EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

5-Amino-4-chloro-o-cresol HCl

COLIPA n° A117

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

The SCCP adopted this opinion at its 14th plenary of 18 December 2007

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 Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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http://ec.europa.eu/health/ph_risk/risk_en.htm

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1. BACKGROUND

Submission I and II for 5-Amino-4-chloro-o-cresol hydrochloride were submitted by COLIPA[1] in May 1994 and in April 2001.

The Scientific Committee on Consumer Products and Non Food Products intended for Consumers (SCCNFP) adopted at its 23rd plenary meeting of 18 March 2003 opinion SCCNFP/0659/03 which concluded that "*the information submitted is insufficient to allow an adequate risk assessment to be carried out. Accordingly, the SCCNFP considers that it is not possible to assess the safe use of the substance. Before any further consideration, the following information is required:*

> *data on the genotoxicity/mutagenicity following the SCCNFP-opinion "Proposal for a Strategy for Testing Hair Dye Cosmetic Ingredients for their Potential of Genotoxicity / Mutagenicity", doc. n° SCCNFP/0566/02 of 4 June 2002, and in accordance with the Notes of Guidance, regularly updated by the SCCNFP (doc. n° SCCNFP/0321/00)".*

According to the current submission III, submitted by COLIPA in July 2005, 5-Amino-4-chloro-o-cresol hydrochloride is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye. The reaction can be accelerated by addition of an oxidizing agent (e.g. hydrogen peroxide). The final concentration on the scalp is proposed to be up to 1.5% (calculated for the hydrochloride salt).

Submission III 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 5-Amino-4-chloro-o-cresol hydrochloride safe for consumers, when used as a precursor in any hair dye formulation with a concentration on the scalp of maximum 1.5% taking into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of 5-Amino-4-chloro-o-cresol hydrochloride in any hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

5-Amino-4-chloro-o-cresol HCl (INCI)

3.1.1.2. Chemical names

Phenol, 5-amino-4-chloro-2-methyl-, hydrochloride (9CI)
2-Methyl-4-chloro-5-aminophenol hydrochloride

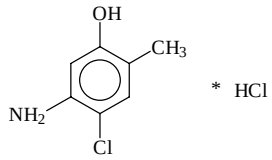
3.1.1.3. Trade names and abbreviations

Ro 934

3.1.1.4. CAS / EINECS number

CAS: 110102-85-7
ELINCS: / (applied for)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C_7H_8ClNO . HCl

3.1.2. Physical form

Beige to light brown amorphous powder

3.1.3. Molecular weight

Molecular weight: 194.06 as hydrochloride

3.1.4. Purity, composition and substance codes

Batch Kn-Gi 8956/88 = SAT 050780

Identification and Characterisation:	NMR, IR and UV and elemental analysis
Purity:	NMR 98% (w/w)
Purity:	HPLC 99.9% (peak area)
Chloride:	18% (w/w)
Sulphate:	0.1% (w/w)

Solvent content:	water	< 0.1% (w/w)
	ethanol	< 0.3% (w/w)
Sulphated ash:	0.6% (w/w)	

Ref.: 2

The chemical characterisation of Batch 3279/54 was performed by NMR, IR, UV/Vis spectroscopy and elemental analysis. Purity: not stated.

Ref.: 3

Chemical characterisation of Batch 2395/56 was performed by NMR, MS, elemental analysis, and TLC. Purity: not stated, water content 0.23%.

Ref.: 4

Chemical characterisation of Batch 3279/89 was based on UV-Vis spectroscopy, TLC, and elemental analysis. Purity: ca. 98% (teratogenicity study), water content 2.5% (w/w)

Ref.: 5

General description of the purity:
NMR > 97 % (W/W)
HPLC > 99% (peak area)

Declaration by the applicant (included in the Summary of Submission III)

The batch of COLIPA A117 (Batch 2257/185) used in the acute oral toxicity test is not fully analytically described. However, information is available from the laboratories that have synthesized this batch concerning the identity and purity of the material produced at that time. From this information it can be concluded that the former not fully described batch is representative and its specification is quite similar to the fully characterized batch Kn-Gi 8956/88.

