Scientific Committee on Consumer Products

SCCP

OPINION ON

tetra-Aminopyrimidine sulfate

COLIPA n° A53

The SCCP adopted this opinion at its 18th plenary of 16 December 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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(ISSN)

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

ACKNOWLEDGMENTS

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Keywords: SCCP, scientific opinion, hair dye, A53, tetra-aminopyrimidine sulfate, directive 76/768/ECC, CAS 5392-28-9, EINECS 226-393-0

Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on tetra-aminopyrimidine sulfate, 16 December 2008

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1. BACKGROUND

Submission I for 2,4,5,6-Tetraaminopyrimidine sulphate was submitted in July 1997 by COLIPA[1, 2].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its 25th plenary meeting of 20 October 2003 following opinion SCCNFP/0695/03, final:

"Most of the investigations/experiments which are reported in Submission I (Aug. 1997) have been carried out in the early eighties (1978-1983). For an appropriate evaluation a number of details are missing. The identification of the test substance is ambiguous. The respective test substance is not specified as to its chemical form in several experiments. TAP showed in different studies alone or in formulations a low median lethal dose (LD50) of > 5.000 mg/kg. The sub-chronic oral toxicity study lead to a NOAEL of 600 mg/kg bw. There were no signs of teratogenicity and embryo-toxicity; maternal toxicity between 500 to 1000 mg/kg bw. The NOAEL for foetal development has been determined at 500 mg/kg bw. TAP was "not irritating" when applied in different species and in appropriate doses on the skin. It was also "not irritating" on mucous membranes. No incompatibilities were observed in human tests. TAP was classified as "not-sensitizing". A number of toxicokinetic studies have been carried out, using radio labelled TAP but in concentrations mostly far below the applied in use concentration of 5 %. TAP has been tested in prokaryotic cells for gene mutation, and in mammalian cells for chromosomal aberration in vitro. One in vivo test has been performed (bone marrow micronucleus). The in vitro test for gene mutation in bacteria is unsuitable for genotoxicity and/or mutagenicity evaluation (test substance, purity, batch not characterised; no dose range finding data, no repeat experiment). The in vitro test for clastogenicity in Chinese Hamster V79 cells is negative. The in vivo micronucleus test in mice is unsuitable for genotoxicity/clastogenicity/aneugenicity evaluation due to the protocol followed, the absence of test agent characterisation, and lack of batch and purity description, no demonstration that the test substance has reached the target cells. The test substances are not specified as to their chemical form. The mutagenicity/genotoxicity data are insufficient."

According to the current submission II, submitted by COLIPA in July 2005, 2,4,5,6-Tetraaminopyrimidine sulphate is used as a precursor for hair colours. It reacts with couplers to form the final dye. The reaction can be accelerated by addition of an oxidizing agent, but it can also be achieved by air oxidation. The substance is used as an ingredient in hair dye formulations, which may or may not contain a hydrogen peroxide based developer mix up to a final on-head-concentration of 3.4% (calculated for the 2,4,5,6-Tetraaminopyrimidine sulphate corresponding to 2.0% calculated for the free base). Under the intended conditions of use the exposure is terminated 30 minutes after application of the mixture to the hair by shampooing and thoroughly rinsing with water.

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.

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

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 2,4,5,6-Tetraaminopyrimidine sulphate safe for use in any hair dye formulation with an on-head concentration of maximum 2.0% (as the free base) taking into account the scientific data provided?*

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

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Tetraaminopyrimidine sulfate (INCI)

3.1.1.2. Chemical names

Pyrimidinetetramine, sulfate (1:1) (CAS name)
2,4,5,6-Tetraaminopyrimidine sulfate
2,4,5,6-Tetraaminopyrimidine sulfate hemihydrate
2,4,5,6-Tetraaminopyrimidine x H_2SO_4
Pyrimidine-2,4,5,6-tetramine sulfate
Pyrimidinetetramine

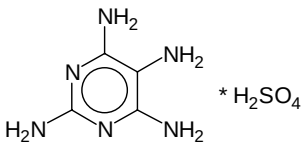
3.1.1.3. Trade names and abbreviations

Ro 1
COLIPA n° A53

3.1.1.4. CAS / EINECS number

CAS: 5392-28-9
EINECS: 226-393-0

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_4H_8N_6 \cdot H_2SO_4$

3.1.2. Physical form

Light yellow, fine powder

3.1.3. Molecular weight

Molecular weight: 238.33 (as sulfate)

3.1.4. Purity, composition and substance codes

The structural identity of the test sample Pyrimidine-2,4,5,6-tetramine Sulfate, batch 111201A395 has been confirmed by ^{1}H- and ^{13}C- NMR-spectra and is additionally supported through the IR- and UV-spectra and the elemental analysis. The purity of the test sample is displayed by the good match of measured and theoretical figures of the elemental analysis. The purity determination by HPLC with UV-detection results in a purity of 97.1 area%.

