Scientific Committee on Consumer Safety

SCCS

OPINION ON

2,5,6-Triamino-4-pyrimidinol sulfate (Colipa No. A143)

The SCCS adopted this Opinion at its 10th plenary meeting

on 25 June 2015

About the Scientific Committees

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

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

SCCS

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

Contact
European Commission
Health and Food Safety
Directorate C: Public Health
Unit C2 – Health Information and Scientific Committees
Office: HTC 03/073
L-2920 Luxembourg
SANTE-C2-SCCS@ec.europa.eu

ISSN 1831-4767 ISBN 978-92-79-56132-0

Doi:10.2875/339295 EW-AQ-16-009-EN-N

ACKNOWLEDGMENTS

SCCS Members
Dr. Qasim Chaudhry
Prof. P.J. Coenraads (chairman)
Prof. M. Dusinska
Dr. W. Lilienblum
Dr. E. Nielsen
Prof. T. Platzek
Dr. S.C. Rastogi
Dr. C. Rousselle
Dr. J. van Benthem (rapporteur)

External experts
Prof. A. Bernard
Dr. L. Bodin
Dr. J. Duus-Johansen
Dr. J. Ezendam
Prof. A.M. Giménez-Arnau
Dr. E. Mirkova
Dr. E. Panteri
Prof. T. Vanhaecke

Keywords: SCCS, scientific opinion, 2,5,6-Triamino-4-pyrimidinol sulphate, A143, Regulation 1223/2009, CAS 1603-02-7, EC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 2,5,6-Triamino-4-pyrimidinol sulphate (A143), 25 June 2015, SCCS/1561/15

TABLE OF CONTENTS

1. BACKGROUND

Submission I and II for the hair dye 2,5,6-Triamino-4-pyrimidinol sulfate (CAS 1603-02-7) was submitted in October 1999 and July 2005 respectively by COLIPA.

The hair dye 2,5,6-Triamino-4-pyrimidinol Sufate is an oxidative hair colouring agent used at on-head concentrations of up to 0.5 %.

In September 2008, the Scientific Committee on Consumer Products (SCCP) adopted an opinion on the hair dye 2,5,6 Triamino-4-pyrimidinol sulphate concluding that:

"... *the safe use of 2,5,6-triamìno-4-pyrìmìdìnol sulfate as an ingredient in oxidative hair dye formulations at a maximum concentration of 0.5% on the head cannot be assessed. The potential for induction of gene mutations has to be clarified. Furthermore, 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.*
A skin sensitising potential of 2,5,6-triamino-4-pyrimidinol sulfate cannot be excluded." (SCCP/1122/07)[1]

COLIPA has transmitted the attached submission III that provides new information and scientific results of a newly conducted HPRT assay for mammalian gene mutation.

2. TERMS OF REFERENCE

(1) In light of the new data provided, does SCCS consider 2,5,6-Triamino-4-pyrimidinol sulfate as safe for use as an oxidative hair with an on-head concentration of 0.5%?

(2) Does the SCCS have any further scientific concerns with regard to the use of 2,5,6-Triamino-4-pyrimidinol sulfate in cosmetic products?

[1] http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_144.pdf

3. OPINION

3.1 Chemical and Physical Specifications

Taken from SCCP/1122/07

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

2,5,6-Triamino-4-pyrimidinol sulfate (INCI)

3.1.1.2 Chemical names

4-Hydroxy-2,5,6-triaminopyrimidine sulfate
4-OH-2,5,6-triamino-pyrimidine (sulfate)
2,4,5-Triamino-6-hydroxypyrimidine-sulfate
2,5,6-Triamino-4-pyrimidinol sulfate
2,5,6-triaminopyrimidin-4-ol
4(1H)-Pyrimidinone, 2,5,6-triamino-

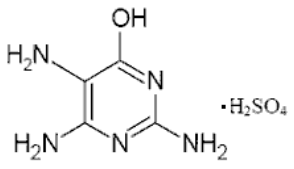
3.1.1.3 Trade names and abbreviations

TRAP
COLIPA n° A143

3.1.1.4 CAS / EC number

CAS: 1603-02-7
EINECS: 216-500-9

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_4H_7N_5O \cdot H_2SO_4$

3.1.2 Physical form

Off-white to yellow or beige, odourless powder

3.1.3 Molecular weight

Molecular weight: 239.21 g/mol

3.1.4 Purity, composition and substance codes

Chemical Characterisation

Batch T0312151 was fully characterised by H^1-NMR, C^{13}-NMR, MS, IR and HPLC.

Ref.: 19 (Submission II)

The following data are simply "stated" in the SUMMARY Submission II July 2005 (COLIPA), not accompanied by raw data and without any reference to respective study-numbers.