3.1.5. Impurities / accompanying contaminants

Batch No. Kn-Gi 8956/88
4-Amino-2-hydroxytoluene 640 ppm (COLIPA A27)

Ref.: 2

Batch 3279/64
4-Amino-2-hydroxytoluene <0.1%

Ref.: 3

Batch 3279/89
4-Amino-2-hydroxytoluene 1-2%

Ref.: 5

General description of impurities
Heavy Metal Content

Pb	<20 ppm
Sb and Ni	<10 ppm
As and Cd	< 5 ppm
Hg	<1 ppm

3.1.6. Solubility

Water: < 1 g/l room temperature

Ethanol:	50 – 200 g/l	room temperature
DMSO:	> 100 g/l	room temperature

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: -1.90 (calculated Syracuse Vers. 1.66)

3.1.8. Additional physical and chemical specifications

Melting point:
Boiling point: > 240 °C (decomposition)
Flash point:
Vapour pressure:
Density:
Viscosity:
pKa:
Refractive index:
pH:
UV_Vis spectrum:

3.1.9. Stability

5-Amino-4-chloro-o-cresol HCl dissolved in bi-distilled water (1.8-28.8 mg/ml) was stable (deviation -1 to + 1%) up to two hour at room temperature (20 ± 2 °C).
The homogeneity varied in the range of -1% to +1% of the mean concentration.

Ref.: 16

General Comments to physico-chemical characterisation

* Purity of several batches of test material is not reported.
* Concentrations of 4-Amino-2-hydroxytoluene as an impurity in the test material varied from 0.06% to 2%.
* Water solubility of the test material is not determined by the EU method
* Log P_{ow}: calculated values cannot be accepted as estimates of the true physical constant without justification.
* Stability of 5-Amino-4-chloro-o-cresol HCl in the marketed products is not reported.
* UV_Vis spectrum of 5-Amino-4-chloro-o-cresol HCl was not submitted.

3.2. Function and uses

5-Amino-4-chloro-o-cresol HCl is used as a precursor for hair colours. It reacts with primary intermediates to form the final dye-stuff. The reaction can be accelerated by addition of an oxidizing agent (e.g. hydrogen peroxide), but can also be achieved by air oxidation.

The final concentration of 5-Amino-4-chloro-o-cresol HCl on head can be up to 1.5% (calculated for the hydrochloride salt).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	/
Species/strain:	Wistar rats
Group size:	4, 2 male and 2 female
Test substance:	Ro 934
Batch:	2257/185
Purity:	Not reported
Dose:	Aliquots of 10 ml/kg body weight, doses of 1184, 1539 and 2000 mg/kg bw. Vehicle was distilled water.
Route:	oral gavage
Observation period:	2 weeks
GLP:	not in compliance

Acute oral toxicity of Ro 934 was tested in Wistar rats, 6 male and 6 female rats were used. A group of 4 rats (2 male and 2 female) was administered via gavage a single dose of 1184, 1539 or 2000 mg 5-Amino-4-chloro-o-cresol HCl (batch 2257/185) per kg bw. During a two weeks observation period, mortalities and clinical-toxicological observations were recorded daily, body weight was noted weekly. A *post mortem* examination was carried out in all animals.

Results
One male rat of the 2000 mg/kg dose group died after the treatment.
Clinical symptoms observed at all dose levels, and included abdominal position, apathy, piloerection, cyanosis, tremor, crouching, diarrhoea, semi-closed eyes and reduced acoustic reactions. In 7/12 animals no substance related pathological findings were registered. For the remaining animals pathological investigations revealed brightened coloration of the liver and the kidneys, ulcerations in the glandulous fundus stomach, hydrometra, ileum and brown-red coloured hydrocele in the intestine. In the dead male also lung emphysema was observed.