Content	> 98 weight% (UV-spectroscopy, relative to standard)
Purity determination by HPLC	> 95 area% (batch 111201A395: 97.1 area%)

3.1.5. Impurities / accompanying contaminants

	Raw Material Description	batch 111201A395
2,5,6-Triaminopyrimidine-4-ol:	< 2.5 weight%	1.7 weight%
Unknown impurity:	< 2.5 area%	1.8 area%
Solvent content (water):	< 1 weight%	< 0.1 weight%
Sulphated ash:	< 0.5 weight%	< 0.1 weight%

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

3.1.6. Solubility

Water	0.3 – 3 g/l
Ethanol	< 1g/l
DMSO	1 – 10 g/l

3.1.7. Partition coefficient (Log P_{ow})

Log Pow: - 2.87 (calculated)

3.1.8. Additional physical and chemical specifications

Melting point:	> 260 °C (thermal decomposition)
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum	/

3.1.9. Stability

The stability of the test substance in dose solution was checked in repeat dose experiments at 0 and 4 hours. The recovery was between 94.1 and 95.9% at 0 h and between 91.6 and 92.0% at 4 hours.
No additional data were provided.

General Comments to physico-chemical characterisation

- The information provided on the compound is incomplete.
- No data on stability in cosmetic formulations was provided.
- The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

3.2. Function and uses

tetra-Aminopyrimidine and its salts are used as a precursor for hair colours. They react with couplers to form the final dye-stuff. The reaction can be accelerated by addition of an oxidising agent (e.g. hydrogen peroxide), but it can also be achieved by air oxidation.
The final concentration on head can be up to 3.4%, calculated for tetra-aminopyrimidine sulfate (corresponding to 2.0% calculated for the free base).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: CF 1 male mice (Winkelmann)
Group size: 10 (6 groups)
Test substance: TAP suspended in water (Aqua dest.)
Batch: /
Purity: /
Dose: 1.99; 2.51; 3.16; 5.01; 6.31; 7.94 g/kg bw
Route: oral
Exposure: gavage
GLP: not in compliance
Study period: August 1974

The acute oral toxicity of 2,4,5,6-tetraaminopyrimidine sulphate-hemihydrate was investigated in young adult male mice of CF1 strain. 60 animals were used in the test, ten per dose. The average body weight at the day of application was approx. 23 g.
Aliquots of 40 ml/kg bw of the aqueous suspension were administered by gavage. The resulted oral doses were 1990, 2510, 3160, 5010, 6310 and 7940 mg/kg bw. During a seven day observation period, mortalities and clinical-toxicological observations were recorded.

Results
During the study all test animals showed toxic symptoms, mainly strong apathy which was reversible in surviving animals. The acute lethal dose was calculated according to the method of Litchfield and Wilcoxon to be LD_{50} = 4700 mg/kg bw.

Ref.: 3

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 (2002)
Species: Albino rabbit, New Zealand White (SPF-Quality)
Group: 3 males
Substance: A 053 / SAT 030631
Batch: 111201A395
Purity: 98.2%
Dose: 0.5 g
Vehicle: moistened with water (Milli-U)
GLP: in compliance
Study period: 5 – 21 November 2003

Three rabbits were exposed to 0.5 g of the test substance, applied onto clipped skin for 4 hours using semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.

Results
The 3 rabbits showed neither erythema nor oedema during the observation period (1-72 hours). No staining of the treated skin was observed by the test substance. No mortality and no symptoms of systemic toxicity were found.

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

Ref.: 4

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405 (2002)
Species:	Albino rabbit, New Zealand White (SPF-Quality)
Group:	3 males
Substance:	A 053 / SAT 030631
Batch:	111201A395
Purity:	98.2%
Dose:	102.1 mg
Vehicle:	/
GLP:	in compliance
Study period:	10 November – 1 December 2003

After health inspection, each animal was treated by installation of 102.1 mg of the test substance in the conjunctival sac of one of the eyes; the other eye served as control. The substance remained in contact with the eyes for 24 hours. The eyes were scored for irritation reactions approx. 1, 24, 48, and 72 hours and 7 days after installation of the test substance.

Results
The instillation of the undiluted test substance 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 25% of the corneal area). The corneas injury had resolved within 72 hours in all animals. Iridial grade 1 was observed in one animal at 24 and 48 hours after instillation. The irritation of the conjunctivae consisted of redness and chemosis and had completely resolved within 14 days in all animals. The animals did not show any symptoms of systemic intoxication.

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

Ref.: 5

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species:	CBA/CaOlaHsd mice
Group:	4 females (1 control group, 3 treatment groups)
Substance:	A 053 / SAT 030631
Batch:	111201A395

Purity: 98.2%
Dose: 2.5%, 5% and 10% (w/v) solutions
Vehicle: acetone:olive oil (4:1 v/v)
Positive control: α-hexylcinnamaldehyde at 5, 10 and 25% in acetone:olive oil (4:1 v/v)
Negative control: acetone:olive oil (4:1 v/v)
GLP: in compliance
Study period: 12 – 26 May 2004

Each test group of mice was treated by topical application to the dorsal surface of each ear lobe (left and right) with solutions of 0%, 2.5%, 5% and 10% of the test substance (25 µl) for three consecutive days. 10 % was the highest technically applicable concentration in the vehicle. Five days after the first topical application, all mice were administered radio-labelled thymidine (^{3}HTdR) by intravenous injection via the tail vein.
Approximately five hours after ^{3}HTdR application the lymph nodes were prepared, re-suspended and transferred to scintillation vials. 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). An appropriate reference (α-hexylcinnamaldehyde) was used as positive control.

Results
No signs of local toxicity at the ears of the animals and no systemic findings were observed during the study period. The Stimulation Indices were 0.8, 1.1 and 1.2 for the low, median and high dose group, respectively. A dose response relation was noted, but the increase was well below a factor of 3, which would indicate a positive response.
The positive control caused a stimulation index of 8.4 (at 25%), demonstrating the reliability of the test system.

