rapidly excised and pooled for each experimental group. After preparing a single cell suspension for each mouse, cells were precipitated by TCA and the radioactivity was

determined (incorporation of [H^3] methyl thymidine in the pellets) by means of liquid scintillation counting as disintegration per minute (dpm).

Results
No mortality was observed throughout the study period. No test item related clinical signs were observed in any animals of the control group, Group2 (5%), or Group 3 (10%). On the third application day, slight ear swelling was observed at both doing sites in all animals of group 4 (25%), persisting for the remainder of the in-life phase of the study.
Body weight development was not affected by the treatment.
The mean stimulation indices (SI), shown in the table below, were not affected in a dose-dependent manner by the treatment with Moe-HM-6116-190. EC3 value was not calculated because no test concentration produced a stimulation index of 3 or higher. The concurrent positive control (using alpha-hexyl cinnamic aldehyde) demonstrated the sensitivity of the assay.

	Test item concentration % (w/v)	**SI**
Group 2	5	1.5
Group 3	10	1.3
Group 4	25	1.9

Conclusions
As indicated by SI, the test substance was not a sensitiser in LLNA.

Ref.: 10

3.3.4. Dermal / percutaneous absorption

Taken from SCCP/0923/05

***In Vitro* Percutaneous Penetration Study**

Guideline: Draft OECD 428, 2000
Tissue: Frozen dermatomed skin from two pigs (age 7 and 9 weeks), thickness approximately 0.75 mm (experiment A mean thickness: 0.73mm and experiment B mean thickness: 0.65mm)
Tissue integrity: Transdermal electrical resistance
Method: Static Franz diffusion cells (receptor chamber volume 8 ml), skin membrane application area 1 cm^2
Test substance: Moe-HM-6116-190 (Lot No. Gro-HM-7733-092), purity 99.9% (HPLC peak area) and ^{14}C-Moe-HM-6116-190 (Lot No. 3489-027), ^{14}C-labelling in the ring specific activity 111µCi/mg, radiochemical purity 96.2% (HPLC)
Formulation A: 17.55% cream SAT020796 + 0.75% Natrosol 250 HR + 79.2% water + 2.5 % test substance (2.1% unlabelled and 0.4% ^{14}C-labelled)
Formulation B: 17.55% cream SAT020796 + 0.75% Natrosol 250 HR + 66.7% water +12.5% developer containing H_2O_2 + 2.5 % test substance (2.1% unlabelled and 0.4% ^{14}C-labelled)
Solution C: 2.1% unlabelled and 0.4% ^{14}C-labelled test substance dissolved in water/ethanol (60:40)
Dose applied: A, B and C each approximately 20 mg/cm², i.e. 0.5 mg test substance/cm²
Receptor fluid: Dulbecco's phosphate buffered saline containing 5% v/v ethanol (96%)
Contact: 30 minutes, then washing of the skin surface, monitoring of the diffusion during 48 h hours
Replicates: Two experiments with the application of each A, B and C, each experiment with 6 replicates
Assay: Liquid scintillation counting, dpm
GLP: study in compliance

The skin penetration of Moe-HM-6116-190 was evaluated in a static diffusion cell using pig skin at temperature was 32.2°C ± 0.3°C. 20 mg solution or formulation (with and without H_2O_2) containing 0.5 mg test substance was applied on 1 cm² and after 30 min exposure, skin was rinsed. 48 h after the application, the stratum corneum was removed by repeated stripping with adhesive tapes to determine the adsorbed test substance. The remaining skin was used to determine the absorbed test substance. The mass balance in this study for formulations A, formulation B and solution C were 89.9%, 90.2% and 96.2% respectively.

Results
The absorbed amounts of Moe-HM-6116-190 were as follows (sum of amounts contained in epidermis, dermis and receptor fluid):

- Formulation A (non-oxidative): 1.57 ± 0.75 µg/cm² (Range 0.57-3.28 µg/cm²) or 0.309 ± 0.152 % (0.11-0.67 %) of the applied dose
- Formulation B (oxidative): 0.69 ± 0.13 µg/cm² (0.58-1.02 µg/cm²) or 0.147 ± 0.043 % (0.09-0.23%) of the applied dose
- Solution C (only test substance): 5.41± 2.97 µg/cm² (2.78-14.35 µg/cm²) or 1.116 ± 0.609 % (0.55-2.94 %) of the applied dose

Ref.: 18

Comment
Dermal absorption under oxidative conditions, i.e. 0.95 µg/cm² (mean + 2SD) will be used for the calculation of Margin of Safety.
Dermal absorption under non-oxidative conditions, i.e. 3.07 µg/cm² (mean + 2SD) will be used for the calculation of Margin of Safety.

