



**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**

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

**COLIPA N° A157**

Adopted by the SCCP  
during the 6<sup>th</sup> plenary of 13 December 2005

**TABLE OF CONTENTS**

1.	BACKGROUND	.....	3
2.	TERMS OF REFERENCE	.....	3
3.	OPINION	.....	3
4.	CONCLUSION	.....	18
5.	MINORITY OPINION	.....	18
6.	REFERENCES	.....	18
7.	ACKNOWLEDGEMENTS	.....	20

## 1. BACKGROUND

Submission I 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. *On the basis of provided data the Scientific Committee of Consumer Products (SCCP) is asked to assess the risk to consumer when Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) is used in cosmetic products.*
2. *Does the SCCP recommend any restrictions with regard to its use in cosmetic products?*

## 3. OPINION

### 3.1. Chemical and Physical Specifications

#### 3.1.1. Chemical identity

##### 3.1.1.1. Primary name and/or INCI name

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

##### 3.1.1.2. Chemical names

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

Synonym: 4-Formyl-1-methylquinolinium-p-toluenesulfonate

##### 3.1.1.3. Trade names and abbreviations

Moe-HM-6116-190

##### 3.1.1.4. CAS / EINECS number

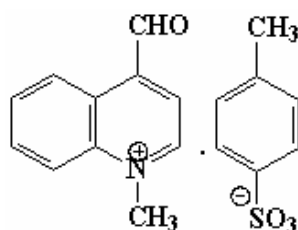
CAS : 223398-02-5

EINECS : /

ELINCS : /

## Opinion on Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) (A157)

## 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

## 3.1.4. Purity, composition and substance codes

Chemical characterisation of Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzene sulfonic acid (Batch No.: 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  $\lambda_{max}$  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 99.0 % (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 <sup>a</sup>	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)

<sup>a</sup> 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.

### 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<sub>ow</sub>)

Log P<sub>ow</sub>: -1.189 (HPLC 23 °C)

### 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: /

#### **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% of an unidentified impurity. However, the NMR spectrometry was not able to identify that as the content of 4-Formyl-1-methylquinolinium-p-toluenesulfonate in the sample was determined to be 97 % by NMR.
- Stability of 4-Formyl-1-methylquinolinium-p-toluenesulfonate in test solutions and in the marketed formulations is not reported.
- pH for P<sub>ow</sub> measurement is not reported.

## 3.2. Function and uses

4-Formyl-1-methylquinolinium-p-toluenesulfonate is used in hair dye formulations 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

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 No.:	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 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<sub>50</sub> is greater than 2000 mg/kg bw.

Ref.: 5

##### 3.3.1.2. Acute dermal toxicity

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 No.:	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 distilled 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<sub>50</sub> is greater than 2000 mg/kg bw in this study.

Ref.: 6

## 3.3.1.3. Acute inhalation toxicity

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**3.3.2 Irritation and corrosivity**

## 3.3.2.1. Skin irritation

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 <sup>2</sup> 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<sup>2</sup> 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-CM-6116-190 is considered to be an irritant to rabbit skin.

Ref.: 7

## 3.3.2.2. Mucous membrane irritation

## Opinion on Quinolinium, 4-formyl-1-methyl-, salt with 4-methylbenzenesulfonic acid (1:1) (A157)

Guideline: 92/69/EEC, B.5, Acute Toxicity – Eye Irritation  
 Species/strain: White guinea pigs, strain Pirbright (SPF)  
 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

#### Buehler test

Guideline: 98/54/EEC, B.6. Skin Sensitisation  
 Species/strain: Male guinea pigs, lbn: 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 <sup>3</sup>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 [<sup>3</sup>H] 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.

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

### Conclusions

Moe-HM-6116-190 causes skin irritation at concentration of 25% (w/v) on the ear dorsum of mice.

As indicated by SI, Moe-HM-6116-190, was not a sensitiser in LLNA.