Conclusion
For male rats the LD_{50} was between 1539 and 2000 mg/kg bw. The LD_{50} value for females was > 2000 mg/kg bw.

Ref.: 6

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 females
Substance:	2-methyl-4-chlor-5-aminophenol hydrochloride
Batch:	3279/64
Purity:	> 99%
Dose:	0.5 g, semi-occlusive
Vehicle:	moistened with water
GLP:	in compliance

Approximately 24 hours before treatment, the dorsal fur was shaved, to expose an area of about 100 cm^2.
An aliquot of 0.5 g of moistened 2-methyl-4-chlor-5-aminophenol hydrochloride was exposed to the intact shaved back skin of each animal. The patch was removed four hours after semi-occlusive contact.
Animals were examined for signs of erythema, eschar and oedema formation. The skin reactions were assessed immediately after exposure, at 1, 24, 48 and 72 hours after termination of the exposure.

Results
The test substance induced some erythema in 2 animals and oedema in the other which were reversible within 24 hours after exposure in all three animals. Brown/yellowish or yellow staining of the skin was observed on the area of application.

Conclusion
The study authors concluded that, under the conditions of the study, the undiluted test substance was not irritating when applied to the intact rabbit skin under semi-occlusive conditions.

Ref.: 7

Comment
The SCCP concluded that the undiluted test substance caused some reversible irritation when applied to the intact rabbit skin under semi-occlusive conditions.

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species:	New Zealand White rabbits
Group:	1 male
Substance:	2-methyl-4-chlor-5-aminophenol hydrochloride
Batch:	3279/64
Purity:	> 99%
Dose:	51 mg instilled into conjunctival sac
Vehicle:	/
GLP:	in compliance

Approximately 51 mg of 2-methyl-4-chlor-5-aminophenol hydrochloride was instilled into the conjunctival sac of one eye of the test animal. The substance remained in permanent contact with the eye. The other eye served as the control.
The eye irritation reactions were scored approximately 1 hour, 24, 48 and 72 hours and 7 days after instillation of the test solution.

Results
The instillation of the test substance immediately caused severe eye irritation. For ethical reasons, a decision was made not to expose additional animals and to terminate the one-animal-test on day 8.
Instillation of 2-methyl-4-chlor-5-aminophenol hydrochloride into the eye affected the cornea, the iris and the conjunctivae. The opacity of the cornea, the injection of the iris and the irritation of the conjunctivae were irreversible within the study period of seven days.

There was evidence of ocular corrosion and staining with fluorescein revealed corneal epithelial damage in the animal.

Conclusion
Under the conditions of the study, the undiluted test substance was extremely irritating to the rabbit eye.

Ref.: 8

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species:	mice; CBA/CaOlaHsd strain
Group:	3 dose groups and a control group (vehicle only) of 4 female mice each
Substance:	A117 / SAT 050780
Batch:	Kn-Gi 8956/88
Purity:	98%
Dose:	5%, 10% and 20%
Vehicle:	ethanol:water (7:3 v/v)
Control:	α-hexylcinnamaldehyde (5, 10, 25%)
GLP:	in compliance

A homogenous dilution of the test item in a mixture of ethanol:water (7:3 v/v) was made shortly before each dosing. The highest technically applicable non-irritating test item concentration was found in a pre-test with two mice. The vehicle was chosen due to the chemical reactivity/instability of the test substance with other organic solvents (acetone, dimethylformamide). Based on test results 5%, 10% and 20% solutions were chosen for the main study.
Each test group of mice was treated by topical application to the dorsal surface of each ear lobe (left and right) with the 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 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 the tail vein.

Approximately five hours after ^{3}HTdR application the 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).

α-Hexylcinnamaldehyde was used as the approximately contemporaneous positive control.

