EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

2-Amino-3-hydroxypyridine

COLIPA n° A132

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

The SCCP adopted this opinion at its 15th plenary of 15 April 2008

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.

Scientific Committee members

Claire Chambers, Gisela Degen, Ruta Dubakiene, Bozena Jazwiec-Kanyion, Vassilios Kapoulas, Jean Krutmann, Carola Lidén, Jean-Paul Marty, Thomas Platzek, Suresh Chandra Rastogi, Jean Revuz, Vera Rogiers, Tore Sanner, Günter Speit, Jacqueline Van Engelen, Ian R. White

Contact
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

(ISSN)

ACKNOWLEDGMENTS

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

External experts

Dr. M.-L. Binderup	Danish Institute for Food & Veterinary Research, Denmark
Dr. H. Norppa	Institute of Occupational Health, Finland
Prof. K. Peltonen	Finnish Food Safety Authority, EVIRA, Finland
Dr. J. van Benthem	RIVM, the Netherlands

Keywords: SCCP, scientific opinion, hair dye, A132, 2-amino-3-hydroxypyridine, directive 76/768/ECC, CAS 16867-03-1, EINECS 240-886-8

Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on 2-amino-3-hydroxypyridine, 15 April 2008

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1. BACKGROUND

Submission I for 2-Amino-3-hydroxypyridine was submitted in November 1995 by COLIPA[1]. Submission II was submitted in September 2001. No opinion was issued by the Scientific Committee.

According to the current submission III, submitted by COLIPA in July 2005, 2-Amino-3-hydroxypyridine is used as a precursor for hair dyes. It reacts with primary intermediates to form the final dye. The reaction can be accelerated by the addition of an oxidizing agent (e.g. hydrogen peroxide), but can also be achieved by air oxidation. The final concentration on the scalp can be up to 1%.

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 the use of 2-Amino-3-hydroxypyridine safe for consumers, when used as a precursor in any hair dye formulations with a concentration on the scalp of maximum 1.0% taking into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of 2-Amino-3-hydroxypyridine in 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

2-Amino-3-hydroxypyridine (INCI)

3.1.1.2. Chemical names

3-Pyridinol, 2-amino-
2-Amino-3-hydroxypyridine
2-Amino-3-pyridinol
2-Aminopyridine-3-ol
3-Hydroxy-2-aminopyridine
3-Hydroxy-2-pyridinamine

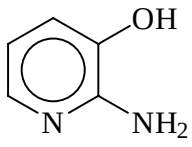
3.1.1.3. Trade names and abbreviations

2-Amino Rhodol	Colorex 2A3HP
HS-P6	Jacocol 2A3HP
2A 3 PYR	Rodol 2A3PYR
COLIPA A 132	

3.1.1.4. CAS / EINECS number

CAS: 16867-03-1
EINECS: 240-886-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_5H_6N_2O$

3.1.2. Physical form

White – light grey, amorphous powder (beige-greyish crystals, *Submission 1*)

3.1.3. Molecular weight

Molecular weight: 110.12

3.1.4. Purity, composition and substance codes

General

Batch n° 02-03-01 (= SAT 030634 = SAT 030383 = SAT 040294)

Identification:	verified by NMR-, IR- and UV-spectrometry
Purity by NMR assay:	> 95.1% (w/w)
Purity by HPLC assay:	> 95% (area%)

Ref.: 2

Batch n° 200410043 (= SAT 050153)

Identification: and	verified by NMR-, IR-, UV-spectrometry and qualitative quantitative HPLC
Purity by NMR assay:	99.9 weight%
Purity by HPLC assay:	99.4 peak area%
Solvent content (water):	< detection limit (0.1 weight%)

Ref.: 3

Batch n° 305711/1

Identification:	HPLC, UV (200-300 nm)
Purity:	99.96% (HPLC peak area)
HPLC content:	98-102%
UV content:	98-102% (at 238 nm)
Sulfate ash:	<1%
Melting point:	172 ± 3 °C (166-176 °C)