Concentration	Stimulation Index
Test item	
2.5%	0.8
5%	1.1
10%	1.2
α-Hexylcinnamaldehyde	
5%	1.5
10%	2.3
25%	8.4

Conclusion
Based on the criteria of the test system, the test substance was found to be a non-sensitizer when tested up to the highest technically applicable concentration of 10% (w/v) in acetone:olive oil (4:1) in mice.

Ref.: 6

Comment
The highest concentration of the test substance (10%) tested is considered too low. Other vehicles should have been tested to obtained possible higher test concentrations.
The test is therefore not considered appropriate. A possible sensitising potential cannot be excluded.

3.3.4. Dermal / percutaneous absorption

Guideline: /
Tissue: Dermatomed pig skin
Group size: Eight membranes (from 2 pigs) per application
Diffusion cells: Glass diffusion cell with an exposed membrane area of 2.54 cm²
Skin integrity: Measured by trans-dermal electrical resistance ($\geq$3kΩ)
Test substance: A 053; tetra-aminopyrimidine sulphate monohydrate, [pyrimidine-2-^{14}C]

Batch:	SAT 030382 (unlabelled); SAT 030671 (radiolabelled)
Purity:	97.8 %
Test item:	Cream formulation with and without hydrogen peroxide and aqueous solution of A 053 (3.4% w/w)
Doses:	Approx. 0.34 mg/cm² skin (test substance), 10 mg formulation/cm²
Receptor fluid:	Physiological saline
Solubility receptor fluid:	/
Stability:	Freshly prepared
Method of Analysis:	Liquid scintillation counting
GLP:	in compliance
Study period:	6 – 14 February 2004

The dermal absorption/percutaneous penetration of [^{14}C]-test substance out of a basic cream (mixed with a developer with and without hydrogen peroxide) and from a solution in water was studied on the clipped excised skin of suckling pigs (aged 6-8 weeks), dermatomed to a mean thickness of 0.40 mm. The integrity of the skin discs was checked by measuring the trans-dermal electrical resistance. The intact, clipped excised pig skin of the flanks area was exposed for 30 minutes to the test substance.
Shortly before application to skin the basic cream was mixed (1:1) with the developer mix with and without hydrogen peroxide as study A and B, respectively. Additionally, a third formulation C was produced by dissolving test substance (traced with [^{14}C] radio-labelled material) in water. The nominal concentration of test substance in all three final application formulations was 3.4%.
The dermal absorption/percutaneous penetration of the test substance was investigated for the open application of about 10 mg formulation per cm² pig skin. Therefore the resulting dose of the test substance was approx. 0.34 mg/cm² skin. Skin discs of 0.79 cm² were exposed to the formulations for 30 minutes, terminated by gently rinsing with a 3% Teepol® solution.
Each of the two formulations and the solution were analysed with eight replicates for adsorbed, absorbed and penetrated amount of the test substance. The receptor fluid used was physiological saline.
In the static system, samples of receptor fluid were taken manually using a positive displacement pipette at recorded intervals (0, 0.5, 2, 4, 6, 12, 24, 30, 36 and 48 hours). The volume of fluid in the receptor chamber was maintained by the addition of an equal volume of fresh receptor fluid to the chamber immediately after the removal of each sample.

Results
The quantities that had penetrated during the 30 minute exposure to test substance containing formulations and within the 48 hours after application are shown in the following table. Both the amounts absorbed and penetrated were taken as systemically available.

ANALYSED SAMPLE	**Formulation A with H_2O_2**		**Formulation B without H_2O_2**		**Solution C in water**	
	[% of dose]	**[µg/cm²]**	**[% of dose]**	**[µg/cm²]**	**[% of dose]**	**[µg/cm²]**
Skin rinsings	113	-	91.9	-	98	-
Adsorption (stratum corneum)	0.082	0.290	0.093	0.731	0.059	0.212
Not Bioavailable	113.1	-	92.0	-	98.1	-
Absorption (epidermis/dermis)	0.207	0.731	0.358	1.30	0.087	0.313
Penetration (receptor fluid)	0.096	0.340	0.019	0.067	0.018	0.063
Bioavailable	0.303±0.109	1.071±0.385	0.377±0.181	1.37±0.656	0.105±0.045	0.376±0.160

ANALYSED SAMPLE	Formulation A with H_2O_2 [% of dose] [µg/cm²]	Formulation B without H_2O_2 [% of dose] [µg/cm²]	Solution C in water [% of dose] [µg/cm²]
Total recovery / mass balance	113 -	92.4 -	98.2 -

Conclusion
In this *in vitro* dermal penetration study, the mean amount of test substance systemically available from a standard cream formulation with or without hydrogen peroxide was found to be 1.07 µg/cm² (0.3%) and 1.37 µg/cm² (0.38%), respectively.
The highest individual values observed for the bioavailable amount of the formulation with or without hydrogen peroxide were 1.64 µg/cm² and 2.21 µg/cm², respectively.

Ref.: 13

Comment
Deviating from the SCCP recommendations, only 8 replicates from 2 donors and 10 mg/cm² formulation were used. According to the function and uses, the substance also can be used without hydrogen peroxide. Therefore, the highest value of 2.21 µg/cm² (formulation without hydrogen peroxide) may be used for the calculation of the Margin of Safety.