The proliferative capacity of pooled lymph node cells was determined by quantifying 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
The Stimulation Index (S.I.) was below 3 in all dose groups. No dose response relation was noted.

Test Item Concentration	**S.I.**
5% (w/v)	1.18
10% (w/v)	0.87
20% (w/v)	0.87

Calculation of the EC3 value was not performed as no test concentration produced a stimulation index of 3 or above. The positive control had a calculated EC3 of 22.2%.

Conclusion
Based on the criteria of the test system, A117 / SAT 050780 was found to be a non-sensitizer when tested up to the highest technically achievable concentration of 20% (w/v) in mice.

Ref.: 9

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428
Tissue:	Pig skin, dermatomed to a mean thickness of 0.75 mm
Group size:	one male, one female; 4 chambers from each
Diffusion cells:	static Franz; skin discs of 1.0 cm²
Skin integrity:	trans-dermal electrical resistance; at least 7kΩ
Test substance:	A117 / SAT 050780
Batch:	Kn-Gi 8956/88
Purity:	98%
Radiolabel	[14C]-labelled A117; SAT 051002
Radiolabel batch	3560-097
Radiolabel purity	99%
Test item:	Cream formulation TM0045-1 containing 3% A117
Doses:	20 mg formulation per cm² pig skin. Dose of the test substance was approximately 0.32 mg/cm² skin
Receptor fluid:	Dulbecco´s phosphate buffered saline (pH 7.35)
Solubility receptor fluid:	< 1g/L in water
Stability:	1mg/L stable in saline
Method of Analysis:	liquid scintillation counting
GLP:	in compliance

The dermal absorption/percutaneous penetration of [^{14}C]-A117 out of a basic cream mixed with a developer containing hydrogen peroxide was studied. The skin integrity of frozen (at -20 °C) 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. The composition of the formulations used is shown in the tables below.

Ingredient of basic cream	**Concentration in %**
A117	3.00
Toluene-2,5-diamine	2.325
Hydrenol D	9.35
Texapon NSO-UP	15.00
Dehyton K	12.50
Lorol techn.	2.20
Eumulgin B2	0.75
Sodium sulphite	0.20
Ammonium sulfate	0.40
Ascorbic acid	0.20
Citric acid	for pH adjustment

Ingredient of developer mix with H_2O_2	**Concentration in %**
Dipicolinic acid	0.10
Sodium pyrophosphate, acid	0.03
Turpinal SL	1.50
Texapon NSO-UP	2.00
Ammonia, 25%	for pH adjustment
Tartaric acid	for pH adjustment
Aculyn 33	15.00
Hydrogen peroxide (50% H_2O_2 solution)	12.00
Water	ad 100
	pH 3.82

Ingredient of basic cream	Concentration in %
Ammonia	for pH adjustment
Water	ad 100
	pH 9.03

Ingredient of developer mix with H_2O_2	Concentration in %

Shortly before topical application to skin, the basic cream was mixed (1:1) with the hydrogen peroxide containing developer mix and then traced with [^{14}C] radio-labelled A117. The content of A117 in the final application formulation was 1.6%.
The dermal absorption/percutaneous penetration of the test substance was investigated for the open application of about 20 mg formulation per cm² pig skin. Therefore the resulted dose of the test substance was approx. 0.32 mg/cm² skin. Skin discs of 1.0 cm² were exposed to the formulations for 30 minutes, terminated by gently rinsing with a 0.01% Tween 80 solution and water.
The formulation was analysed in two experiments with four replicates per experiment for adsorbed, absorbed and penetrated amount of the test substance. The receptor fluid used was Dulbecco´s phosphate buffered saline (pH 7.35). In the static system, samples of the receptor fluid were drawn before the application of the test substance formulation and 0.5, 1, 2, 4, 6, 24, 28 and 48 hours after application. The removed volume was replaced by fresh receptor fluid.