Ref.: 4

3.1.5. Impurities / accompanying contaminants

General

Heavy Metal Content: Pb < 20, Sb and Ni < 10, As and Cd < 5, Hg 1ppm

Batch n° 02-03-01 (= SAT 030634 = SAT 030383 = SAT 040294)

2,3-Dihydroxypyridine:	5.5% (w/w)
Solvent content (water):	< detection limit (0.1%, w/w)

Ref.: 2

Batch n° 200410043 (= SAT 050153)

2,3-Dihydroxypyridine:	< detection limit of 100 ppm
Solvent content (water):	< detection limit (0.1 weight%)

Ref.: 3

Batch n° 305711/1

3-Hydroxy-2-pyridine:	< 500 ppm
Iron:	< 50 ppm
Water:	< 1%

Ref.: 4

3.1.6. Solubility

Submission I

Water: 3 g /100 ml in water at 20 °C

Submission III

Water:	10 – 100 g/l at room temperature
Ethanol:	10 – 100 g/l at room temperature
DMSO:	50 – 200 g/l at room temperature

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.05 (calculated)

3.1.8. Additional physical and chemical specifications

Melting point:	172 °C (166-176°C)
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum:	Absorption at 208 nm, 238 nm and at 307 nm (λ_{max})

3.1.9. Stability

The test solutions of 2-Amino-3-hydroxypyridine in water (30-125 mg/10 ml) were stable (deviation from nominal concentration -8.3 to +5.5%) for 4 h at room temperature.

Ref.: 14

General Comments to physico-chemical characterisation

- Water solubility of 2-Amino-3-hydroxypyridine is not determined according to EEC method A6.
- Log P_{ow}: calculated values cannot be accepted as estimates of the true physical constant without justification.
- The content of 2,3-dihydroxypyridine varied from 0 to 5.5% (w/w) in the three reported batches.
- Stability of 2-Amino-3-hydroxypyridine in the test vehicles other than water and in marketed products is not reported.

3.2. Function and uses

2-Amino-3-hydroxypyridine 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 the addition of an oxidizing agent (e.g. hydrogen peroxide), but can also be achieved by air oxidation.

The final concentration of 2-amino-3-hydroxypyridine on head can be up to 1.0%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 423
Species/strain:	Rat, strain Wistar
Group size:	6 females, 3 males
Test substance:	2-amino-3-hydroxypyridine in propylene glycol
Batch:	02-03-01
Purity:	> 95% (HPLC)
Dose levels:	three males 300 mg/kg bw; three females 300 and 1000 mg/kg bw
Route:	Oral, by gavage
Exposure:	Single administration
GLP:	In compliance
Date:	25 May – 30 June 2004

2-Amino-3-hydroxypyridine was suspended in propylene glycol and administered once by gavage to 6 female and 3 male Wistar rats at doses of 300 and 1000 mg/kg bw. All three females treated with 1000 mg/kg bw died and one out of three females treated with 300 mg/kg bw also died. No mortality occurred among male rats.

Results
Mortalities occurred within several minutes post-treatment. Clinical signs at 1000 mg/kg bw exposure were tremor, cramped posture, abnormal gait, salivation and chromodacryorrhoea. In females at 300 mg/kg bw the signs were mainly lethargy, tremor, laboured respiration, salivation and chromodacryorrhoea. In males at 300 mg/kg bw no clinical signs were noted. Recovery of survivors was complete. Macroscopic post mortem examination of all females that were found dead after a dose of 1000 mg/kg bw showed enlarged lungs with many dark red foci. Reddish discolouration of mucosa in stomach was noted in a female died at 300 mg/kg bw group.

Conclusion
The acute median lethal oral dose (LD_{50}) of 2-amino-3-hydroxypyridine in male Wistar rat is higher than 300 mg/kg bw and in female Wistar rat less than 1000 mg/kg bw. Based on the OECD 423 the LD_{50} cut-off value is 500 mg/kg bw.