SPECIFICATION

Overall Purity (HPLC)	> 98%
Solvent Content	< 1%
Sulphated Ash	< 1%
Arsenic	< 5 ppm
Lead	< 20 ppm
Antimony	< 5 ppm
Cadmium	< 10 ppm
Mercury	< 5 ppm

BATCH COMPARISON

Lot. No.	Specification	Study	Study No.	Submission
T0312151	Purity: 98.67%	Chemical Characterisation	RCC 853019	II
T0312151	Purity: 98.67 %	Mouse Lymphoma	RCC-CCR 822202	II
T0312151	Purity: 98.67 %	Ames Test	RCC-CCR 822201	II
T0312151	Purity: 98.67 %	Micronucleus Test	RCC-CCR 869900	II
8157329	Purity > 97%	Subacute 28-day oral toxicity (rat)	RCC 336407	I
67346	Purity 100.4 %	Subchronic 13-week oral toxicity (80 rats)	RCC 376255	I
Iqv-54	^{14}C-TRAP	Toxicokinetics	RCC 378437	I
-	Purity > 98%	Embryotoxicity and Teratogenicity	RCC 634320	I
-	Specification not defined	Skin irritation	RCC 286222	I
-	Specification not defined	Eye irritation	RCC 336385	I
-	Specification not defined	Eye irritation	RCC 336385	I
-	Specification not defined	Contact Hypersensitivity	RCC 286536 RCC 298743	I
-	Specification not defined	Acute Toxicity oral	RCC 336363	I
-	Specification not defined	Acute Toxicity dermal	RCC 336374	I

3.1.5 Impurities / accompanying contaminants

-

3.1.6 Solubility

Water: > 0.2%
DMSO: < 0.1%
Ethanol: < 0.1%

3.1.7 Partition coefficient (Log P_{ow})

Log P (ACD): - 0.88 ± 0.39 (free base)

3.1.8 Additional physical and chemical specifications

Melting point:	> 300 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum:	λ_{max} 208nm (ε = 15177) and 262 nm (ε =4524) at pH 7, λ_{max} 262 nm (ε =13535) at pH 1 and λ_{max} 276 nm (ε =2285) at pH 13

3.1.9 Homogeneity and Stability

2,5,6-Triamino-4-Pyrimidinol Sulfate (TRAP) is stable in water up to 24 hours after mixing with a suitable antioxidant.

General comments to physico-chemical characterisation

- Only one batch is fully characterised. There is no other documentation regarding the stated quantitative data on the composition of batches.
- The stability of 2,5,6-triamino-4-pyrimidinol sulfate in the marketed products is not reported. Furthermore, the stability in water (section 3.1.9.) is reported after mixing with a suitable antioxidant without any additional explanation.
- The reported solubility data lack accuracy and are indefinite in relation to pH.
- Log P_{ow}: calculated values cannot be accepted as an estimate of the true physical constant without justification.

3.2 Function and uses

2,5,6-Triamino-4-pyrimidinol sulfate (TRAP) is used as an oxidative hair colouring agent. The final concentration of 2,5,6-triamino-4-pyrimidinol sulfate in oxidative hair colouring formulations, after mixing with hydrogen peroxide (1:1 or 1:2), can be up to 0.5% on the head.

3.3 Toxicological evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Taken from SCCNFP/0710/03

Guideline:	OECD 401 (1981) and EEC 84/449/EEC Part B.1
Species/strain:	HanIbm: WIST rat
Group size:	5 males + 5 females
Test substance:	4-OH-2,5,6-triamino-pyrimidine sulphate homogenised in corn oil
Batch:	/
Purity:	/
Dose:	2000 mg/kg bw by gavage
Observation period:	14 days
GLP:	in compliance

5 male (body weight 205-214 g) and 5 female (body-weight 171-179 g) Wistar rats were treated with 2000 mg/kg bw of the test substance by gavage.

Results
No mortality occurred. No clinical signs of toxicity were observed. The macroscopic examination at terminal necropsy revealed no organ alterations. The body-weight gain was not affected adversely during the study period. The LD50 of the test substance administered to rats by the oral route was >2000 mg/kg bw.

Ref.:1 (Submission I)

3.3.1.2 Acute dermal toxicity

Taken from SCCNFP/0710/03

Guideline:	OECD 402 (1987) and EEC 84/449/EEC Part B.3
Species/strain:	HanIbm: WIST rat
Group size:	5 males + 5 females
Test substance:	4-OH-2,5,6-triamino-pyrimidine sulphate homogenised in corn oil
Batch:	/
Purity:	/
Dose:	2000 mg/kg bw applied on the intact skin (semi-occlusive, 24 h)
Observation period:	14 days
GLP:	in compliance

5 male (body-weight 219-239 g) and 5 female (body-weight 202-215 g) Wistar rats were treated with 2000 mg/kg bw of the test substance on the clipped skin. The treated skin was covered with a semi-occlusive dressing. After 24 h the dressing was removed and the skin was washed with lukewarm tap water.

Results

No mortality occurred. With the exception of scales and erythema at the site of application, no clinical signs of toxicity were observed. The macroscopic examination at terminal necropsy revealed no organ alterations.
The LD50 of the test substance administered to rats by the dermal route was > 2000 mg/kg bw.

Ref.: 2 (Submission I)

3.3.1.3 Acute inhalation toxicity

No data submitted

3.3.1.4 Acute intraperitoneal toxicity

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Taken from SCCNFP/0710/03

Guideline:	OECD 404 (1981)
Species/strain:	New Zealand albino rabbit
Group size:	1 male, 2 females
Test substance:	TRAP, a yellow solid
Batch:	/
Purity:	/
Dose:	0.5 ml of test article solution
GLP:	In compliance

The dorsal fur was clipped and the test article was dissolved in distilled water to yield a final concentration of 3.6% (w/v). Sodium sulphite was present to prevent oxidation. The pH was adjusted to 9.5 using a 25% ammonium water solution. To initiate treatment, 0.5 ml of this solution was applied to approximately 6 cm² of the intact skin of the clipped area, covered with a surgical gauze pad and semi-occlusively dressed. Treatment was terminated after 4 hours by removing the tape and washing with lukewarm water. Skin reactions were assessed at 1, 24, 48 and 72 hours after removal of the dressing and test article.