3.3.5. Repeated dose toxicity

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

Taken from SCCP/0923/05

Dose range finding study in rats

Guideline:	OECD 407
Species/strain:	Wistar rats
Group size:	5 animals per sex and dose
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch:	Moe-HM-6116-190
Purity:	99.3 %
Dose level:	0, 100, 300 and 1000 mg/kg bw/day
Route:	Oral, gavage
Exposure period:	28 days (males), 29 days (females)
GLP:	in compliance

4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water was administered, by gavage, once daily to Wistar rats (5/sex/per dose) for 28 (males) or 29 (females) days. The test substance was administered at dosage levels of 0, 100, 300 and 1000 mg/kg bw/day. The control group received the vehicle (distilled water) only. All animals were observed twice daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly. At the end of the study urinalysis was performed and the animals were subjected to a complete necropsy.

Results
No treatment related effects were found with regard to mortality, clinical signs, feed consumption, organ weights, urinalysis and pathology.

Ref.: 15

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

Taken from SCCP/0923/05

Guideline:	OECD 408
Species/strain:	Wistar rats
Group size:	10 animals per sex and dose and two recovery groups
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch:	Gro-HM-7733-092
Dose level:	0, 62.5, 250 and 1000 mg/kg bw/day
Route:	Oral, gavage
Exposure period:	13 weeks
GLP:	in compliance

4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water was administered, by gavage, once daily to Wistar rats (10/sex/per dose) for 90 days. The dosages were chosen based on a 28 day dose range finding study. The test substance was administered at dosage levels of 0, 62.5, 250 and 1000 mg/kg bw/day. The control group received the vehicle (distilled water) only. The animals were sacrificed at the end of the study while the animals of the high dose recovery group were observed for a further 4 week-period. All animals were observed daily for mortality, clinical signs and water consumption. Body weights and feed consumption were monitored as well as neurobehaviour in weekly intervals. Ophthalmological examination was performed prior to treatment and to sacrifice. Haematology and clinical chemistry was conducted in week 12 and 4 of the recovery period. Organ weights were measured and macroscopy and histopathology was performed, on all animals.

Results
No mortality and no clinical signs of toxicity were observed and feed consumption and body weight was not affected. Ophthalmoscopy and neurobehavioural observations did not show substance-related toxicity and motor activity and sensory reactivity were comparable to controls.

Changes in haematological parameters in high dose females were found reversible in the recovery group. Clinical chemistry and urinalysis showed no treatment related changes.

The NOAEL, according to the applicant, is 250 mg/kg bw/day.

Ref.: 16

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 (1997)
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA102, TA1535 and TA1537
Replicates:	triplicate cultures
Test substance:	Moe-HM-6116-190
Batch:	Moe-HM-6116-190
Purity:	97.9 % (HPLC)

Vehicle: deionised water
Concentrations: 33, 100, 333, 1000, 2500 and 5000 μg/plate without and with S9-mix
Treatment: direct plate incorporation method
GLP: in compliance
Study period: 16 September 1999 – 15 November 1999

Moe-HM-6116-190 was investigated for the induction of gene mutations in strains of *S. typhimurium*. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment with TA98 and TA100. Toxicity was evaluated up to the prescribed maximum concentration of 5000 μg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. The results of the pre-experiment are reported as part of the main experiment since no relevant toxicity was observed and it comprised five evaluable concentrations for the strains used. The experiments were performed according to the direct plate-incorporation test. Negative and positive controls were in accordance with the OECD guideline.

Results
Moe-HM-6116-190 treatment resulted in weak toxic effects evident as a reduction in the number of spontaneous revertants in strains TA98 and TA1535 in the absence of S9. All plates incubated with Moe-HM-6116-190 showed normal background growth.
A biologically relevant and concentration dependent increase in the number of revertant colonies was observed in strain TA1537 with S9 and in strains TA100 and TA102 with and without S9-mix.