Ref.: 5

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:	rabbits, New Zealand White strain
Group:	3 male Albino

Substance:	A132 / SAT 030634
Batch:	02-03-01
Purity:	> 95% (HPLC)
Dose:	0.5g
Vehicle:	water (Milli-U)
GLP:	in compliance
Date:	18 - 28 November 2003

An aliquot of 0.5 g of the moistened test substance was applied under semi-occlusive conditions 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 approximately 1 hour, 24, 48 and 72 hours after termination of the exposure.

Results
Under the conditions of the study, the undiluted test substance produced no reaction at any observation point. Brownish staining did not hamper scoring.

Conclusion
Under the conditions of the study, the test substance was not irritant to rabbit skin.

Ref.: 6

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species:	rabbits, New Zealand White strain
Group:	3 male Albino
Substance:	A132 / SAT 030634
Batch:	02-03-01
Purity:	> 95% (HPLC)
Dose:	60 mg (equivalent to about 0.1 ml)
Vehicle:	/
GLP:	in compliance
Date:	24 November – 16 December 2003

The equivalent of 0.1 ml of A132 was instilled into the conjunctival sac of one eye of the test animals. The substance remained in permanent contact with the eyes until rinsing with water, 24 hours after instillation. The other eyes served as controls. 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 undiluted A132 into the eyes resulted in effects on the cornea, iris and conjunctivae. The corneal injury consisted of opacity (maximum grade 1) and epithelial damage (maximum 50% of the corneal area). The corneal injury had resolved within 48 or 72 hours, or 7 days in the respective animals. Iridial irritation grade 1 was observed in one animal 1 and 24 hours after instillation. The irritation of the conjunctivae consisted of redness and chemosis and had completely resolved within 14 days in all animals.

Ref.: 7

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

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species:	mice; CBA strain
Group:	3 dose groups (with a control group, receiving the vehicle only, being used from another study – Notox 398115) of 5 female mice each
Substance:	A132 / SAT 030634
Batch:	02-03-01
Purity:	> 95% (HPLC)
Dose:	5%, 25% and 50%
Vehicle:	ethanol:water (7:3 v/v)
Control:	α-hexylcinnamaldehyde; 85% purity; non-contemporaneous (9 months earlier)
GLP:	in compliance
Date:	5- 26 January 2004

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 non-irritating test item concentration was found in a pre-test with four mice. Based on these test results 5%, 25% and 50% solutions were chosen for the main study.
Each test group of mice was treated by topical (epidermal) 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 all mice were killed. The draining lymph nodes were excised and pooled for each animal. 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).
Results from a non-contemporaneous experiment with α-hexylcinnamaldehyde (August 2003) were used as the 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)	2.9
25% (w/v)	1.7
50% (w/v)	1.9

The non-contemporaneous positive control gave calculated EC3 value of 5.5% by linear extrapolation (Comment: this control was undertaken 9 months before the present study and therefore was outside OECD guidelines).

Conclusion
Based on the criteria of the test system, A132 was determined to be a non-sensitizer when tested up to the highest achievable concentration of 50% (w/v) in ethanol:water (7:3) in mice.

Ref.: 8

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428
Tissue:	pig skin, dermatomed to 0.4mm
Group size:	3 pigs (sex not stated); 2 membranes from each
Diffusion cells:	static;
Skin integrity:	trans-dermal electrical resistance; >3KΩ
Test substance:	A132 (supplied at 2% in formulation TM0034-1a, batch SAT 050321)
Batch:	200410043
Purity:	/
Radiolabel:	[^{14}C]-2-amino-3-hydroxypyridine
Radiolabel batch	EPPS-05-043-29-27
Radiolabel purity	99%
Test item:	Formulation mixed with oxidative developer; final concentration of A132: 1.04%
Doses:	20 mg formulation per cm² pig skin (test substance was approximately 0.2 mg/cm² skin)
Receptor fluid:	Dulbecco's phosphate buffered saline (pH 7.35)
Solubility receptor fluid:	/
Stability:	/
Method of Analysis:	liquid scintillation
GLP:	in compliance
Date:	18 – 24 November 2005