Results
A skin irritation score of 0.22 was found, indicating that the test article was classified as non-irritant to rabbit skin.

Ref.: 4 (Submission I)

3.3.2.2 Mucous membrane irritation / Eye irritation

Taken from SCCNFP/0710/03

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand albino rabbit
Group size:	1 male, 2 females
Test substance:	4-OH-2,5,6-triaminopyrimidine (sulphate), solid, light yellow
Batch:	/
Purity:	/
Dose:	0.1 g

GLP: In compliance

0,1 ml of the test substance was applied once to the left eye of the rabbits, without rinsing. The right eye served as control and was untreated. Ocular reactions were recorded at 1, 24, 48, 72 hours and 7 days after installation.

Results
The substance showed a primary irritation score of 1.08. No staining or corrosion was observed. Based on these observations, the test article was not irritating to the eye.

Ref.: 3 (Submission I)

3.3.3 Skin sensitisation

Taken from SCCNFP/0710/03

Study 1 (Guinea pig maximisation test)

Guideline:	OECD 406 (1981)
Species/strain:	Himalayan spotted albino guinea pigs
Group size:	20 females in test group, 10 female controls and 6 females for pre-test
Test substance:	TRAP, prepared in water in an approximately 3.6% concentration. Sodium sulphite was present to prevent oxidation and pH was adjusted to 9.5 using a 25% ammonium water solution
Batch:	/
Purity:	/
Concentration:	- Intradermal induction: a 5% solution of the test article was injected intra-cutaneously with and without Freund's Complete Adjuvant. - Topical induction: undiluted test article (base solution containing ca. 3.6 % TRAP) for 48 hours, occluded - Challenge: A non-irritant concentration of the test article, 75% in distilled water for 24 hours, occluded.
GLP:	In compliance

Induction treatment was given according to the protocol. Control animals were treated with vehicle during the induction phase and challenged with a 75% test article dilution. The skin reactions were evaluated according to a ranking scale 24 and 48 hours after removal of the patch.

Results
One guinea pig in the test group was killed for ethical reasons. After first challenge all controls were negative, and one of nineteen test animals was positive. A second challenge was performed two weeks after the first challenge, using the same treatment procedure, and no reactions were seen. The test substance was not considered to be a sensitiser under the experimental conditions.

Ref.: 5 (Submission I)

Comment
It cannot be excluded that a higher induction concentration could be applied for both intradermal and topical induction. Pre-treatment with SLS prior to topical induction was not performed.

Study 2 (Guinea pig maximisation test)

Guideline:	OECD 406 (1981)
Species/strain:	Himalayan spotted albino guinea pigs

Group size:	10 female test animals, 5 female controls, 6 female animals for pre-test
Test substance:	4.48% TRAP solution in water was made. Sodium sulphite was added to prevent oxidation, and a small amount of 25% ammonium water was added to adjust the pH. This solution was filtered and incorporated in petrolatum oil (ratio of 61 g TRAP solution pr. 35 g petrolatum oil). This preparation was performed to make the undiluted test article named TRAP.1
Batch:	Not given
Purity:	Not given
Concentration:	- Intradermal induction: a 5% solution of the test article was injected intra-cutaneously with and without Freund's Complete Adjuvant. - Topical induction: undiluted test article for 48 hours occluded - Challenge: A non-irritant concentration of the test article, 75% in distilled water for 24 hours, occluded.
GLP:	In compliance

During pre-test the test article was applied intradermally in three concentrations 5%, 3% and 1%. Minimal oedema and erythema was seen for all 3 concentrations, hence the 5% concentration was selected for intradermal induction.
For epidermal application the test article (TRAP.1) was applied in 4 concentrations 100%, 75%, 50% and 25%. One animal showed minimal erythema at 24 hours after 100% concentration. Hence the undiluted TRAP.1 was selected for topical induction and 75% dilution for the challenge procedure.
In the main study the induction treatment was given according to the protocol, and controls were treated with vehicle alone. Challenge was performed with occluded patches applied for 24 hours. Skin reactions were evaluated according to a ranking scale 24 and 48 hours after removal of the patch.

Results
No reactions were seen in the test groups or in controls. TRAP.1 was not a sensitiser at the concentration tested.

Ref.: 6 (Submission I)

Comment
The test report does not establish that the test material was tested at an appropriate induction concentration.

3.3.4 Dermal / percutaneous absorption

Taken from SCCNFP/0710/03

Guideline:	EPA (1993)
Species/strain:	Rats, male, female, Sprague Dawley, SPF-quality
Test substance:	ring-labelled 14C-TRAP (1 mg/ml, specific activity 105 µCi/ml)
Dose levels:	50 µl/cm² of a 0.075% of 2,5,6-Triamino-4-pyrimidol sulfate in a mixture for hair dyeing (with developer) on a total of 9 cm² per animal
Exposure time:	group A: 30 min exposure and 72 h follow up group B: 30 min exposure and 24 h follow up
GLP:	in compliance

20 male and 20 female rats were used for this assay and assigned to the following groups:

Group A: 0.5 h dermal exposure, sacrifice after 72 h
Group B: 0.5 h dermal exposure, sacrifice after 24 h
Group C: 72 h oral exposure
Group D: 24 h oral exposure

Results
group A males: 1.57% absorbed in 72 h
group A females: 3.16% absorbed in 72 h
group B males: 2.25% absorbed in 24 h
group B females: 2.98% absorbed in 24 h

When taking the highest value of 3.16% absorption into account a total percutaneous absorption 0.52 µg/cm² would pertain, which results in an exposure of 0.006 mg/kg bw TRAP.