Ref.: 10

3.3.4. Dermal / percutaneous absorption
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***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 (0.45-1.0 mm)
Tissue integrity:	Transdermal electrical resistance
Method:	Static Franz diffusion cells (receptor chamber volume 8 ml), skin membrane application area 1 cm <sup>2</sup>
Test substance:	Moe-HM-6116-190 (Lot No. Gro-HM-7733-092), purity 99.9% (HPLC peak area) and <sup>14</sup> C-Moe-HM-6116-190 (Lot No. 3489-027), <sup>14</sup> C-labelling in the ring specific activity 111 μCi/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% <sup>14</sup> C-labelled) Formulation B: 17.55% cream SAT020796 + 0.75% Natrosol 250 HR + 66.7% water + 12.5% developer containing H <sub>2</sub> O <sub>2</sub> + 2.5 % test substance (2.1% unlabelled and 0.4% <sup>14</sup> C-labelled) Solution C: 2.1% unlabelled and 0.4% <sup>14</sup> C-labelled test substance dissolved in water/ethanol (60:40)
Dose applied:	A, B and C each approximately 20 mg/cm <sup>2</sup> , i.e. 0.5 mg test substance/cm <sup>2</sup>
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<sub>2</sub>O<sub>2</sub>) containing 0.5 mg test substance was applied on 1 cm<sup>2</sup> 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: 1.57 ± 0.75 μg/cm<sup>2</sup> (Range 0.57-3.28 μg/cm<sup>2</sup>) or 0.309 ± 0.152 % (0.11-0.67 %) of the applied dose
- Formulation B: 0.69 ± 0.13 μg/cm<sup>2</sup> (0.58-1.02 μg/cm<sup>2</sup>) or 0.147 ± 0.043 % (0.09-0.23%) of the applied dose
- Solution C (only test substance): 5.41 ± 2.97 μg/cm<sup>2</sup> (2.78-14.35 μg/cm<sup>2</sup>) or 1.116 ± 0.609 % (0.55-2.94 %) of the applied dose

**Conclusion**

As the content of the test substance in the formulation B will decrease during the experimental period, due to reaction with H<sub>2</sub>O<sub>2</sub> followed by dye formation, the results of the study using formulation B are not suitable for safety evaluation. Therefore, the worst case of the dermal absorption of Moe-HM-6116-190 for formulation A, i.e 3.28 µg/cm<sup>2</sup>, will be considered for the calculation of Margin of Safety.

Ref.: 18

**3.3.5. Repeated dose toxicity****3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity****Dose range finding study in rats**

Guideline:	OECD 407
Species/strain:	Wistar rats
Group size:	10 animals per sex and dose and a recovery group
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch No.:	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**

Guideline:	OECD 408
Species/strain:	Wistar rats
Group size:	10 animals per sex and dose and a recovery group
Test substance:	4-Formyl-1-methylquinolonium-p-toluenesulfonate in distilled water
Batch No.:	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 is 250 mg/kg bw/day.

Ref.: 16

3.3.5.3. Chronic (> 12 months) toxicity
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<b>3.3.6. Mutagenicity / Genotoxicity</b>
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3.3.6.1. Mutagenicity / Genotoxicity <i>in vitro</i>
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### Bacterial gene mutation assay

Guideline:	OECD 471
Species/strain:	<i>Salmonella typhimurium</i> , TA98, TA100, TA102, TA1535 and TA1537
Replicates:	Two independent tests (plate incorporation assay)
Test substance:	COLIPA A 157
Batch No.:	Moe-HM-6116-190
Concentrations:	33 - 5000 µg/plate without and with metabolic activation
GLP:	Quality Assurance Statement included

A 157 has been investigated for the induction of gene mutations in *Salmonella typhimurium*. Liver S9 fraction from rats induced with phenobarbital and β-naphthoflavone was used as the exogenous metabolic activation system. A preliminary study revealed no toxicity and therefore the concentration range was based on the recommended maximum of 5000 µg/plate. Negative and positive controls were in accordance with the OECD guideline.

### Results

A 157 did not induce toxic effects. It induced mutations in the absence and presence of S9 mix in TA 100 and TA 102. Positive results were also obtained in TA 1537 in experiments with S9 mix. A 157 is mutagenic in the bacterial gene mutation assay.

Ref.: 11

### ***In vitro* chromosome aberration test**

Guideline: OECD 473  
 Cells: Chinese hamster V79 cells  
 Replicates: 2 independent tests with and without S9 mix  
 Test substance: COLIPA A 157  
 Batch No.: Moe-HM-6116-190  
 Concentr. scored: 56.3 - 900 µg/ml without metabolic activation  
 56.3 - 1350 µg/ml with metabolic activation  
 GLP: Quality Assurance Statement included

4-formyl-1-methylquinolonium-p-toluenesulfonate (A157) has been investigated for induction of chromosomal aberrations in V79 cells with a harvest time of 18 hours (after start of treatment for 4 hours). Liver S9 fraction from rats induced with phenobarbital and β-naphthoflavone was used as the exogenous metabolic activation system. Test concentrations were selected on the basis of pre-experiments on toxicity. Negative and positive controls were in accordance with the OECD guideline.

#### **Results**

In both experiments, in the absence and the presence of S9, a significant increase in the frequency of chromosome aberrations was measured.

Under the experimental conditions used, A 157 was clastogenic in mammalian cells (V79 cells) *in vitro*.