Results
Both the amounts absorbed and penetrated were taken as systemically available.
In this *in vitro* dermal penetration study, the amount of A117 systemically available from a standard cream formulation mixed with a developer containing hydrogen peroxide was found to be 12.47 ± 1.82 µg/cm² (range 10.60 to 16.47 µg/cm²) or 3.90 ± 0.69 % (range 3.12 to 5.29 %) of the applied dose.

Ref.: 17

Comment
As too few chambers were used, the A_{max} of 16.47 µg/cm² in an oxidising formulation (or 5.29% of the applied dose in a final application formula containing 1.6% active) is used for the calculation of the MOS.
This evaluation applies to oxidative conditions only; no data on absorption in non-oxidative conditions has been made available.

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:	/
Species/strain:	Sprague-Dawley (CD) rats
Group size:	20 (10/sex) and 10 rats (5/sex) in a recovery study
Test substance:	Ro 934
Batch:	2395/56
Purity:	Not reported (although in submission stated: comparable to Kn-Gi 8956/88)
Dose:	0, 20, 60 and 180 mg/kg bw diluted in 10 ml/kg bw of distilled water
Route:	oral gavage
GLP:	in compliance

The test substance was given as an aqueous solution for 90 consecutive days in daily doses of 0, 20, 60 and 180 mg/kg bw by oral gavage to groups of Sprague Dawley rats (10/sex).

Additionally, 5/sex in both a control and a high dose group, were assessed for recovery of treatment-related effects, four weeks after the last administration. During the study mortality, signs of intoxication, body weight, food and water consumption, haematological and biochemical parameters were recorded. In addition, ophthalmoscopy was performed. At the end of the study, the animals were sacrificed and subjected to pathological investigations.

Results
Ro 934 was well tolerated by rats after repeated oral administration of up to 180 mg/kg bw/day. Food consumption was comparable to control during the course of study in all male test animals. In female rats of the mid dose group, an increased food consumption was observed. However, this was not considered a treatment related effect. Water consumption was not affected. Ophthalmoscopy revealed no toxicologically relevant effects. No change in body weight gain was found in the treated animals. Minor deviations of a few biochemical and haematological parameters were recorded but these findings were not dose-related and were not correlated with organ toxicity and therefore are not regarded as indication of systemic toxicity. Macroscopy did not reveal any effect. Histopathology was only performed on the control and the high dose group, and did also not reveal any effect.

Conclusions
On the basis of the these results, the No-Observed-Adverse Effect-Level (NOAEL) in rats is 180 mg/kg bw/day

Ref.: 4, 14

Comment
For hazard identification, the test concentration used was too low. However, the study is useful for evaluation.

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 Reverse Mutation Test

Guideline:	OECD 471
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102
Replicates:	triplicates both in the presence and absence of metabolic activation.
Test substance:	A117
Solvent:	deionised water
Batch:	Kn-Gi 8956/88
Purity:	98 %
Concentrations:	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
Treatment:	direct plate incorporation with 48 h incubation, without and with S9-mix
GLP:	In compliance

A117 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a pre-experiment with all strains both without and with S9-mix. Toxicity was measured as a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn. The pre-experiment was reported as main experiment. The

test compound was evaluated up to the prescribed maximum concentration of 5000 µg/plate without and with metabolic activation with the direct plate incorporation method. Negative and positive controls were in accordance with the guideline.

Results
No precipitation of the test compound occurred up to the highest dose investigated. Toxic effects at the higher concentrations were found in the absence of metabolic activation for TA98, TA100, TA102 and TA1537, in the presence of metabolic activation for all strains.
A moderate but dose dependent increase in revertant colonies was found in strain TA98 in the presence of metabolic activation. The reduction in revertant colonies at 5000 µg/plate might be caused by toxic effects. The test compound did not induce an increase in the number of revertant colonies in the other strains at any concentration tested both in the presence or absence of metabolic activation

Conclusion
Under the experimental conditions used A117 was genotoxic (mutagenic) in this gene mutation tests in TA98 with metabolic activation.