Ref.: 9 (Submission I)

Comment
In submission II, the applicant has reduced the intended on-head concentration to 0.5%. According to the SCCP Notes of Guidance and assuming 100% absorption, the worst case assumption for dermal absorption would result in an exposure of 0.83 mg/kg bw.

3.3.5 Repeated dose toxicity

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

Taken from SCCNFP/0710/03

Guideline:	OECD 407 (1981) and EEC 84/449/EEC Part B.7
Species/strain:	HanIbm: WIST rat
Group size:	5 males + 5 females
Test substance:	4-OH-2,5,6-triamino-pyrimidine sulphate homogenized in corn oil
Batch:	815 7329
Purity:	97 %
Dose levels:	0, 50, 200 and 1000 mg/kg bw/day by gavage
Exposure period:	28 days, once daily, 7 days per week
GLP:	in compliance

40 rats (20 males, 145.0-156.0 g bw and 20 females, 145.7-158.2 g bw) were used. The test substance was administered, by gavage, once daily 7 days per week for 28 days at dosage levels of 0, 50, 200 and 1000 mg/kg bw/day, application volume 10 ml/kg bw/day. The control group received the vehicle (corn oil) only. All animals were observed daily for clinical signs and mortality. Bodyweights, food and water consumption were recorded individually in weekly intervals. Ophthalmoscopic examination was performed at week 4 on all animals. At week 4, blood and urine samples were taken of all animals for haematological (17 parameters), clinical chemistry (22 parameters) investigations as well for urinalysis (13 parameters). All animals were sacrificed at the end of the study. Organ weights were recorded. Macroscopy and histopathology were performed on all animals.

Results
No animal died during the study. One female of the 1000 mg/kg bw/day group showed clinical signs (sedation, ruffled fur and body weight loss). No changes in food consumption or bodyweight gain were observed related to the test substance. No abnormal findings were noted at ophthalmoscopy. No relevant changes were found in haematology, clinical biochemistry, absolute and relative organ weights. A discoloration of the urine was observed in the dose groups 200 mg/kg bw/day (deep-yellow) and 1000 mg/kg bw/day (deep-brown). Discoloration or discoloured foci were observed in some organs in all test substance treated groups. 2 females of the 1000 mg/kg bw/day group had abnormalities of the kidneys: tubular basophilia and brownish pigment intratubular or in the pelvis. The NOAEL is 200 mg/kg bw/day.

Ref.: 7 (Submission I)

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

Taken from SCCNFP/0710/03

Guideline:	OECD 408 (1981)
Species/strain:	HanIbm: WIST rat
Group size:	10 males + 10 females
Test substance:	4-OH-2,5,6-triamino-pyrimidine sulphate homogenized in corn oil
Batch:	67346
Purity:	100.4%
Dose levels:	0, 50, 200 and 1000 mg/kg bw/day by gavage
Exposure period:	at least 13 weeks, once daily, 7 days per week
GLP:	in compliance

The test substance was administered, by gavage, once daily 7 days/week, to Wistar rats (10 per sex at each dosage) (bw males 60-80 g; bw females 50-69 g) for at least 13 weeks at the dosage levels of 0, 50, 200 and 1000 mg/kg bw/day, respectively. The control group received the vehicle (corn oil) only. All animals were observed daily for clinical signs and mortality. Body-weights and food consumption were recorded individually in weekly intervals. Ophthalmoscopic examinations were performed on all animals at pre-test and on all animals of dose groups 0 and 1000 mg/kg at week 13. Blood and urine samples were collected from all animals for haematological and clinical chemistry investigations and urinalysis, after week 13. All animals were necropsied, organ weights and macroscopic abnormalities were recorded and histopathology was performed.

Results
No treatment-related signs of toxicity were observed. Food consumption, body-weight change and ophthalmoscopy revealed no treatment-related effect.
In all dose groups, urine discoloration was observed, accompanied by turbidity at 1000 mg/kg bw/day (both sexes) and 200 mg/kg bw/day (females), which may be related to the substance or a metabolite. At the highest dose some significant changes of biochemical and haematological parameters were found (RBC, HP, HCT, MCV, MCH, reticulocyte count). Organ weight changes (kidney) and brownish pigment deposition associated with epithelial degeneration in kidney and rectum were confined to the highest dose group. The NOAEL is considered to be 200 mg/kg bw/day.

Ref.: 8 (Submission I)

3.3.5.3 Chronic (> 12 months) toxicity

No data submitted

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCP/1122/07, but modified

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537
Replicates:	Triplicates in four independent experiments

Test substance:	2,5,6-Triamino-4-pyrimidinol sulfate (TRAP)	
Solvent:	The test item was mixed with sodium sulfite (5:1) and the mixture was dissolved in deionised water	
Batch:	TO312151	
Purity:	98.67%	
Concentrations:	Experiment I:	TA98 and TA100: 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix TA102, TA1535 and TA1537: 0, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
	Experiment II:	0, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
	Experiment IIA:	TA1535 and TA1537: 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
Treatment:	Experiment I:	standard plate incorporation method without and WithS9-mix
	Experiment II:	pre-incubation method with 60 minutes pre-incubation and at least 48 h incubation without and with S9-mix
GLP:	in compliance	
Date:	26 March 2004– 27 May 2004	