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 (1998)
Species/strain: Wistar rat (HsdBrlHan:WIST)
Group size: 10 rats per sex; high dose groups: 12 rats per sex
Test substance: A 053
Batch: 111201A395
Purity: 98.2 area% (HPLC)
Dose: 0, 150, 300 and 1000 mg/kg bw/day
Route: oral (gavage)
Exposure: 90 consecutive days, daily doses in corn oil
GLP: in compliance
Study period: 22 December 2004 – 2 May 2005

During the study the mortality, signs of intoxication, the body weight and the food consumption were recorded. The animals of the recovery groups were additionally examined during the 4-week treatment-free period. At the end of the study, the animals were sacrificed and subjected to pathological investigations.

Results
No mortality was observed in any of the groups during the course of experiment. Ophthalmological examinations did not reveal any ocular abnormalities.
Mean body weights of treatment group animals remained comparable with the respective control group animals in rats belonging to either sex. No treatment related variations were observed in mean food consumption of treatment group animals as compared to respective control groups.
Neuro-behavioural observations/detailed clinical observations performed at weekly intervals did not reveal any treatment related changes. No treatment related variations were observed in motor activity, hind limb and forelimb grip strength, and hind limb foot splay

values in any of the treatment group animals. Sensory reactivity tests did not reveal any treatment related abnormalities.
Haematological parameters evaluated at the end of week 13 revealed a significant reduction in mean values of RBC, Hb and HCT at 1000 mg/kg bw/day dose level (high dose group) in both sexes. This moderate anaemic effect of test substance observed at 1000 mg/kg bw/day dose level is considered to be treatment related. Further, a significantly reduced mean platelet count in high dose group males and significantly increased MCH in high dose group females as compared to respective control group animals were observed. Post 28 days of recovery period only the mean value of RBC count in high dose recovery group males lasted to be significantly decreased as compared to control recovery group males.
Results of clinical chemistry analysis revealed significantly increased phosphorus in males of all treated groups and increased sodium and chloride in high dose group males as compared to control group males. In females, significantly increased mean chloride values in all treated groups, increased phosphorus and sodium in low and mid dose groups and increased calcium in mid dose group, were observed as compared to control group females. The alterations of these electrolytes were found to be not strictly dose related and corresponding histopathological changes were seen in high dose animals and some mid dose animals. Animals of the low dose group were found to be without any conspicuous alterations in kidneys. Based on the observed massive excretion of the orally applied test substance via urine (discoloration) effects on the diuresis process seem to be likely. This slight impact in low dose animals is considered to be not adverse.
Post 28 days of recovery period the mean values of sodium, chloride and calcium in high dose recovery group males were significantly increased as compared to control recovery group males. High dose recovery group females revealed significant increase in mean value of phosphorus and chloride as compared to control recovery group females.
Statistical analysis for tests of urinalysis revealed decreased pH and urobilinogen in mid and high dose group males as compared to control group males. The high dose group females revealed significantly decreased mean value of pH as compared to control group females. Post 28 days of recovery period the mean values of pH and urobilinogen of high dose recovery group animals returned to normal values.
No treatment related changes were observed in the microscopic evaluation of urine sediment in animals from either sex.
The absolute weight of adrenals in high dose group and spleen in high dose and high dose recovery group males were significantly increased as compared to respective control group males. Relative weight of adrenals and kidneys in high dose, spleen in mid and high dose group males were significantly increased as compared to control group males. In females no statistical significant variations were noticed.
Visceral examination of carcasses belonging to different treatment groups at the end of 90-day exposure period did not reveal clear cut treatment related changes as compared to control groups.
The treatment related histopathological lesion, viz. degeneration and necrosis of tubular epithelial cells in inner – outer medulla and cortex of kidneys were observed in animals belonging to the 1000 mg/kg bw/day group. Four males and four females belonging to mid dose group also showed these types of lesions. Mild degenerative changes in tubular epithelial cells in outer medulla of kidneys of two male animals of the low dose group were observed, compared to one male of the control group (Table). After 28 days of recovery period the treatment related lesions persisted in kidneys of high dose recovery group animals: the incidences were 10/12 in males and 03/12 in females.
Dose-dependent histopathological changes were observed in thymus, spleen, adrenals and lymph nodes; this might be suggestive of stress. The changes in thymus (starry sky appearance) were also found in lowest dose groups (5/10 in males and 6/10 females: Table). A clear NOAEL was also absent for the effects in spleens (males) and in adrenals (females). After the recovery period these treatment related changes were only partly reversed. The histopathological changes were substantiated by increases in spleen and adrenal weights and a slight decrease in thymus weights. Histopathological examination of other organs did not reveal treatment related adverse effects.

Table: Histopathological Findings (Summary of critical findings)

Organ: Lesion	**Dose (mg/kg bw/day)**			
	0	**150**	**300**	**1000**
Kidney: Focal / multifocal degeneration and necrosis of tubular epithelial cells in inner, outer medulla and cortex	1 (m) 1 (f)	2 (m) 0 (f)	4 (m) 4 (f)	12 (m) 12 (f)
Thymus: Starry sky appearance	1 (m) 1 (f)	5 (m) 6 (f)	6 (m) 7 (f)	7 (m) 7 (f)
Spleen: Decrease cellularity in white pulp / decreased cellularity and size of white pulp	2 (m) 3 (f)	4 (m) 3 (f)	6 (m) 5 (f)	9 (m) 8 (f)
Adrenals: Sinusoidal congestion	1 (m) 1 (f)	0 (m) 3 (f)	2 (m) 4 (f)	4 (m) 8 (f)

Conclusion
The 90-day repeated dose oral exposure of rats to the test item affected haematological parameters at the highest dose level (1000 mg/kg bw/day). Signs of nephrotoxicity were observed at 300 and 1000 mg/kg bw/day. The study authors derived a NOAEL of 150 mg/kg bw/d.