The dermal absorption/percutaneous penetration of [^{14}C]-A132 from a standard hair dye formulation was studied on the clipped, dermatomed skin of three young pigs.
The skin integrity of frozen (at -20 °C) skin discs was checked by measuring the trans-dermal electrical resistance.
Shortly before topical application to skin, the basic cream was mixed (1:1) with the developer mix containing hydrogen peroxide. The concentration of A132 in the final applied formulation was 1.04%.
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 resulting dose of the test substance was approx. 0.2 mg/cm² skin. Skin discs of 2.54 cm² were exposed to the formulations for 30 minutes, terminated by gently rinsing with a 3% Teepol® solution.
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, 29 and 48 hours after application. The removed volume was replaced by fresh receptor fluid. The formulation was analysed with six replicates for adsorbed, absorbed and penetrated amount of the test substance.

Ingredient of basic cream	Concentration %
A132	2.00
Toluene-2,5-diamine (COLIPA A 005)	2.20
Hydrenol D	9.35
Texapon NSO-UP	15.00
Dehyton K	12.50
Lorol techn.	2.20
Eumulgin B2	0.75

Ingredient of basic cream	Concentration %
Sodium sulphite	0.20
Ammonium sulfate	0.40
Ascorbic acid	0.20
Citric acid	for pH adjustment
Ammonia	for pH adjustment
Water	ad 100
	pH 10.02

This formulation was traced with [^{14}C] radio-labelled A132 shortly before application.

Ingredient of developer mix with H_2O_2	[%]
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

Results
Both the amounts absorbed and penetrated were taken as systemically available.
In this *in vitro* dermal penetration study the amount of A132 systemically available from a standard cream formulation with hydrogen peroxide (final concentration of A132 1.04%) was found to be 1.26 ± 0.218 µg/cm² (range 1.02 to 1.61) or 0.606 ± 0.104 % (range 0.490 to 0.774).

Conclusion
An A_{max} of 1.61 µg/cm² may be used for calculating the MOS as too few test chambers were used.

Ref.: 17

Comment
No data were provided on the dermal absorption of A132 from a standard cream formulation without hydrogen peroxide.

3.3.5. Repeated dose toxicity

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

No data submitted

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

Guideline:	OECD 408
Species/strain:	Rat, strain Wistar
Group size:	10 per sex per dose 5 per sex (satellite recovery control and high dose groups)
Test substance:	2-amino-3-hydroxypyridine
Batch:	02-03-01
Purity:	> 95% (HPLC)
Dose levels:	0, 30, 60 and 120 mg/kg bw/day, aqueous solution
Route:	Oral, by gavage
Exposure:	Once daily over a period of 13 weeks
GLP:	In compliance

Date: 19 October 2004 – 2 May 2005

Aliquots of 2-amino-3-hydroxypyridine (10 ml/kg bw) were administered in a single dose by gavage. Animals were exposed for 91 days in daily doses of 0, 30, 60 and 120 mg/kg bw. Control animals received the vehicle only. During the study mortality, signs of intoxication, body weight gain and the food consumption were recorded. The animals in the satellite groups were additionally examined during the 4 weeks treatment free period.

Results
Four animals were found dead during the study. Two animals were accidentally killed and two females died in the high exposure group. Oedema of the lungs and the subcutis were noted post mortem in these particular cases.
Some animals in both sexes at the high exposure group demonstrated clinical signs like clonic convulsion, salivation and vocalization. In addition to this disturbed locomotion, chromodakryorrhoea were noted in the mid and high doses. The ophthalmoscopical findings were all normal. The body weights were significantly reduced in both sexes at the high exposure groups. The haematological investigations showed significant differences in mean corpuscular haemoglobin and mean cell volume. However, parameters were not dose dependently related. Biochemical parameters like potassium, protein and alanine aminotransferase levels were increased at the high dose male group. In high dose female group aspartate aminotransferase was increased and cholesterol was decreased.
The differences in kidney and liver weights at the end of exposure were noted. However, the histopathological findings gave no indication of the cause of the weight change. In the liver the histopathology changes were necrosis in mid and high dose groups.