Ref.: 10

Comment
Since a positive result was obtained, a second experiment was not performed.

In Vitro Mammalian Cell Gene Mutation Test (*tk* locus*)*

Guideline:	OECD 476	
Cells:	L5178Y Mouse lymphoma cells	
Replicates:	duplicate cultures in 2 independent experiments	
Test substance:	A117	
Solvent:	deionised water	
Batch:	Kn-Gi 8956/88	
Purity:	98 %	
Concentrations:	Experiment 1:	62.5, 125, 250, 375 and 500 µg/ml without S9-mix 31.3, 62.5, 125, 250 and 375 µg/ml with S9-mix
	Experiment 2:	31.3, 62.5, 125, 250, and 375 µg/ml without S9-mix 100, 200, 250, 300 and 350 µg/ml with S9-mix
Treatment	Experiment 1:	4 h treatment without and with S9-mix; expression
period		72 h; selection period of 10-15 days
	Experiment 2:	24 h treatment without S9-mix; expression period 48
h;		selection period of 10-15 days 4 h treatment with S9-mix; expression period 72 h; selection period of 10-15 days
GLP:	in compliance	

A117 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. 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 measuring relative suspension growth and precipitation. In the main test, cells were treated for 4 h (experiment I, experiment II with S9-mix) or 24 h (experiment II without S9-mix) followed by an expression period of 72 h (4 h treatment) or 48 h (24 h treatment) to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as relative suspension growth and/or relative total growth of the treated cultures relative to that of the solvent control cultures. The number of colonies was counted manually; colony size distribution was determined in the controls and in all treated concentrations of A117. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test precipitation was observed at 500 µg/ml and above in the absence or presence of S9-mix at both treatment intervals. The appropriate level of toxicity (10-20% survival after the highest dose) was reached in experiment I but not in experiment II. In experiment I without S9-mix an increase in the mutant frequency (MF) was seen at an intermediate dose. Since this increase was not reproducible and the MF was normal again at the higher doses, it was considered not biologically relevant. With S9-mix in experiment I an increase in MF was observed at the highest concentration tested. Since this increase was within the historical control range and not reproducible in experiment II, this increase was also considered not biologically relevant.

Conclusion
Under the experimental conditions used, A117 was not genotoxic (mutagenic or clastogenic) in the mouse lymphoma assay at the *tk* locus.

Ref.: 11

In vitro Micronucleus Test

Guideline:	draft OECD 487 (2004)
Cells:	V79
Replicates:	duplicates cultures
Test substance:	A117
Solvent:	deionised water
Batch:	Kn-Gi 8956/88
Purity:	98 %
Concentrations:	0, 62.5, 125, 250, 500 and 1000 µg/ml without S9-mix 0, 62.5, 125, 250 and 500 µg/ml with S9-mix
Treatment	4 h treatment; harvest 24 h after beginning of treatment
GLP:	In compliance

The test was performed in accordance with the draft OECD 487 (2004) and OECD 473 (1998) (*in vitro* mammalian chromosome aberration test).

A117 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in V79 cells. Dose selection was performed according OECD 473. The highest concentration chosen for the evaluation of genotoxictity should produce clear cytotoxicity with reduced cell growth by 60%, determined by the mean of cell count prior to cell seeding on slides, and/or the occurrence of precipitation. V79 cells were treated with A117 for 4 h without and with S9-mix. Harvest times were 24 hours after the beginning of treatment. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the draft guideline.

Results
Precipitation was observed at 250 µg/mL and above both in the absence and presence of S9-mix.
A117 had no effect on osmolarity or pH as compared to concurrent vehicle controls.
Clear toxic effects were observed at the highest concentrations both without and with S9-mix. Both without and with S9-mix a clear dose dependent increase in cells with micronuclei was found for the remaining concentrations tested.