Ref.: 11

Comment
Based on dose-dependently increased incidences of histopathological effects in thymus, spleen and adrenals observed in all treatment groups, the SCCP considers the Lowest Observed Adverse Effect Level (LOAEL) of TAP in rats to be 150 mg/kg bw/day.

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:	*Salmonella typhimurium* TA 98, 100, 102, 1535 and 1537
Replicates:	Two experiments with triplicate plates
Test substance:	A 053
Solvent:	deionised water
Batch:	111201A395
Purity:	98.2%
Concentrations:	Experiment I and II with and without S9-mix: 33, 100, 333, 1000, 2500 and 5000 µg/ml
Treatment:	plate incorporation assay and pre-incubation assay
GLP:	in compliance
Study period:	28 August – 15 September 2003

A direct plate incorporation assay, as well as a pre-incubation experiment as the second run of the main test, were performed. Sodium azide (10 µg/plate) served as a positive control for TA 100 and TA 1535, 4-nitro-o-phenylene-diamine (10 µg/plate) for TA 1537 and TA 98 and methyl methane sulfonate (4 µl/plate) for TA 102 without S9-mix. The solvent was deionised water.

Results
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9-mix in all strains used. A minor toxic effect, evident as a

reduction in the number of revertants, was observed at 5000 µg/plate in strain TA 1537 without S9-mix in experiment II.
No biologically relevant or reproducible increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance, neither in the presence nor absence of metabolic activation. The mutagens used as positive controls showed a distinct increase of induced revertant colonies.

Conclusion
The test substance is not mutagenic in this *in vitro* bacterial mutagenicity test.

Ref.: 7

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

Guideline:	OECD 476 (1998)
Species/strain:	mouse lymphoma cell line L5178Y
Replicates:	two independent experiments with parallel cultures
Test substance:	A 053
Solvent:	culture medium containing 0.5% DMSO
Batch:	111201A395
Purity:	98.2%
Concentrations:	Experiment I without S9-mix: 75, 150, 300, 600, 900 µg/ml with S9-mix: 75, 150, 300, 600 µg/ml Experiment II without S9-mix: 18.8, 37.5, 75, 150, 600 µg/ml
Treatment:	4 h with and without S9-mix, 24 h without S9-mix (2nd experiment)
GLP:	in compliance
Study period:	29 June – 9 August 2004

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without metabolic activation (S9-mix) and a treatment period of 4 hours. The second experiment was solely performed in the absence of metabolic activation with a treatment period of 24 hours. Appropriate references (methyl methane sulfonate 13 µg/ml without S9-mix and cyclophosphamide 6.0 µg/ml with S9-mix) were used as positive controls.

Results
In experiment I relevant toxicity (relative cloning efficiency 1 and / or relative total growth of less than 50% survival) was detected in both parallel cultures at 600 µg/ml and above in the presence and absence of metabolic activation. However, the desired range of 10 – 20% of survival was not reached due to heavy precipitation at higher concentrations. In the second experiment (24 hour treatment without metabolic activation) severe toxic effects occurred at precipitating concentrations of 600 µg/ml and above.
No substantial reproducible increase of the mutant frequency was observed with and without metabolic activation. A minor increase of the mutant frequency was observed in the first culture of the first experiment without metabolic activation (induction factor of 1.8). The historical control range was exceeded at 300 – 900 µg/ml. However, no comparable effect occurred in the parallel culture under identical conditions. Therefore, this increase was judged as biologically irrelevant fluctuation. In the second experiment without S9-mix no relevant increase in mutant frequency was observed. In the presence of metabolic activation a minor increase of the mutant frequency was observed at 1200 µg/ml. The threshold of twice the mutant frequency of the solvent control was slightly exceeded in the second culture (factor: 2.1) but not in the first culture (factor: 1.4) for which the mutant frequency of the solvent control was high (slightly above the historical control range). Precipitation was observed at 1200 µg/ml so this minor increase might be an artefact based on precipitation. No repeat experiment with S9-mix was performed.

The positive control mutagens used showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large colonies.

Conclusion
In the study described and under the experimental conditions reported, the test article did not induce a biologically relevant or reproducible increase in mutant colonies and, therefore, is considered to be not mutagenic in this mouse lymphoma assay.

Ref.: 8

***In vitro* chromosome aberration test**

Guideline:	OECD 473 (1998)
Species/strain:	Chinese Hamster V79 cells
Replicates:	One experiment with duplicate cultures
Test substance:	A 053
Solvent:	culture medium
Batch:	111201A395
Purity:	98.2%
Concentrations:	without S9-mix: 25, 50 and 100 µg/ml, with S9-mix: 125, 250 and 500 µg/ml
Treatment:	4 hours
GLP:	in compliance
Study period:	3 September - 6 November 2003

The harvest time was 18 hours after start of treatment. The treatment interval was four hours with and without metabolic activation. 100 metaphases per culture were scored for structural chromosome aberrations. Appropriate references (ethyl methane sulfonate 200 µg/ml without S9-mix and cyclophosphamide 0.7 µg/ml with S9-mix) were used as positive controls to show distinct increases in cells with structural chromosome aberrations.