2,5,6-Triamino-4-pyrimidinol sulfate 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 results of a pre-experiment for toxicity and mutation induction with strains TA98 and TA100 both without and with S9-mix. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Since in this pre-experiment evaluable plates were obtained for five concentrations or more in all strains used, the pre-experiment is reported as experiment I.
Experiment I was performed with the direct plate incorporation method; experiment II with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
The plates incubated with 2,5,6-triamino-4-pyrimidinol sulfate showed normal background growth up to 5000 µg/plate. Toxic effects, evident as a reduction in the number of revertants, occurred in strain TA98 at ≥ 1000 µg/plate in experiment I.
There were no indications of biologically relevant increases in revertant colony numbers in any of the five strains at any concentration tested neither in the presence nor in the absence of metabolic activation.
Strains TA1535 and TA1537 both with and without metabolic activation showed a weak increase at all concentrations tested that was not concentration-related and within the range of the historical control data. The only exception was observed in TA1537 in the absence of S9-mix in experiment II where the number of colonies exceeded the threshold of thrice the number of the corresponding solvent control at concentrations of 1000 and 5000 µg/plate. Therefore, a confirmatory experiment (experiment IIA) was performed with both strains. A biological relevant increase in the number of relevant colony numbers at any concentration tested was not observed.

Conclusion
Under the test conditions used 2,5,6-triamino-4-pyrimidinol sulfate was not mutagenic in this gene mutation test in bacteria.

Ref.: 2 (submission II)

Taken from SCCP/1122/07, but modified

***In vitro* Gene Mutation Assay (mouse lymphoma assay (*tk*$^{+/-}$ locus)**

Guideline:	OECD 476 (1997)	
Species/strain:	Mouse lymphoma cell line L5178Y (*tk*$^{+/-}$)	
Replicates:	Duplicates in two independent experiments	
Test substance:	2,5,6-Triamino-4-pyrimidinol sulfate (TRAP)	
Solvent:	Test item was mixed with sodium sulfite (5:1) and the mixture was dissolved in deionised water	
Batch:	TO312151	
Purity:	98.67%	
Concentrations:	Experiment I:	0, 75, 150, 300, 600 and 1200 µg/ml
	Experiment II:	0, 37.5, 75, 150, 300 and 600 µg/ml
Treatment	Experiment I:	4 h treatment with and without S9-mix; expression period 72 h and selection period of 10-15 days
	Experiment II:	24 h treatment without S9-mix; expression period 72 h and selection period of 10-15 days
GLP:	In compliance	
Date:	16 March – 3 May 2004	

2,5,6-Triamino-4-pyrimidinol sulfate 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 suspension grow at the end of the growth period.
In the main tests, cells were treated for 4 h (experiment I without and with S9-mix) or 24 h (experiment II without S9-mix) followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as percentage total growth of the treated cultures relative to the total growth of the solvent control cultures. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. Negative and positive controls were in accordance with the OECD guideline.

Results
On the pre-test, toxic effects were observed at ≥ 600 µg/ml (4 and 24 hour treatment) in the absence of metabolic activation and at ≥ 1200 µg/ml (4 h treatment) in the presence of metabolic activation. In the first experiment, precipitation was observed at ≥ 600 µg/ml in the absence and at 1200 µg/ml in the presence of metabolic activation. In the second experiment, precipitation occurred at ≥ 600 µg/ml. Toxic effects were observed at precipitating concentrations of both main experiments without S9-mix; no substantial toxic effect was observed with S9-mix at any tested concentration. However, the recommended toxic range of approximately 10-20% survival compared to the concurrent negative controls was covered in experiment II but not always in experiment I.
The quality of the performance of experiment I did not allow a conclusion. Consequently the results of experiment I are considered inconclusive. In the second experiment with 24 h treatment without S9-mix, an increase in mutant frequency at the highest tested concentration, 3.1 and 1.8 fold above the control values in the two cultures respectively, was observed. This increase, however, was not considered valid since it occurred at precipitating concentrations. Particularly, during long-term treatment precipitates tend to damage cells by mechanical shear forces.

Conclusion
Under the test conditions used, the results for 2,5,6-triamino-4-pyrimidinol sulfate in this *in vitro* gene mutation test in mammalian cells is considered inconclusive.

Ref.: 1 (Submission II)

SCCS comments
In experiment I, the result without S9-mix is equivocal and an intermediate concentration should be tested for clarification (*i.e.* repetition with narrow spacing of the higher concentrations). In experiment I, sufficient toxicity at the maximum concentration tested was not reached.