Conclusion
Under the experimental conditions used A117 did induce micronuclei and, consequently, is genotoxic (clastogenic and/or aneugenic) in V79 cells.

Ref.: 12

Comment
According to the international guidelines only one experiment was performed, since a positive result was obtained in this experiment.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474
Species/strain:	CFW 1 (Winkelmann) mice
Group size:	7 mice/sex
Test substance:	Ro 934 (2395/56)
Batch:	2395/56
Purity:	not stated
Dose level:	0 and 500 mg/kg bw
Route:	intraperitoneal
Vehicle:	distilled water
Sacrifice times:	24, 48 and 72 h after treatment
GLP:	not in compliance

Ro 934 has been investigated for the induction of micronuclei in bone marrow cells of mice. The test concentration was based on a dose range finding assay with concentration up to the prescribed maximum concentration of 2000 mg/kg bw. In the main experiment mice were exposed intraperitoneally to a single dose of 0 and 500 mg/kg bw Ro 934. Bone marrow cells were collected 24, 48 and 72 h after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE ratio). Negative and positive controls were included.

Results
Mortality due to treatment did not occur. Slight toxic reactions were seen, reduced activity, ruffled fur, and abdominal position. After about 20 h orange coloured urine was observed. Exposure to A117 only resulted in a slightly decreased PCE/NCE ratio 48 h after treatment. A117 did not induce a biologically relevant increase in micronucleated erythrocytes in any of the groups treated.

Conclusion
Under the experimental conditions used A117 did not induce micronuclei in bone marrow cells of treated mice and, consequently, A117 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 13

Comment
Although only a slight decrease in the PCE/NCE ratio was observed, intraperitoneal administration of a test compound ensures sufficient systemic exposure of the target bone marrow cells. Systemic exposure was also evident by the observations of toxicity.

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

Guideline:	OECD 414
Species/strain:	Wistar IHAN (Kfm: WIST, outbred, SPF) rats, pregnant
Group size:	25 dams/group
Test substance:	Ro 934
Batch:	3279/89
Purity:	ca. 98%
Stability:	pure substance: not given in vehicle: at least 2h
Vehicle:	distilled water
Dose:	0, 20, 100, or 500 mg/kg bw dissolved in water
Route:	oral, gavage
GLP:	in compliance

Groups of Wistar rats (25 dams/group) were treated daily with 0, 20, 100, or 500 mg/kg bw of Ro 934 (dissolved in water) during GD 6-15. The mortality and the body weight gain were observed daily. The dams were sacrificed on day 20 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, placentae and the sex of foetuses were recorded. Approximately one-half of the foetuses were selected at random and examined for visceral alterations. The remaining foetuses were examined for skeletal malformations, variations and retardation of the normal organo-genesis after appropriate staining.

Results

1. Maternal toxicity

The evaluation of food consumption and body weight gain revealed no treatment related effects. There were no mortalities, behavioural changes or necropsy findings in the dams of all dose groups considered to be related to administration of the test substance. One female of the mid dose group died due to an intubation error.

The only treatment related finding was yellow-brownish discoloration of the urine in all dose groups.

None of the maternal reproduction parameters - pregnancy rate, numbers of corpora lutea and implantations, pre- and post-implantation loss and number of live foetuses - were affected by treatment with Ro 934. An increase in post implantation loss was observed in the low dose group. Although this increase was slightly above historical control values (historical data were included in the test report) this increase was not considered relevant, since there was no dose-relation, there were no other signs of maternal toxicity observed in this dose group and there were no signs of foetotoxicity in any other dose group.

2. Foetal toxicity

No differences were observed in any of the foetal parameters - sex ratios, mean foetal body weight and type or frequency of abnormal findings noted during external, visceral and skeletal examination - which were attributed to administration of the test article.