Results
In the absence of S9-mix, neither reduced mitotic indices nor reduced cell numbers were observed up to the highest evaluated concentration of the test item.
In the presence of S9-mix, toxic effects indicated by reduced mitotic indices of below 50% of control were observed after treatment with 500 µg/ml.
In the absence of S9-mix, no statistically significant and biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item were similar or near the value of the negative control and within the range of the historical control data.
In the presence of S9-mix, dose-related increases in the number of aberrant cells (3%, 9% and 11.5%) and in the number of cells carrying exchanges (0.5%, 4% and 7.5%) were observed in the concentration range evaluated (at 125, 250 and 500 µg/ml). The aberration rates were significantly increased after treatment with 250 and 500 µg/ml compared to the corresponding negative control (1.5%). These values clearly exceeded the historical control data range (0 – 4% aberrant cells, exclusive gaps). Also, the number of cells carrying exchanges (4% and 7.5%) was distinctly increased with 250 and 500 µg/ml as compared to the negative control (0%) and gave additional evidence for a clastogenic potential of the test item in this test system. Therefore, these observations were regarded as biologically relevant.
No biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item as compared to the values of the negative controls.
In both experiments, the positive control used showed distinct increases in cells with structural chromosome aberrations.

Conclusion

The test article induced structural chromosome aberrations in the V79-cells in the presence of metabolic activation and is considered to be clastogenic in this *in vitro* assay.

Ref.: 9

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Bone marrow micronucleus test in mice

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	Five mice per dose and sex
Test substance:	A 053
Batch:	111201A395
Purity:	98.2 %
Dose level:	18.75, 37.5 and 75 mg/kg bw
Route:	i.p.
Vehicle:	aqueous DMSO (30%)
Sacrifice times:	24 h and 48 h (high dose only)
GLP:	in compliance
Study period:	16 September – 9 December 2004

The test article, dissolved in aqueous DMSO (30%), was administered intraperitoneally to 6 NMRI mice per dose and sex in a single dose of 18.75, 37.5 and 75 mg/kg bw (10 ml/kg). Bone marrow of femurs was prepared 24 and 48 (only for the high dose level) hours after application of the test substance. For each animal at least 2,000 polychromatic erythrocytes (PCE) obtained from femoral bone marrow were examined. The frequency of cells with micronuclei was calculated for each animal and dose group.
Cyclophosphamide monohydrate (40 mg/kg bw) and the vehicle, respectively served as positive and negative controls.

Results
The highest dose of the test article (75 mg /kg bw) was without significant effects on the survival rates, but with clear signs of toxicity. At a higher dose (100 mg/kg) one of four animals died. The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of polychromatic erythrocytes (PCEs) of the vehicle control indicating that the test item had no cytotoxic properties in the bone marrow. The bioavailability of the test item was, however, confirmed by chemical analysis (HPLC) of the blood of the treated animals.
In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of micronucleated bone marrow cells at any preparation interval and dose level after administration of the test item. The mean number of cells with micronuclei observed after treatment with test item was below or near to the value of the vehicle control group.
The positive control substance caused cytotoxicity and produced micronuclei in polychromatic erythrocytes, thus demonstrating the sensitivity of the test system used for the endpoints investigated in this study.

Conclusion
From the results obtained in this study, it was concluded that the test item had no clastogenic or aneugenic potential in mice.

Ref.: 10

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 (2001)
Species/strain:	Wistar rats (Harlan Hsd SD).
Group size:	Twenty-five sperm positive females per dose group
Test substance:	A 053
Batch:	111201A395
Purity:	98.2%
Dose:	0, 375, 750 and 1500 mg/kg bw per day (day 5-19 of pregnancy)
Route:	Gavage
Exposure:	24 h and 48 h (high dose only)
GLP:	in compliance
Study period:	7 June – 28 July 2004

The females were paired with male rats of the same strain three to one with an accurate day of mating (G0), fixed by the presence of sperm in the vaginal smear.
The test item was suspended in corn oil at dose levels of 0, 375, 750 and 1500 mg/kg bw and aliquots of 4 ml/kg bw were daily administered (day 5-19 of pregnancy) by gavage. Dosages were based on the results of a previously performed dose range-finding study (0, 300, 600, 1200 mg/kg bw/day).
Clinical signs of toxicity were recorded daily. Body weight and feed consumption were recorded on day 0, 3, 5, 8, 11, 14, 17 and 20 of gestation.
The dams were sacrificed on day 20 post-coitum by carbon dioxide asphyxiation and subjected to necropsy. The number of alive and dead foetuses, their distribution and site in the uterus, early and late resorption, implantation and number of *corpora lutea* was determined. The weight of the foetuses, gravid uteri, uteri without foetuses, placentae and the sex of foetuses was recorded. Approximately one-half of the foetuses were selected at random and examined for visceral alterations. The remaining foetuses were examined for skeletal malformations, variations and retardation of the normal organo-genesis after appropriate staining.

Results
1. Maternal Response to Treatment
No mortality was observed in the rats. Treatment did not exhibit any affect on pregnancy rate. No clinical observations were made apart from salivation in a number of animals of the mid and high dose group. The symptom was exhibited immediately post dosing and the treated rats recovered within 1 hour post dosing.
No treatment related changes were observed in the mean maternal weight, body weight gain and feed consumption. The gross pathological changes/lesions observed during necropsy were mild, inconsistent and could not be attributed to the treatment.
No treatment related effects were observed in the mean prenatal reproductive and foetal data indices up to the highest dose level of 1500 mg/kg bw.

