Scientific Committee on Consumer Products

SCCP

OPINION ON

2,5,6-Triamino-4-pyrimidinol sulfate

COLIPA n° A143

The SCCP adopted this opinion at its 17th plenary of 30 September 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.

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1. BACKGROUND

Submission I of 2,5,6-Triamino-4-pyrimidinol sulfate (CAS 1603-02-7) was submitted in October 1999 by COLIPA[1, 2].

The Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) in its 24th plenary meeting on 24-25 June 2003 adopted an opinion (SCCNFP/0710/03) requesting further information on:

- *Proper identification of the compound or confirmation of the identity reported in the dossier*
- *Proper analytical and physico-chemical data*
- *Data on the genotoxicity/mutagenicity*

Submission II was submitted by COLIPA in July 2005. According to this submission 2,5,6-Triamino-4-pyrimidinol sulfate is used as an oxidative hair colouring agent. The oxidative hair colouring formulations are used after mixing with hydrogen peroxide, either 1:1 or 1:2, which leads to an on-head concentration of up to 0.5%. As common practice, 100 ml of the mixed formulation is applied. The application time covers a period of 30 minutes followed by washing off with water and shampoo. It is assumed that application may be repeated monthly. This use pattern means that the concentration in the finished cosmetic product could be either 1.0 or 1.5%.

Submission II 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 SCCP consider 2,5,6-Triamino-4-pyrimidinol sulfate safe for use as an oxidative hair dye with a concentration of 0.5% on the head taken into account the scientific data provided?*

2. *Does the SCCP recommend any restrictions with regard to the use of 2,5,6-Triamino-4-pyrimidinol sulfate in oxidative hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association
[2] According to records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

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-

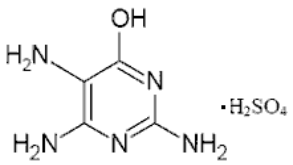
3.1.1.3. Trade names and abbreviations

TRAP
COLIPA n° A143

3.1.1.4. CAS / EINECS 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 characterized by H^1-NMR, C^{13}-NMR, MS, IR and HPLC.

Ref.: 19

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 Characterization	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% I	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. 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

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 homogenized 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 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

3.3.1.3. Acute inhalation toxicity

No data submitted

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 cm^2 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

3.3.2.2. Mucous membrane 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

3.3.3. Skin sensitisation

Taken from SCCNFP/0710/03

Study 1 (Guinea pig maximization 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 sensitizer under the experimental conditions.

Ref.: 5

Comment
It can not 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 maximization 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 neither in the test groups nor in controls. TRAP.1 was not a sensitizer at the concentration tested.

Ref.: 6

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: 30min exposure and 72h follow up group B: 30min exposure and 24h 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 72h
group A females:	3.16% absorbed in 72h
group B males:	2.25% absorbed in 24h
group B females:	2.98% absorbed in 24h

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

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 0.12 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. Body weights, 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 and body weight 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

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

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline: OECD 471 (1997)
Species/strain: TA1535, TA1537, TA98, TA100 and TA102
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 1 (TA98, TA100) and Experiment 2 and 2A: 0, 33, 100, 333, 1000, 2500 and 5000 μg/plate
Experiment 1(TA1535, TA1537, TA102): 0, 10, 33, 100, 333, 1000, 2500 and 5000 μg/plate
Treatment: Experiment 1: Standard plate incorporation assay
Experiment 2: Pre-incubation method
Both experiments were performed with and without phenobarbital / naphtoflavone induced S9-mix
GLP: in compliance
Date: 26 March – 27 May 2004

A pre-experiment for toxicity was performed with strains TA98 and TA100 with and without metabolic activation using eight concentrations between 3 and 5000 μg/plate. The plates 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.

Results
In all strains the plates showed normal background growth up to 5000 μg/plate. In the first experiment, toxic effects were only observed in strain TA98 without metabolic activation at ≥ 1000 μg/plate. There were no indications of any increases in revertant numbers in any strains and at any concentration tested.

In experiment two using the pre-incubation assay, strains TA98, TA100 and TA102 did not show any increases in revertant numbers. Strains TA1535 both with and without metabolic activation and strain TA1537 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. However, a confirmatory experiment was performed with both strains. Due to irregular bacteria growth in the first confirmatory experiment no data were evaluated and the experiment had to be repeated. In this experiment there were no indications of any increases in revertant numbers at any concentration tested.

Conclusion
Under the test conditions used 2,5,6-triamino-4-pyrimidinol sulfate did not induce gene mutations in bacteria.