Conclusions

Daily oral administration of Ro 934 during organogenesis (day 6-15 of gestation) did not cause any adverse effects in dams up to the highest dose tested. Also up to the highest dose tested, the test chemical was neither embryo-lethal, embryotoxic nor teratogenic. Therefore the *No-Observed-Adverse Effect-Level* (NOAEL) for both maternal and foetal toxicity was 500 mg/kg bw/day.

Ref.: 5, 15, 16

Comment
For hazard identification, the test concentration used was too low. However, the study is useful for evaluation.

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

(5-Amino-4-chloro-o-cresol HCl)
(oxidative / permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**16.47 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**11.53 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.19 mg/kg**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**180 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**947**

3.3.14. Discussion

Physico-chemical properties
5-Amino-4-chloro-o-cresol HCl is used as a precursor in oxidative and non-oxidative hair colouring formulations. Its final concentration on head can be up to 1.5% (as hydrochloride).
The purity of several batches of test material was not reported. Concentration of 4-Amino-2-hydroxytoluene as an impurity in the test material varied up to 640 ppm. Water solubility of the test material is not determined by an EU method. A calculated value of Log P_{ow} cannot be accepted as an estimate of the true physical constant without justification. The stability of 5-Amino-4-chloro-o-cresol HCl in the marketed products is not reported.

General toxicity
For male rats the LD_{50} was between 1539 and 2000 mg/kg bw. The LD_{50} value for females was > 2000 mg/kg bw.
On the basis of the results of a 90-day study in rats, the No-Observed-Adverse Effect-Level (NOAEL) was set at 180 mg/kg bw/day.
In a teratogenicity study in rats, the NOAEL for both maternal and foetal toxicity was set at 500 mg/kg bw/day.

Irritation / sensitisation
Under the conditions of the study, the undiluted test substance caused some reversible irritation when applied to the intact rabbit skin under semi-occlusive conditions. The undiluted test substance was extremely irritating to the rabbit eye.
Based on the criteria of the test system, A117 / SAT 050780 was found to be a non-sensitizer when tested up to the highest technically achievable concentration of 20% (w/v) in mice (LLNA).

Dermal absorption
The amount of A117 systemically available from a standard cream formulation mixed with a developer containing hydrogen peroxide was found to be 12.47 ± 1.82 µg/cm² (range 10.60 to 16.47 µg/cm²) or 3.90 ± 0.69 % (range 3.12 to 5.29 %) of the applied dose.
As too few chambers were used, the A_{max} 16.47 µg/cm² in an oxidising formulation (or 5.29% of the applied dose in a final application formula containing 1.6% active) will be used in calculating the MOS.
This evaluation applies to oxidative conditions only; no data on absorption in non-oxidative conditions has been made available.

Mutagenicity / genotoxicity
Overall, the genotoxicity of A117 is sufficiently investigated in valid genotoxicity tests for the three types of mutation: gene mutation, structural chromosome mutation and aneuploidy. A117 did induce gene mutations in bacteria but not in mammalian cells. An increase in V79 with micronuclei was induced in an *in vitro* micronucleus test. The latter result was not confirmed in an *in vivo* micronucleus test.
Since only a weak positive results was found in the bacterial gene mutation test (only in TA98 with metabolic activation) which was not confirmed in a mammalian *in vitro* gene mutation assay and since the clastogenic effect found *in vitro* was not confirmed *in vivo*, A117 itself can be considered to have no relevant mutagenic potential. Additional tests are not necessary.

To reach a definitive conclusion, appropriate tests with A117 in combination with hydrogen peroxide have to be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

This risk assessment relates to the use of 5-amino-4-chloro-o-cresol HCl in oxidative hair dye formulations only.

The SCCP is of the opinion that the use of 5-amino-4-chloro-o-cresol HCl, at a maximum concentration of 1.5% (calculated for the hydrochloride salt) on the head, does not pose a risk to the health of the consumer.

5-amino-4-chloro-o-cresol HCl itself has no mutagenic potential.
However, studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

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