2. Examination of Foetuses
No significant differences in the incidences of malformation of birth defects were recorded during external, visceral, head razor and skeletal examination of foetuses from the control or treated groups. Increased incidences of incomplete ossification of interparietal bone, 1st sternebra unossified and incomplete ossification of xiphisternum in treated rats were reported to be within the range of historical controls in Wistar rats and, therefore, these findings were considered to be incidental.

Conclusion
With respect to the mentioned slight maternal effects noticed in the mid and high dose group (salivation), the maternal No-Observed-Effect-Level (NOEL) was considered to be 375 mg/kg/d. The test chemical was neither embryo-lethal, embryotoxic nor teratogenic up to the highest dose tested (1500 mg/kg bw/day).

Ref.: 12

3.3.9. Toxicokinetics

Taken from SCCNFP/0695/03

Percutaneous penetration / dermal absorption of a hair dye formulation *in vivo*

Guideline: /
Species/strain: Wistar rats (SPFCpb)
Test substance: radioactive labelled TAP (0.226%) + non labelled TAP; total 0.451% in a cream preparation (without developer)
Radioactive purity: > 91%
Batch: no data
Dose: see below
GLP: not in compliance

The percutaneous penetration/dermal absorption of 2,4,5,6-tetraamino-(2-[^{14}C])pyrimidine sulphate hemihydrate was studied in ten rats (five per sex) of Wistar (SPFCpb) strain, with a mean body weight of 295 g (males) respectively 233 g (females). The formulation applied consisted of:

- [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate / unlabelled
- sodium sulphite and ammonium sulphate
- basic emulsion *Bth 66 B* (mix of fatty alcohols and fatty alcohol polyglycol sulphate)
- water, ammonia.

The concentration of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate (adjusted to pH 9.6) in the test was 0.451% before 1:1 dilution with CO_2-minimized water. The percutaneous penetration/dermal absorption of [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate during a period of 48 hours, was investigated after application to a skin area of 10 cm². The diluted (1:1) formulation was exposed to the intact, clipped skin of rats under semiocclusive conditions without rinsing. Finally 200 mg of the formulation were applied per animal, which resulted in a test substance dose of 0.045 mg/cm².

Faeces and urine were analysed daily. After two days the animals were sacrificed and the treated skin as well as the carcass were analysed for remaining radioactivity. Two animals were chosen to analyse the exhalation rate of [^{14}C]-CO_2 during the study.

Results
The mean percutaneous penetration/dermal absorption of the test substance was 2.65% (males) and 2.83% (females) corresponding to the described test conditions. 2,4,5,6-Tetraaminopyrimidine sulphate hemihydrate was excreted mainly *via* urine (83% males / 88% females) and to a lesser extent *via* faeces (7.7% males / 4.6 females). The exhaled [^{14}C]-concentrations of both animals were below the detection limit.

Ref.: 14

Percutaneous penetration / dermal absorption of a complete hair dye formulation *in vivo*

Guideline: /
Species/strain: Wistar rats (SPF-TNO)
Test substance: radioactive labelled TAP; 0.12% after combination with H_2O_2 (6%)
Batch: not data
Dose levels: see below
GLP: not in compliance

The percutaneous penetration / dermal absorption of 2,4,5,6-tetraamino-(2-[^{14}C])pyrimidine sulphate hemihydrate was studied in 16 rats (eight per sex) of Wistar (SPF-TNO) strain, with a body weight of 165-225 g (males) respectively 142-167 g (females). The formulation applied consisted of :

- [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate / unlabelled
- sodium sulphite and ammonium sulphate
- basic emulsion *Bth 66 B* (mix of fatty alcohols and fatty alcohol polyglycol sulphate)
- 2,7-dihydroxynaphthalene as a coupler
- water, ammonia

The concentration of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate (adjusted to pH 9.5 -10.0) in the test was 0.117% after dilution with six per cent aqueous H_2O_2. The percutaneous Penetration/dermal absorption of [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate during a period of 24 hours, was investigated after semi-occlusive exposure for 30 minutes to a clipped skin area of 8 cm2. Finally 400 mg of the formulation were applied per animal, which resulted in a test substance dose of 0.058 mg/cm². Faeces and urine were analysed at the beginning and at the end of the study. After 24 hours the animals were sacrificed and the treated skin was analysed for remaining radioactivity.

Results
The mean percutaneous penetration / dermal absorption of the test substance was 0.25% (0.150 µg/cm², male rats) and 0.27% (0.153 µg/cm², female rats) corresponding to the described test conditions. 2,4,5,6-Tetraaminopyrimidine sulphate hemihydrate was almost completely excreted *via* urine. The [^{14}C]-concentration of the faeces and carcasses was below the detection limit.

Ref.: 15

Excretion after oral absorption in rats

The intestinal absorption of 2,4,5,6-tetraamino-(2-[^{14}C])-pyrimidine sulphate hemihydrate was studied in 16 rats (eight per sex) of Wistar (SPF-TNO) strain, with a body weight of 190-210 g (males) respectively 143-162 g (females).

A 0.067% aqueous dilution of [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was used. The excretion of [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was investigated after orally administration of a single dose of 10-13 mg/kg bw. Faeces and urine were taken as daily fractions over four days. The amount of radioactivity in the carcass and in the gastrointestinal tract at the end of the observation period (96 hours) was also measured.