Conclusions
Based on the histopathological changes in the liver the NOAEL was set to 30 mg/kg bw/day.
Ref.: 14

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 (1997)
Species/strain:	TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	Triplicates in two independent experiments
Test substance:	A 132
Solvent:	DMSO
Batch:	Lot 02-03-01
Purity:	> 99.9% (area%, HPLC)
Concentrations:	0, 33, 100, 333, 1000, 2500, 5000 μg/plate
Treatment:	Experiment one: Standard plate incorporation assay Experiment two: Pre-incubation assay Both assays with and without Phenobarbital/β-Naphthoflavone induced rat liver S9-mix
GLP:	in compliance
Date:	28 August – 25 September 2003

A pre-study was performed with strains TA 98 and TA 100 to evaluate the toxicity of the test item. The cultures showed normal background growth up to 5000 μg/plate and there were no signs of toxicity, evident as a reduction in the number of revertants.

Results
In both experiments the plates incubated with A 132 showed normal background growth up to 5000 μg/plate with and without S9-mix in all strains used. In both experiments there were no indications of an increase in the mutant frequency at any concentration in the tester strains either with or without metabolic activation. The only exception was TA 100 in both experiments without metabolic activation.
A concentration-related increase was observed. However, it was weak and the number of revertants at the highest concentrations was within the range of historical control. Thus, the weak increase is not considered of biological importance.

Conclusion
Under the test conditions used, A132 did not induce gene mutations in bacteria.

Ref.: 9

In Vitro Mammalian Cell Gene Mutation Assay (*tk*$^{+/-}$ locus)

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma cell line L5178Y
Replicates:	Duplicate cultures in one experiment
Test substance:	A 132
Solvent:	Deionised water
Batch:	02-03-01
Purity:	> 99.9% (area%, HPLC)
Concentrations:	0, 150, 300, 600, 900 and 1200 μg/ml
Treatment:	With and without Phenobarbital/β-Naphthoflavone induced rat liver S9-mix: 4-hour treatment and 72 h expression period.
GLP:	In compliance
Date:	29 November – 27 December 2004

The test substance was examined for its genotoxic (mutagenic/clastogenic) activity in the L5178Y *tk*$^{+/-}$ mouse lymphoma test in the absence and presence of metabolic activation. A preliminary toxicity test was conducted with 4 and 24 hours without metabolic activation and 4 hours with metabolic activation with 8 concentrations between 4.7 and 600 μg/ml (maximum solubility in DMSO). Following 4 h treatment without metabolic activation no relevant toxicity was observed up to the maximum concentration of 600 μg/ml. After 4 h treatment with metabolic activation toxic effects leading to RSG (relative suspension growth) values below 50 % were observed at 150 μg/mL and above. However, the gradient of toxicity was shallow and the RSG was reduced to 33.3 % at 600 μg/ml. Following 24 h treatment (without metabolic activation) toxic effects as described above occurred at 75 μg/mL and above. The test medium was checked for precipitation at the end of each treatment period (4 or 24 hours) before the test item was removed. No precipitation was observed after 4 and 24 hours treatment up to the maximum concentration in the presence and absence of metabolic activation.

Results
Based on the toxicity test the maximum concentration of the main experiment was raised to 1200 μg/ml (10 mM) by using water as solvent instead of DMSO. A treatment time of four hours was used. Toxic effects in both parallel cultures indicated by a relative total growth of less than 50 % occurred at 600 μg/ml and above in the absence of metabolic activation (26 and 21% at the highest tested concentration). In the presence of metabolic activation relevant toxic effects occurred already at 300 μg/ml and above (25% and 15 % at the highest tested concentration).

A substantial and reproducible dose dependent increase in mutant colony numbers was observed in the absence of metabolic activation. The threshold of two times the corresponding solvent control was reached and exceeded at 1200 µg/ml in both parallel cultures. The historical range of negative and solvent controls was exceeded at the maximum concentration in culture I.
The ratio of small versus large colonies was shifted towards small colonies, indicating clastogenic activity. With metabolic activation no relevant increase in mutation frequency was observed.

Conclusion
Under the test conditions used A 132 was genotoxic (mutagenic and/or clastogenic) in the absence of metabolic activation in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y.