New in submission III

***In vitro* Mammalian Cell Gene Mutation Test**

Guideline:	OECD 476 (1997)	
Cells:	L5178Y mouse lymphoma cells (*hprt* locus)	
Replicates:	duplicate cultures in two independent experiments	
Test substance:	2,5,6-Triamino-4-pyrimidinol (sulfate salt)	
Solvent:	deionised water	
Batch:	712171	
Purity:	100% (HPLC)	
Concentrations:	Experiment I:	0, 21.9, 43.8, 87.5, 175 and 350 µg/ml without and with S9-mix
	Experiment II:	0, 21.9, 43.8, 87.5, 175 and 350 µg/ml without S9-mix 0, 87.56, 175, 350, 525 and 700 µg/ml with S9-mix
Treatment	Experiment I:	4 h treatment with and without S9-mix; expression period 72 h and selection period of 10-15 days
	Experiment II:	4 h treatment with S9-mix; expression period 72 h and selection period of 10-15 days 24 h treatment without S9-mix; expression period 72 h and selection period of 10-15 days
GLP:	in compliance	
Study period:	4 May 2009 – 3 July 2009	

2,5,6-Triamino-4-pyrimidinol (sulfate salt) was assayed for gene mutations at the *hprt* 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 suspension grow at the end of the growth period with concentrations up to 1400 µg/ml limited by the solubility of 2,5,6-triamino-4-pyrimidinol (sulfate salt).
In the main tests, cells were treated for 4h without and with S9-mix (experiment I and II) or 24 h (experiment II) without S9-mix followed by an expression period of 72 h to fix the DNA damage into stable *hprt* mutations. Toxicity was measured in the main experiments as percentage suspension growth and total growth of the treated cultures relative to the suspension growth and total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD test guideline.

Results
Precipitation of 2,5,6-triamino-4-pyrimidinol (sulfate salt) was observed at 350 µg/ml and above in experiment II with S9-mix. The appropriate levels of toxicity (10-20% relative survival after the highest concentration) were reached in experiment I in both cultures with S9-mix only and in experiment II in one culture without S9-mix and in both cultures with S9-mix.
In both experiments, a biologically relevant increase in the mutant frequencies was not observed, both in the presence and absence of S9-mix.
In experiment II, the induction factor of three times the mutant frequency of the corresponding solvent control was reached in one culture treated with 350 µg/ml with S9-mix. However, this increase was not reproduced in the second culture and was therefore

considered as biologically irrelevant. The mean value of both cultures did not reach the threshold of 3 times the value of the concurrent control.

Conclusion
Under the experimental conditions used, 2,5,6-triamino-4-pyrimidinol (sulfate salt) was not mutagenic in this gene mutation assay with mouse lymphoma cells using the *hprt* locus as reporter gene.

Ref.: 1 (Submission III)

3.3.6.2 Mutagenicity / Genotoxicity in vivo

Taken from SCCP/1122/07, but modified

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	5 males and 5 females in each group
Test substance:	2,5,6-Triamino-4-pyrimidinol sulphate (TRAP)
Lot no:	TO312151
Purity:	98.67%
Dose level:	0, 312.5, 625 and 1250 mg/kg bw
Route:	Oral, single dose
Vehicle:	Deionised water
Sacrifice times:	24 h and 48 h (highest dose only)
GLP:	In compliance
Date:	1 February 2005 – 25 February 2005

2,5,6-Triamino-4-pyrimidinol sulphate has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on a pre-experiment on acute toxicity with 2 mice per sex. The mice were treated orally and examined for acute toxic symptoms at various intervals of 1, 2-4, 6, 24, 30 and 48 h after start of treatment.
In the main experiment, mice were exposed orally to 0, 312.5, 625 and 1250 mg/kg bw. The mice of the highest dose group were examined for acute toxic symptoms at intervals of around 1, 2-4, 6 and 24 h after treatment. Bone marrow cells were collected 24 h or 48 h (high dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/TE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test at 1500 mg/kg bw one female mouse died after 24 hours. Moreover there were clear toxic reactions such as reduction of spontaneous activity and ruffled fur, and occasionally also abdominal position, eyelid closure, apathy and tremors. At 1250 mg/kg bw toxic reactions such as reduction of spontaneous activity and ruffled fur were also observed; at 750 mg/kg bw only ruffled fur was observed. Based on these findings 1250 mg/kg bw was chosen as the maximum dose.
In the main experiment almost all mice treated with 1250 mg/kg bw showed reduction of spontaneous activity and ruffled fur. Moreover, the bedding of the animals showed signs of orange urine, indicating that the test item was bioavailable. At 312.5 and 625 mg/kg bw no toxic reactions were observed but after 24 h the bedding of the animals showed signs of orange urine.
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 2,5,6-triamino-4-pyrimidinol sulfate had no cytotoxic properties in the bone marrow.

A biologically relevant increase in the number of cells with micronuclei was not found at any concentrations tested in either males or females at 24 h and 48 h harvest time-points.

Conclusion
Under the test conditions used 2,5,6-triamino-4-pyrimidinol sulphate did not induce micronuclei in the micronucleus test in the bone marrow cells of mice and, consequently, 2,5,6-triamino-4-pyrimidinol sulphate is not clastogenic and/or aneugenic in bone marrow cells of mice.

Ref.: 3 (Submission II)

3.3.7 Carcinogenicity

No data submitted

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

No data submitted

3.3.8.2 Teratogenicity

Taken from SCCNFP/0710/03

Guideline:	OECD 414 (1981)
Species/strain:	HanIbm: WIST rat
Group size:	25 females mated per dose group
Test substance:	4-Hydroxy-2,5,6-triaminopyrimidine sulphate homogenized in corn oil
Batch:	not given
Purity:	> 98%
Dose levels:	0 and 1000 mg/kg bw/day by gavage
Treatment period:	Day 6 - 15 of gestation
GLP:	in compliance

The test substance was administered, once daily by gavage, from day 6 to 15 of gestation a group of 25 pregnant rats at the limit dose 1000 mg/kg bw/day. The control group received the vehicle (corn oil) only. All mated females were sacrificed at day 20 of gestation. The animals were observed at least twice daily for mortality and clinical signs. Individual bodyweights were recorded daily from day 0 to 21 post coitum. Food consumption was measured for the day-intervals 0-6, 6-11, 11-16, and 16-21. Immediately following sacrifice, macroscopic examination of the maternal organs was carried out. The uterus was removed and weighed, the number of corpora lutea, early and late resorptions, total implantations and viable foetuses were recorded. All foetuses were individually weighed and the sex of the foetuses was determined. One half of the foetuses was examined for skeletal defects and variations of the ossification process by Alizarin Red staining and one half was evaluated for visceral alterations.