Conclusions
Under the test conditions used, it is concluded that Moe-HM-6116-190 is mutagenic in this gene mutation test in bacteria.

Ref.: 11

In vitro chromosome aberration test

Guideline: OECD 473 (1997)
Cells: Chinese hamster V79 cells
Replicates: duplicate cultures in 2 independent tests
Test substance: Moe-HM-6116-190
Batch: Moe-HM-6116-190
Purity: 97.9 % (HPLC)
Vehicle: deionised water
Concentrations: experiment I: 225, 450, 675 and 900 μg/ml without S9-mix
225, 450 and 900 μg/ml with S9-mix
experiment II: 600, 675 and 750 μg/ml without S9-mix
900, 1125 and 1350 μg/ml with S9-mix
Treatment: 4 h without and with S9-mix; harvest time 18 h after start of treatment.
GLP: in compliance
Study period: 19 August 1999 – 8 December 1999

Moe-HM-6116-190 was investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in V79 cells. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on toxicity, performed identically to the main experiment, with concentrations up to 1800 μg/ml in the absence and presence of S9-mix measuring cell counts and cell morphology. In the main experiment cells were treated for 4 h without and with S9-mix and harvested 18 h after the start of

treatment. Approximately 2 h before harvest, each culture was treated with Colcemid® (final concentration 0.2 µg/ml) to block cells at metaphase of mitosis.
Additionally, 2 cultures per group, not treated with Colcemid®, were set up in parallel, to determine microscopically the cell number; the % reduction in cell number is reported relative to the untreated control. To describe a cytotoxic effect the mitotic index was determined. Chromosome (metaphase) preparations were 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 on toxicity, clear toxic effects were observed after 4 h treatment with 900 µg/ml and above both in the absence and presence of S9-mix. Reduced mitotic indices and reduced cell counts were found both in the absence and presence of S9-mix,
In both experiments, a concentration dependent and statistically significant increase in the number of V79 cells with chromosomal aberrations was observed both without and with S9-mix. An increase in the number of polyploid cells was not observed.

Conclusion
Under the experimental conditions used, Moe-HM-6116-190 was genotoxic (clastogenic) in this chromosome aberration test with mammalian cells (V79 cells).

Ref.: 12

Comment
The required longer treatment period of 18 h in the absence of S9-mix was not performed.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	OECD 474 (1997)
Species/strain:	Crl:NMRI BR mice,
Group size:	5 mice/sex/group
Test substance:	Moe-HM-6116-190
Batch:	Moe-HM-6116-190
Purity:	97.9% (HPLC)
Vehicle:	0.5% carboxymethylcellulose, sodium salt in deionised water
Dose levels:	0, 1000, 1500 and 2000 mg/kg bw
Route:	oral gavage
Sacrifice time:	24 and 48 hours (negative control and high dose only) after the treatment
GLP:	in compliance
Study period:	16 November 1999- 4 February 2000

Moe-HM-6116-190 has been investigated for induction of micronuclei in bone marrow cells of male or female mice. Dose levels were based on the results of a range-finding study in male and female mice on toxicity and mortality recorded over a period of 2 days. In the main experiment mice were exposed orally to 0, 1000, 1500 and 2000 mg/kg bw. Erythrocytes were collected 24 h or 48 h (negative control and high dose only) after dosing. All mice were observed at least once daily for toxic effects and for behavioural changes starting at the day of administration. Composition of the bone marrow was evaluated by the ratio between nucleated cells and erythrocytes. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Bone marrow preparations were examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
No pronounced toxicity was observed in any of the treated groups.

In treated males, the PCE/NCE ratio was slightly lower than in the negative control group but the difference was not statistically significant and in the range of historical negative control data. Yet the average ratios for the different concentrations tested were always lower compared to the negative controls with one exception: in females dosed with 1500 mg/kg bw. Therefore, this result may be indicative for slight cytotoxicity and thus bioavailability of Moe-HM-6116-190.
A biological relevant and dose dependent increase in the number of polychromatic erythrocytes with micronuclei over the concurrent vehicle control was not observed. The positive control substance gave the expected result.