Ref.: 10

In Vitro Mammalian Cell Gene Mutation Assay (*hprt*-locus)

Guideline:	OECD 476 (1997)
Species/strain:	Chinese hamster cell line V79
Replicates:	Duplicate cultures in two different experiments
Test substance:	A 132
Solvent:	deionized water
Batch:	02-03-01
Purity:	99.9% (area%, HPLC)
Concentrations:	Experiment I: without S9-mix: 0, 75, 150, 300, 450, 600 µg/ml with S9-mix: 0, 75, 150, 300, 600, 1200 µg/ml Experiment II: without S9-mix: 0, 37.5, 75, 150, 300, 450 µg/ml
Treatment	With and without Phenobarbital/β-Naphthoflavone induced rat liver S9-mix: four hours treatment in experiment one and 24 hours in experiment two
GLP:	in compliance
Date:	8 March – 28 April 2005

A pre-test on toxicity was performed in order to determine the concentration range for the mutagenicity experiments. The highest concentration used in the pre-test was chosen with regard to the purity and the molecular weight of the test item. Test item concentrations between 9.4 and 1200 µg/mL (10 mM) were used to evaluate toxicity in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Following 4 and 24 h treatment toxic effects were observed at 300 µg/mL in the absence of metabolic activation. At higher concentrations the cell growth was completely inhibited. In the presence of metabolic activation no relevant toxicity occurred up to the maximum tested concentration. Up to the highest concentration with and without metabolic activation no precipitation occurred at both treatment intervals.

Results
Toxic effects in both parallel cultures were observed in the first experiment at 300 µg/mL and above in the absence of metabolic activation indicated by a relative cloning efficiency reduced below 50 %. In the presence of metabolic activation no toxic effect occurred up to the maximum concentration. In experiment II, toxic effects were observed at 150 µg/mL and above in both cultures. No significant reproducible increase in mutant colony numbers/10^6 cells was observed in experiment I up to the maximum concentration with and without metabolic activation. The induction factor exceeded the threshold of three times the corresponding solvent control in culture I of experiment I with metabolic activation at 600 µg/mL. However, the total number of mutant colonies/10^6 cells remained well within the range of the historical solvent control data and no increase was observed in the parallel culture.

In experiment II the threshold was exceeded at 75 µg/mL in culture I and at 75.0 to 450 µg/mL in culture II. The absolute mutation frequencies remained within the historical range. The increase of the induction factor was not dose dependent. Since the increase was only reproducible at the lowest concentration (75 µg/mL) and the increase in culture II was not dose dependent and within historical control data, the increase was not considered to be of biological relevance. The positive controls in both experiments showed distinct increases.

Conclusion
Under the test conditions used, the test substance did not induce gene mutations in Chinese Hamster cells.

Ref.: 11

***In Vitro* Mammalian Chromosome Aberration Test**

Guideline:	OECD 473 (1997)
Species/strain:	Chinese hamster cells V79
Replicates:	Two replicates in one experiment
Test substance:	A 132
Solvent:	deionised water
Batch:	02-03-01
Purity:	> 99.9% (area%, HPLC)
Concentrations:	With and without S9-mix: 0, 800, 1000 and 1100 µg/ml.
Treatment:	With and without Phenobarbital/β-Naphthoflavone induced rat liver S9-mix: 4-hours treatment and harvest time 18 hours after start of treatment.
GLP:	in compliance
Date:	10 September – 14 October 2003

A pretest for toxicity was performed with and without metabolic activation using 4-hours treatment. Eight concentrations between 8.6 and 1100 µg/ml were tested. The same concentrations were used without metabolic activation using 24-hours treatment time. Using 4-hours treatment time the reduction in cell numbers at the highest concentration was 40 and 52% with and without metabolic activation, respectively. Using 24-hours treatment time the reduction was 55% at 550 µg/ml and 100% at the highest concentration of 1100 µg/ml.

Results
Without metabolic activation the reductions in cell numbers were 14, 32 and 51% at the tested concentrations of 800, 1000 and 1100 µg/ml, respectively. With metabolic activation, the reductions in cell numbers were 34, 32 and 52% at the three tested concentrations.