Ref.: 12

### **3.3.6.2 Mutagenicity/Genotoxicity *in vivo***

#### **Mouse bone marrow micronucleus test**

Guideline: OECD 474  
 Species/strain: Mouse, CrI:NMRI BR  
 Group size: 5 males + 5 females  
 Test substance: COLIPA A 157  
 Batch No.: Moe-HM-6116-190  
 Dose levels: 1000, 1500 and 2000 mg/kg bw  
 Sacrifice time: 24 and 48 (high dose only) hours after the treatment  
 GLP: Quality Assurance Statement included

A 157 has been investigated for induction of micronuclei in the bone marrow cells of mice. 2000 mg/kg bw was selected as the top dose-level. 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. This result does not clearly indicate toxicity to the bone marrow and relevant exposure of the target cells. The mean MNPCE frequencies were not significantly increased in any of the groups treated with the test substance. The positive control substance gave the expected result.

The study was conducted appropriately. A 157 did not induce chromosome aberrations or damage to the mitotic apparatus in bone marrow cells of mice after oral treatment under the test conditions used.

Ref.: 13

### Rat liver *in vivo/in vitro* UDS assay

Guideline:	OECD 486
Species/strain:	Sprague-Dawley SD rats
Group size:	3 males
Test substance:	COLIPA A 157
Batch No.:	Moe-HM-6116-190
Dose levels:	500, 1000 and 2000 mg/kg bw, by gavage
Sacrifice times:	14 hours and 2 hours after treatment
GLP:	Quality Assurance Statement included

A 157 has been investigated for induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* following *in vivo* dosing. 2000 mg/kg bw was selected as the upper dose for the UDS study on the basis of acute toxicity data of the test substance. Negative and positive controls were in accordance with the OECD guideline. Animals were sacrificed after 14 hours in the first experiment and after 2 hours in the second experiment. Hepatocytes from three animals were evaluated per group.

### Results

No clinical signs were observed in any of the animals treated with the test substance. In none of the groups treated with the test substance there was a significant induction of UDS compared to the control group. The results met all the pre-defined criteria for a negative response and therefore the test substance was not found to induce UDS. The positive control substance gave the expected result. The negative test result indicates that A 157 does not induce DNA damage that is detectable with the UDS test.

Ref.: 14

### Conclusion

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 test substance did not induce damage to chromosomes or to the mitotic apparatus in the *in vivo* micronucleus test and did not induce DNA damage detectable with the *in vivo* UDS test. Thus, the mutagenic potential of A157 seen *in vitro* does not lead to genotoxic or mutagenic effects *in vivo* under standard test conditions. However, it has not been demonstrated that there is a relevant exposure of the target cells in the *in vivo* tests under the test conditions applied.

### 3.3.7. Carcinogenicity

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**3.3.8. Reproductive toxicity****3.3.8.1. Two generation reproduction toxicity**

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**3.3.8.2. Teratogenicity**

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 100 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**

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**3.3.10. Photo-induced toxicity****3.3.10.1. Phototoxicity / photoirritation and photosensitisation**

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**3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity**

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**3.3.11. Human data**

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**3.3.12. Special investigations**

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**3.3.13. Safety evaluation (including calculation of the MoS)**

Not applicable

**3.3.14. Discussion**

4-Formyl-1-methylquinolinium-p-toluenesulfonate is used in hair dye formulations 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 an unknown impurity (12.8%), detected at 468 nm by HPLC. 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.

*Toxicity*

The LD<sub>50</sub> of 4-formyl-1-methylquinolinium-p-toluenesulfonate is found to be >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).

*Irritation, sensitisation*

4-Formyl-1-methylquinolinium-p-toluenesulfonate is an irritant to rabbit skin and, at a concentration of 25%, to the ear dorsum of mice. It is an irritant to rabbit eye. The substance is a non-sensitiser in the Buehler test and the LLNA. However, the vehicle used may not be suitable.

#### *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 3.28 µg/cm<sup>2</sup> (worst case formulation A in the absence of H<sub>2</sub>O<sub>2</sub>).

#### *Mutagenicity*

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 test substance did not induce damage to chromosomes or to the mitotic apparatus in the *in vivo* micronucleus test and did not induce DNA damage detectable with the *in vivo* UDS test. Thus, the mutagenic potential of A157 seen *in vitro* does not lead to genotoxic or mutagenic effects *in vivo* under standard test conditions. However, it has not been demonstrated that there is a relevant exposure of the target cells in the *in vivo* tests under the test conditions applied.

## 4. 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.

## 5. MINORITY OPINION

Not applicable

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Dr. C. Chambers  
Prof. R. Dubakiene  
Prof. V. Kapoulas  
Prof. C. Lidén  
Prof. J.-P. Marty  
Prof. T. Platzek (chairman)  
Dr. S.C. Rastogi (rapporteur)  
Prof. T. Sanner  
Prof. G. Speit  
Dr. J. van Engelen  
Dr. I.R. White