Conclusions
Under the experimental conditions used, Moe-HM-6116-190 did not induce an increase in the number of micronucleated polychromatic erythrocytes and, consequently, Moe-HM-6116-190 is not genotoxic (clastogenic and/or aneugenic) in polychromatic erythrocytes of mice

Ref.: 13

Unscheduled DNA Synthesis (UDS) Test

Guideline:	OECD 486 (1997)
Species/strain:	Sprague-Dawley SD rats
Group size:	3 males
Test substance:	Moe-HM-6116-190
Batch:	Moe-HM-6116-190
Purity:	97.7% (HPLC)
Vehicle:	0.5% carboxymethylcellulose in distilled water
Dose levels:	0, 500, 1000 and 2000 mg/kg bw
Route:	oral gavage
Sacrifice times:	14 hours and 2 hours after treatment
GLP:	in compliance
Study period:	1 December 1999 – 23 February 2000

Moe-HM-6116-190 was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on in formation of the acute toxicity of Moe-HM-6116-190 provided by the sponsor indicating that the oral LD50 in rats is >2000 mg/kg bw. In the main experiment rats were exposed orally to 0, 1000, 1500 and 2000 mg/kg bw. Clinical observations were carried out approximately 30 minute after dosing and prior to perfusion. The animals were starved for 10-14 h before treatment.
The liver was removed and cleaned. The capsule of the liver was opened and the cells were combed out. The cell suspension was then filtered through sterile gauze and diluted. The hepatocytes were separated from the fibroblasts by slow centrifugation. Cell viability was determined by the trypan blue dye exclusion method. At least 90 minutes after plating the cells were incubated for 4 h with 10 µCi/ml ^{3}H-thymidine followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 11-14 days.
UDS was reported as nuclear and cytoplasmic grain counts (average of three areas in the cytoplasm each having the size of the nucleus) as well as the net grain counts (nuclear minus cytoplasmic grains). The percentage cells in repair was calculated for each animal. Unscheduled synthesis was determined in 50 randomly selected hepatocytes on 2 replicate slides per rat. Negative and positive controls were in accordance with the OECD guideline.

Results
No clinical signs were observed in any of the animals treated with Moe-HM-6116-190. At all dose-levels, red to yellow spots were observed in the cage litter. This observation was considered to be related to the excretion of discouloured urine indicating to systemic availability of Moe-HM-6116-190.

No cytotoxic effects were observed in hepatocyte preparations even at the highest dose of 2000 mg/kg bw. In none of the groups treated with the test substance there was a biologically relevant induction of UDS compared to the control group.

Conclusion
Under the experimental conditions used, Moe-HM-6116-190 did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 14

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

Taken from SCCP/0923/05

Guideline:	OECD 414
Species/strain:	Wistar rat
Group size:	20 pregnant females per dose group
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch no:	Moe-HM-6116-190 (report) or Gro-HM-7733-092 (dossier)
Purity:	99.3 % (report)
Dose levels:	0, 100, 316, 1000 mg/kg bw/day
GLP:	In compliance

4-Formyl-1-methylquinolonium-p-toluenesulfonate was administered, by gavage, to 3 groups of 20 or 21 pregnant Wistar rats, a control group received distilled water. Administration was performed daily at dosage levels of 0, 100, 316, or 1000 mg/kg bw (based on a dose-range finding study) from day 5 to 19 of gestation. All mated females were sacrificed at day 20 post coitum. The animals were observed daily for clinical signs. Individual body weights were recorded at days 0, 5, 10, 15 and 20. Feed consumption was measured for the day-intervals 0-5, 5-8, 8-11, 11-14, 14-17, and 17-20. Immediately following sacrifice, the uterus was removed, weighed and the number of (non)viable foetuses, early and late resorptions and the number of total implantations and corpora lutea was recorded. A macroscopic examination of the organs was carried out. All foetuses were individually weighed and the sex of the foetuses was determined. One half of the foetuses were examined for skeletal defects and variations of the ossification process by Alizarin Red staining and one half was evaluated for visceral anomalies.
One animal in the 100 and 316 mg/kg group and 2 animals in 1000 mg/kg group died during the study which may be of incidental nature. No maternal clinical signs of toxicity were reported, feed consumption and body weight gain change was not affected significantly. No gross pathological lesions were found on necropsy.
Variations in pregnancy and litter data were not considered dose-related. A significant decrease in foetal weight was reported for the dose group 1000 mg/kg bw. Significant incidences of haemorrhage in the thymus and cerebral oedema were observed in the middle and high dose groups.
The NOAEL of embryo/foetotoxicity was 100 mg/kg bw, for maternal toxicity the NOAEL was 1000 mg/kg bw.