Both with and without metabolic activation, statistically significant increases in the number of cells with structural chromosome aberrations were observed at all three concentrations. The observed increases were at the same level or even higher compared to the positive control.
A concentration dependent increase of polyploid cells was observed, both with and without metabolic activation, but this increase was not statistically significant.

Conclusion
Under the test conditions used, A 132 induced structural chromosome aberrations both in the presence and absence of metabolic activation in V79 cells.

Ref.: 12

Comment
The historical solvent and positive control data for polyploid metaphases were not provided.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	5 males and 5 females
Test substance:	A 132
Lot no:	02-03-01
Purity:	> 99.9% (area%, HPLC)
Dose level:	0, 12.5, 25 and 50 mg/kg bw
Route:	intraperitoneal
Vehicle:	2.5% carboxymethylcellulose
Sacrifice times:	24 hours and 48 hours (only for the high dose level)
GLP:	In compliance
Date:	30 August – 7 December 2004

A pre-experiment for acute toxicity was performed using two males and two females. The mice were examined for acute toxic symptoms at intervals around, 1 h, 2-4 h, 6 h, 24 h, 30 h and 48 h after administration of the test item. This showed that 50 mg A 132 per kg bw was the highest applicable dose without significant effects on the survival rates, but with clear signs of toxicity. At a higher dose (75 mg/kg bw) all 4 treated animals died. In the main study at least 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei.

Results
The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that A 132 had no cytotoxic effect on the bone marrow. The analysis of the blood samples treated with 50 mg test item per kg bw showed that the test item could be quantified in the blood of the treated animals (data of only 2 males per test group reported), whereby the concentration in blood samples taken 1 h after the treatment were higher than in those taken at the 4 h interval, indicating the bioavailability of the test item.

There were no indications of an increase in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with A 132 were below or near to the value of the vehicle control group. The positive control (40 mg/kg bw cyclophosphamide) administered i.p.) showed a statistically significant increase of induced micronucleus frequency.

Conclusion
Under the test conditions used the test article COLIPA 132 was not clastogenic and/or aneugenic in the bone marrow cells of mice.

Ref.: 13

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:	Rat, strain Wistar
Group size:	24 to 25 females per dose group
Test substance:	2-amino-3-hydroxypyridine
Batch:	305711/1
Purity:	99%
Dose levels:	0, 15, 45, 135/90 mg/kg bw/day
Vehicle:	distilled water
Route:	Oral, by gavage
Exposure:	daily from day 6 of gestation until day 15
GLP:	In compliance
Date:	14 June – 6 July 1993

2-Amino-3-hydroxypyridine was administered daily at the doses of 0, 15, 45, 135/90 mg/kg bw/day. The highest dose was reduced to 90 mg/kg bw/day after 5 days due to the mortality of the animals. Distilled water was used as a vehicle for the test substance. Mortality and clinical signs were observed on a daily basis. Body weight gain was recorded on days 0, 6, 11, 16 and 20 of gestation. The dams were sacrificed on the day 20 post-coitum 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. About half of the foetuses were examined for visceral alterations. The remaining foetuses were examined for skeletal malformations, variations and retardation of normal organo-genesis.

Results
Two dams died in the high dose group dosed with 135 mg/kg bw/day after one or two days of dosing. Convulsion and hypersalivation were noted before deaths. Hypersalivation was also noted with some other dams at high dose groups. Statistically significant reduction of food consumption and body weight gain was seen in high dose group animals compared to control group.

The occurrence of a statistically significant increase in incompletely ossified caudal vertebrae in the mid dose group and statistically significant increase in incompletely ossified pelvis in the low and mid dose group were noted. However, there was no dose response on these variations. Statistically significant increase in the incidence of rudiment lumbar ribs and variations were observed in the highest dose group.

Conclusions
Because of the death of dams and reduced body weight gain at the highest dose group, the NOAEL of maternal toxicity is set to 45 mg/kg bw/day. Based on the increase in the incidence of rudimentary lumbar ribs and other variations in the highest exposure group, the NOAEL for embryotoxicity is also determined to be 45 mg/kg bw/day.