Results
The bedding material in the cages was discoloured orange in the treated group. No maternal toxicity was found. No substance-related changes of reproduction data (number of implantations, resorptions and foetuses, foetal weight and external abnormalities) was noted. No substance-related changes in the incidence of visceral and skeletal abnormalities was found. The NOAEL of maternal and embryo/foetotoxicity was 1000 mg/kg bw/day in this study.

Ref.: 10 (Submission I)

3.3.9 Toxicokinetics

No data submitted

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

No data submitted

3.3.10.2 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

(2,5,6-triamino-4-pyrimidinol sulfate)
(oxidative)

Amount of formulation applied	**A (g/day)**	**= 100 g**
Concentration on head of the ingredient	**C (%)**	**= 0.5%**
Dermal absorption per treatment	**DAp (%)**	**= 100%**
Retention factor	**RF**	**= 0.1**
Typical body weight of human		**= 60 kg**
Systemic exposure dose (SED)	**A x C x DAp x RF/60**	**= 0.83 mg/kg bw/d**
No observed adverse effect level (mg/kg) (90-day, oral, rat)	**NOAEL**	**= 200 mg/kg bw/d**
Bioavailability* 50%		**=100 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**= 120**

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

3.3.14 Discussion

Physico-chemical properties
2,5,6-Triamino-4-pyrimidinol sulfate is used as an oxidative hair colouring agent at a concentration up to 0.5% on the head.
Only one batch is fully characterised. There is no other documentation regarding the stated quantitative data on the composition of the batches.
The stability of 2,5,6-triamino-4-pyrimidinol sulfate in the marketed products is not reported. Furthermore, the stability in water (section 3.1.9.) is reported after mixing with a suitable antioxidant without any additional explanation. The reported solubility data lack accuracy and are indefinite in relation to pH. Calculated values of Log P_{ow} cannot be accepted as an estimate of the true physical constant without justification.

General toxicity
The LD_{50} of the 2,5,6-Triamino-4-pyrimidinol sulfate administered to rats by the oral and dermal route was >2000 mg/kg bw.
In the repeated dose oral toxicity in rats study, abnormalities of the kidneys were noted in the dose group 1000 mg/kg bw/day: tubular basophilia and brownish pigment intratubular or in the pelvis. In the study on subchronic oral toxicity in rats urine, discoloration was observed in all dose groups, accompanied by turbidity at 1000 mg/kg bw/day (both sexes) and 200 mg/kg bw/day (females) which may be related to the substance or a metabolite. At the highest dose some significant changes of biochemical and haematological parameters were found. Organ weight changes (kidney) and brownish pigment deposition associated with epithelial degeneration in kidney and rectum were confined to the highest dose group. The NOAEL is considered to be 200 mg/kg bw/day. In the teratogenicity study, the limit dose 1000 mg/kg bw/day 2,5,6-Triamino-4-pyrimidinol sulfate exhibited no maternal and embryo/foetotoxicity.

Irritation / Sensitisation
2,5,6-Triamino-4-pyrimidinol sulfate was considered as non-irritant to rabbit skin and eye.
The substance was not a sensitiser in the concentration tested. However, the study is considered inadequate as the concentrations tested were too low.

Dermal absorption
Though the *in vivo* study is performed *lege artis*, it is unsuitable for the intended safety calculation, since a 7-fold lower dosage (0.075%) is applied than claimed (0.5%). Since it cannot be excluded that higher percutaneous absorption rates occur under use conditions, a worst case calculation is made, assuming 100% absorption from a 0.5% formulation. According to the SCCS Notes of Guidance (100 ml, retention factor 0.1), a dose of 0.83 mg/kg bw would be estimated as a worst case.

Mutagenicity / genotoxicity
2,5,6-Triamino-4-pyrimidinol sulfate was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations. 2,5,6-Triamino-4-pyrimidinol sulfate did not induce mutations in bacteria. The results in an *in vitro* gene mutation test using the *tk* locus of mammalian cells were inconclusive. A second gene mutation test, submitted for submission III, using the *hprt* locus in mammalian cells was negative. 2,5,6-Triamino-4-pyrimidinol sulfate was negative in an *in vivo* micronucleus assay in bone marrow cells of mice.
Consequently, on the basis of these tests, 2,5,6-triamino-4-pyrimidinol sulfate can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

(1) In light of the new data provided, does SCCS consider 2,5,6-Triamino-4-pyrimidinol sulfate as safe for use as an oxidative hair with an on-head concentration of 0.5%?

In the light of the new data provided, the SCCS considers the use of 2,5,6-triamino-4-pyrimidinol sulfate as an ingredient in oxidative hair dye formulations at a maximum concentration of 0.5% on the head is safe.

(2) Does the SCCS have any further scientific concerns with regard to the use of 2,5,6-Triamino-4-pyrimidinol sulfate in cosmetic products?

A skin sensitising potential of 2,5,6-triamino-4-pyrimidinol sulfate cannot be excluded.