Ref.: 17

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

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma cell line L5178Y (tk locus for trifluorothymidine (TFT) resistance
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 1: 0, 75, 150, 300, 600 and 1200 μg/ml Experiment 2: 0, 37.5, 75, 150, 300 and 600 μg/ml
Treatment	Experiment 1: 4 hour treatment with and without metabolic activation: Phenobarbital/β-naphthoflavone induced S9-mix Experiment 2: 24 hour treatment without metabolic activation
GLP:	In compliance
Date:	16 March – 3 May 2004

The test substance was examined for its mutagenic activity in the L5178Y $tk^{+/-}$ mouse lymphoma test in the absence and presence of metabolic activation. A preliminary toxicity test was conducted using 8 concentrations between 18.8 and 2400 μg/ml in the presence (4 h treatment) and absence (4 and 24 hour treatment) of metabolic activation. Toxic effects were observed at ≥ 600 μg/ml (4 and 24 hour treatment) in the absence of metabolic activation and at ≥ 1200μg/ml (4 h treatment) in the presence of metabolic activation.

Results
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. In experiment 1 in the absence of metabolic activation a minor toxic effect was observed in culture II at 600 μg/ml indicated by a relative total growth (RTG) of < 50% as compared to the solvent control. In the parallel culture I no relevant toxic effect occurred at this concentration. At the highest tested concentration severe toxic effect was observed in both cultures (RTG was less than 10 %). In the second experiment in the absence of metabolic activation, strong toxic effects were observed at 600 μg/ml in both cultures (RTG was less then 10%.
In the first experiment without metabolic activation the mutant frequency was increased 2.4 –fold above the corresponding control value at the highest toxic concentration. However, the increase was not observed in the second culture and only observed at the extreme toxic level, and therefore not considered biological relevant. No substantial and reproducible increase of the mutation frequency was observed at the other tested concentrations in the first experiment with and without metabolic activation. However, with metabolic activation no relevant toxic effect was observed at any tested concentration.

In the second experiment there was an increase in mutation frequency at the highest tested concentration, 3.1 and 1.8 fold above the control values in the two cultures respectively. However, the increase was only observed at a severe toxic concentration and not considered biological relevant.

MMS (13 μg/ml) and CPA (3.0 μg/ml) were used as positive controls and showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large colonies.

Conclusion
Under the test conditions used 2,5,6-Triamino-4-Pyrimidinol Sulfate is not considered mutagenic in this *in vitro* assay.

Ref.: 16

Comment of the SCCP
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 with S9-mix, sufficient toxicity at the maximum concentration tested was not reached.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	5 male and 5 female in each group
Test substance:	2,5,6-Triamino-4-Pyrimidinol Sulfate (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 – 25 February 2005

Dose selection was based on findings in the dose range finding study for toxicity covering 750, 1250 and 1500 mg/kg bw administered to two males and two females and observed for up to 48 hours for toxic signs and mortality. At the highest concentration (1500 mg/kg bw) one female died after 24 hours. Moreover there were clear toxic reactions such as reduction of spontaneous activity, abdominal position and ruffled fur. At 1250 mg/kg bw

toxic reactions such as reduction of spontaneous activity and ruffled fur were also observed and based on these findings 1250 mg/kg bw were chosen as the maximum dose. At least 2000 PCEs per animal were analysed for the frequency of micronuclei. In addition, the ratio between polychromatic and total erythrocytes was analysed.

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 2,5,6-Triamino-4-Pyrimidinol Sulfate (TRAP) had no cytotoxic properties in the bone marrow. After the highest dose (1250 mg/kg bw) toxic reactions as reductions of spontaneous activity and ruffled fur were observed. 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.

There were no indications of increases in micronucleated PCEs at any concentrations tested in either males or females at 24 h and 48 h harvest time-points.
40 mg/kg bw cyclophosphamide was used as positive control that showed a statistically significant increase of induced micronucleus frequency.

Conclusion
It is concluded that under the test conditions reported in this study, 2,5,6-Triamino-4-Pyrimidinol Sulfate (TRAP) did not induce clastogenic and or aneugenic activity determined by this *in vivo* assay.

Ref.: 18

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 body weights 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

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

Not applicable

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
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. The NOAEL is 200 mg/kg bw/day. In the study on subchronic oral toxicity in rats 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. 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.
The substance was not a sensitizer 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 under use conditions higher percutaneous absorption rates occur, a worst case calculation is made, assuming 100% absorption from a 0.5% formulation. According to the SCCP 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 aberration. 2,5,6-Triamino-4-pyrimidinol sulfate did not induce mutations in bacteria. The results of the gene mutation test with mammalian cells *in vitro* are inconclusive: in experiment I, the result without S9 is equivocal and an intermediate concentration should have been tested for clarification, i.e. repetition with narrow spacing of the higher concentrations. In experiment I with S9, toxicity or maximum concentration is not reached. It was negative in the *in vivo* micronucleus assay in bone marrow cells of mice. Based on all results a mutagenic potential of 2,5,6-triamino-4-pyrimidinol sulfate cannot be excluded.

Furthermore, to reach a definitive conclusion, appropriate tests with 2,5,6-triamino-4-pyrimidinol sulfate in combination with hydrogen peroxide should be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCP is of the opinion that the safe 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 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.

5. MINORITY OPINION

Not applicable

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