Ref.: 16

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(2-amino-3-hydroxypyridine)
(oxidative - permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**1.61 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.13 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.02 mg/kg bw**
No observed adverse effect level (90-day, rat, oral)	**NOAEL**	**=**	**30 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**1500**

3.3.14. Discussion

Physico-chemical properties
2-Amino-3-hydroxypyridine 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 the addition of an oxidizing agent (e.g. hydrogen peroxide), but can also be achieved by air oxidation. The final concentration of 2-Amino-3-hydroxypyridine on head can be up to 1.0%. The reported values of water solubility and Log P_{ow} of the test material are not adequate. The content of 2,3-dihydroxypyridine varied from 0 to 5.5% (w/w) in the three reported batches. Stability of 2-Amino-3-hydroxypyridine in test vehicles other than water and in marketed products is not reported.

General toxicity
The acute median lethal oral dose (LD_{50}) of 2-amino-3-hydroxypyridine in male Wistar rat is higher than 300 mg/kg bw and in female Wistar rat less than 1000 mg/kg bw.

In a 90-day study on rats, the NOAEL was set at 30 mg/kg bw/day, based on the histopathological changes in the liver.
Because of the death of dams at the highest dose group the NOAEL of maternal toxicity is set to 45 mg/kg bw/day. Based on the increase in the incidence of rudimentary lumbar ribs and other variations in the highest exposure group, NOAEL for embryotoxicity is determined to be 45 mg/kg bw/day.

Irritation / sensitisation
Based on the criteria of the test system, 2-Amino-3-hydroxypyridine was not irritant to rabbit skin. Undiluted 2-Amino-3-hydroxypyridine was irritating to the rabbit eye.
2-Amino-3-hydroxypyridine was determined to be a non-sensitizer when tested up to the highest achievable concentration of 50% (w/v) in ethanol:water (7:3) in mice.

Dermal absorption
As too few chambers were used, the bioavailability of 2-Amino-3-hydroxypyridine in an oxidising formulation containing 1.0% 2-Amino-3-hydroxypyridine should be considered as Amax 1.61 µg/cm². This may be used for calculating the MOS.
No data were provided on the dermal absorption of A132 from a standard cream formulation without hydrogen peroxide.

Mutagenicity / genotoxicity
2-Amino-3-hydroxypyridine has been investigated for genetic endpoints *in vitro*: gene mutations in bacteria, gene mutations and chromosomal aberrations at the *tk* locus in the mouse lymphoma cell line, gene mutations at the *hprt*-locus in V79 cells of Chinese hamster and chromosome aberrations in Chinese hamster ovary cells. 2-Amino-3-hydroxypyridine did not induce gene mutations in bacteria or gene mutations at the *hprt*-locus. 2-Amino-3-hydroxypyridine was a potent clastogene in Chinese hamster ovary cells and clastogenic effect was also indicated by the induction of small colonies in the mouse lymphoma assay with mammalian cells (L5178Y) in the absence of metabolic activation. The genotoxic/clastogenic effects observed in the *in vitro* assays could not be confirmed in an *in vivo* assay.
The discrepancy between the two *in vitro* mammalian gene mutation tests may be explained by the fact that the increase in the mouse lymphoma assay due to the increase in small colonies, which indicate a clastogenic effect.
Based on these results, 2-Amino-3-hydroxypyridine itself is not considered to have *in vivo* genotoxic potential. However, appropriate tests with 2-Amino-3-hydroxypyridine in combination with hydrogen peroxide should be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

Since no data on dermal absorption under non-oxidative conditions were provided, this opinion relates to the use of 2-amino-3-hydroxypyridine in oxidative hair dye formulations only.

The SCCP is of the opinion that the use of 2-amino-3-hydroxypyridine in oxidative hair dye formulations at a maximum concentration of 1.0% on the head does not pose a risk to the health of the consumer.

2-Amino-3-hydroxypyridine 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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