5. MINORITY OPINION

Not applicable

6. REFERENCES

Submission I, 1999

1. A. Mahl, 1992; Acute oral toxicity study with 4-OH-2,5,6-triamino-pyrimidine (sulphate) in rats, RCC Project No. 336363, Test Report, Itingen/CH, November 12, 1992
2. A. Mahl, 1992; Acute dermal toxicity study with 4-OH-2,5,6-triamino-pyrimidine (sulphate) in rats, RCC Project No. 336374, Test Report, Itingen/CH, November 19, 1992
3. A. Mahl, 1992; Primary eye irritation study with 4-OH-2,5,6-triamino-pyrimidine (sulphate) in rats, RCC Project No. 336385, Test Report, Itingen/CH, November 11, 1992
4. L. Ullmann, T. Porricello, N. Hoff, 1990; Primary skin irritation study with TRAP in rabbits, RCC Project No. 286222, Test Report, Itingen/CH, December 5, 1990
5. L. Ullmann, C. Kröling, C. Böni, M. Faroug, 1991; Contact Hypersensitivity to TRAP in albino guinea pigs maximisation test, RCC Project No. 286536, Test Report, Itingen/CH, February 1, 1991
6. L. Ullmann, C. Kröling, C. Böni, N. Hoff, 1991; Contact Hypersensitivity to TRAP.1 in albino guinea pigs maximisation test, RCC Project No. 298743, Test Report, Itingen/CH, July 15, 1991
7. A. Dotti, K. Biedermann, H. Luetkemeier, K. Weber, 1993; Subacute 28-day oral toxicity (gavage) study with 4-OH-2,5,6-triamino-pyrimidine (sulphate) in the rat, RCC Project No. 336407, Test Report, Itingen/CH, April 20, 1993
8. H. Schmid, K. Biedermann, H. Luetkemeier, K. Weber, 1995; Subchronic 13-week oral (gavage) toxicity study with 4-OH-2,5,6-triamino-pyrimidine (sulphate) in the rat, RCC Project No. 376255, Test Report, Itingen/CH, October 31, 1995
9. R. Burri 1995; 14C-TRAP: Absorption, Distribution and Excretion Study (ADE) in rats after single dermal or oral administration, RCC Project No. 378437, Test Report, Itingen/CH, September 11, 1995
10. H. Becker, K. Biedermann, 1997; Limit test of embryotoxicity (including teratogenicity) with 4-OH-2,5,6-triamino-pyrimidine (sulphate) in the rat, RCC Project No. 634320, Test Report, Itingen/CH, February 20, 1997

11. A. Poth 1992; Salmonella typhimurium and Escherichia coli reverse mutation assay with TRAP.1, CCR Project No. 274206, Test Report, Roßdorf/Germany, March 09, 1992
12. A. Poth 1992; Salmonella typhimurium and Escherichia coli reverse mutation assay with FO-TRAP.2, CCR Project No. 274307, Test Report, Roßdorf/Germany, March 13, 1992
13. H. Müllerschön 1992; Gene mutation assay in Chinese hamster V79 cells in vitro with 4-OH-2,5,6-triamino-pyrimidine (sulphate), CCR Project No. 321412, Test Report, Roßdorf/Germany, December 16, 1992
14. A. Heidemann and H. Müllerschön 1993; Chromosome aberration assay in Chinese hamster V79 cells in vitro with 4-OH-2,5,6-triamino-pyrimidine (sulphate), CCR Project No. 321423, Test Report, Roßdorf/Germany, May 25, 1993
15. R. Fautz 1993; Micronucleus Assay in Bone Marrow cells of the Mouse with 4-OH-2,5,6-triamino-pyrimidine (sulphate), CCR Project No. 421806, Test Report, Roßdorf/Germany, August 30, 1993

Submission II, 2005

1. Wollny H-E., Cell mutation assay at the thymidine kinase locus (tk+/-) in mouse lymphoma L5178Y cells with 2,5,6-triamino-4-pyrimidinol sulfate (TRAP). RCC-CCR, Rossdorf, Germany, 14 December 2004. RCC-CCR 822202
2. Sokolowsky A., *Salmonella typhimurium* reverse mutation assay with 2,5,6-triamino-4-pyrimidinol sulfate (TRAP). RCC-CCR, Rossdorf, Germany, 4 February 2005. RCC-CCR 822201
3. Honarvar N., Micronucleus assay in bone marrow cells of the mouse with 2,5,6-triamino-4-pyrimidinol sulfate (TRAP). RCC-CCR, Rossdorf, Germany, 26 April 2005. RCC-CCR 869900
4. Tognucci A., Determination of the NMR-, IR-, UV/VIS absorption, mass spectra and a HPLC chromatograph of TRAP. RCC Ltd, Itingen, Switzerland, 12 July 2005. RCC 853019

Submission III, 2014

1. Moore et al., Mouse Lymphoma Thymidine Kinase Gene Mutation Assay: Follow up Meeting of the International Workshop on Genotoxicity Testing – Aberdeen, Scotland, 2003 – Assay Acceptance Criteria, Positive controls and Data Evaluation
2. Wollny H-E. and Sokolowski A., Cell mutation assay at the hypoxanthine-guanine-phosphoribosyl transferase locus (HPRT) in mouse lymphoma L5178Y cells with TRAP. Harlan Cytotest Cell Research GmbH, Rossdorf , Germany. Harlan study number 1255400, 19 October 2009.