Ref.: 17

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

Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene sulfonic acid (1:1)

Oxidative

Absorption through the skin	**A**	**=**	**0.95 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.551mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.009mg/kg bw/d**
No Observed Adverse Effect Level (Teratogenicity, gavage, rat)	**NOAEL**	**=**	**100 mg/kg bw/d**
Adjusted for 50% bioavailablity*		**=**	**50 mg/kg bw/d**
MOS		**=**	**5555**

Non-oxidative

Absorption through the skin	**A**	**=**	**3.07 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.781 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.0297 mg/kg bw/d**
No Observed Adverse Effect Level (Teratogenicity, gavage, rat)	**NOAEL**	**=**	**100 mg/kg bw/d**
Adjusted for 50% Bioavailability*			**50 mg/kg bw/d**
MOS		**=**	**1685**

* standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation

3.3.14. Discussion

Physico-chemical specifications

4-Formyl-1-methylquinolinium-p-toluenesulfonate is used in hair dye formulations (used in the absence or presence of hydrogen peroxide) up to a maximum concentration of 2.5% on scalp. Chemical analyses of the two batches of 4-Formyl-1-methylquinolinium-p-toluenesulfonate revealed that one of the batches contained 12.8% (HPLC peak area) of an unknown impurity, determined at its λmax 467 nm. However, the applicant has made a declaration that the specification of the material used in products on the market excludes the presence of this impurity. The stability of 4-Formyl-1-methylquinolinium-p-toluenesulfonate in typical hair dye formulations is not reported.

Toxicity

The oral and dermal LD_{50} of 4-formyl-1-methylquinolinium-p-toluenesulfonate were >2000 mg/kg bw. The NOAEL was set at 250 mg/kg bw (90 day oral rat). The NOAEL was set at

1000 mg/kg bw for maternal toxicity and at 100 mg/kg bw for embryo/foetotoxicity (teratogenicity study). The NOAEL derived from embryo/foetotoxicity has been used for the calculation of MoS.
No reproduction toxicity study was submitted.

Irritation, sensitisation
4-Formyl-1-methylquinolinium-p-toluenesulfonate is an irritant to rabbit skin. It is an irritant to rabbit eye.
The substance is a non-sensitiser in the Buehler test and the LLNA.

Percutaneous absorption
Dermal absorption of 4-formyl-1-methylquinolinium-p-toluenesulfonate solution as well as of two formulations, containing the test material with and without hydrogen peroxide, has been performed *in vitro*. The dermal absorption rate was set at 0.95 µg/cm^2 under oxidative conditions, for the calculation of margin of safety and 3.07 µg/cm² (mean + 2SD) non-oxidative

Mutagenicity
Conclusion
Overall, the genotoxicity of quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene-sulfonic acid (1:1) is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) is mutagenic in vitro, it induced gene mutations in bacteria and chromosome aberrations in mammalian cells *in vitro*.
The positive result in the *in vitro* tests could not be confirmed in *in vivo* tests. In a micronucleus test in mice quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) did not induce an increase in the number of cells with micronuclei. Moreover, exposure did not result in unscheduled DNA synthesis in rats.
In these *in vivo* tests demonstration of target cell exposure is problematic. However, in both *in vivo* tests indications for exposure, although weak, are found. In the micronucleus test in treated males, the PCE/NCE ratio indicative for cytotoxicity and thus exposure was slightly lower than in the negative control group but the difference was not statistically significant and in the range of historical negative control data. Yet the average ratios for the different concentrations tested were always lower in males as well as in females compared to the negative controls with the exception of females dosed with the mid dose. Therefore, this result may be indicative for slight toxicity and thus bioavailability of quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene-sulfonic acid (1:1). In the UDS test at all dose-levels, red to yellow spots were observed in the cage litter. This observation was considered to be related to the excretion of discoloured urine indicating to systemic availability of quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene-sulfonic acid (1:1). Together these findings may be sufficient to consider that quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) is systematically available.
Consequently, quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

4. CONCLUSION

Based on the data provided, Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) does not pose a risk to the health of the consumer when used as an oxidative hair dye (used in the absence or presence of hydrogen peroxide) with a maximum on-head concentration of 2.5%.

5. MINORITY OPINION

Not applicable

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