Results
The mean minimum per-oral absorption of the test substance *via* the intestine was 28.2% for the female rats and 41.4% for the male rats. 24.3% (female rats) and 39.6% (male rats) of the administered dose was excreted *via* urine within 24 hours after administration of

the test substance. The amount of radioactivity excreted in the faeces was 70.2% (females) and 64.3% (males) of the applied dose. Only minor amounts of the applied dose were found in the carcass and gastrointestinal tract at the end of the study.

Ref.: 15

Percutaneous absorption of a complete hair dye formulation *in vivo*

Guideline:	/
Species/strain:	Wistar rats (SPF-TNO)
Test substance:	radioactive labelled TAP with and without coupler; 0.24% after combination with H_2O_2 (6%)
Batch:	no data
Dose levels:	see below
GLP:	not in compliance

The percutaneous penetration / dermal absorption of 2,4,5,6-tetraamino-(2-[^{14}C])pyrimidine sulphate hemihydrate was studied in 34 rats (17 per sex) of Wistar (SPFTNO) strain, with a body weight of 170-200 g. The formulation applied consisted of:

- [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate / unlabelled
- sodium sulphite and ammonium sulphate
- basic emulsion *Bth 66* (mix of fatty alcohols and fatty alcohol polyglycol sulphate)
- water, ammonia
- with (form. 1) or without (form. II) 2-methylresorcinol as a coupler

The concentration of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate (adjusted to pH 9.5 -10.0) in the test was 0.24% after dilution with six per cent aqueous hydrogen peroxide. The percutaneous penetration / dermal absorption of [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate during a period of 24 hours, was investigated after semi-occlusive exposure for 30 minutes to a clipped skin area of 8 cm² (form. I) or 10 cm² (form. II). Finally 400 mg (form. I) or 500 mg (form. II) of the formulations were applied per animal, which resulted in a test substance dose of approx. 0.120 mg/cm².
Faeces and urine were analysed at the beginning and at the end of the study. After 24 hours the animals were sacrificed and the treated skin was analysed for remaining radioactivity.

Results
The mean percutaneous penetration / dermal absorption of the test substance was 0.48% (0.58 µg/cm², males) and 0.30% (0.29 µg/cm², females) corresponding to the described test conditions with the coupler. In the absence of the coupler, 0.64% (0.62 µg/cm², male rats) respectively 0.35% (0.33 µg/cm², female rats) were percutaneously absorbed. All per cent values are related to the actual applied [^{14}C]-amounts. 2,4,5,6-Tetraaminopyrimidine sulphate hemihydrate was almost completely excreted via urine.

Ref.: 16

Excretion after subcutaneous / intravenous application in rats

The excretion of 2,4,5,6-tetraamino-(2-[^{14}C])-pyrimidine sulphate hemihydrate after subcutaneous application was studied in 16 rats (eight per sex) of Wistar (SPF-TNO) strain, with a body weight of 170 - 200 g. The excretion after intravenous application was informatively studied in one male rat of the same strain which weighed 280 g.
A 0.2% aqueous dilution of [^{14}C]-2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was used. The excretion was investigated after a single subcutaneous administration of 10 - 12 mg/kg bw. Faeces and urine were taken as daily fractions over seven days. The amount of

radioactivity in the carcass at the end of the observation period (168 hours) was also measured.
Additionally the rate and pattern of excretion was determined 48 hours after intravenous administration of 9.25 mg/kg bw of the prepared suspension (2.59 mg [^{14}C]-TAP in 0.5 ml Tyrode-solution). The residual amounts of radioactivity after 48 hours were measured likewise in the following organs: liver, kidneys, heart, spleen, lungs, stomach, small intestine, large intestine, caecum, muscle, fat (in muscle and intestine) and in blood.

Results
98% (male rats) and 82% (female rats) of the applied radioactivity was excreted *via* urine after subcutaneous administration. The amount of radioactivity excreted with the faeces was in the range of five per cent (male) and 19% (female) of the applied doses. The excretion was practically completed within 48 hours. Radio-thin layer chromatography revealed no parent substance in the urine of treated rats. The identification of metabolites was not intended.
After intravenous administration 94% of the radioactivity was excreted *via* urine, approx. 2.4% with the faeces. Nearly no radioactivity (< 0.1%) was found in exhalation. Marginal radioactivity was measured in specific organs after 48 hours, with the highest level in the stomach and the large intestine (0.18% and 0.11%).

Ref.: 16

Comment
A number of toxicokinetic studies have been carried out, using radio-labelled TAP but in concentrations mostly far below the applied in use concentration of 3.4% (or 2.0% free base).

3.3.10. Photo-induced toxicity

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

tetraaminopyrimidine sulfate

Maximum absorption through the skin	**A (µg/cm²)**	**=**	**2.21 µg/cm²**
Skin Area surface	**SAS (cm²)**	**=**	**700 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.55 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.026 mg/kg**
Lowest observed adverse effect level (subchronic, oral, rats)	**LOAEL x 0.30 ***	**=**	**45 mg/kg bw**
Margin of Safety	**LOAEL / SED**	**=**	**1731 ****

* Following the toxicokinetics study, the oral absorption rate of tetraaminopyrimidine sulfate is considered to be approximately 30%.

** Even though the calculation is based on a LOAEL, the Margin of Safety is considered sufficiently high.

3.3.14. Discussion

Physico-chemical properties
Tetraaminopyrimidine and its salts are used as a precursor for hair colours. The final concentration on head can be up to 3.4%, calculated for tetra-aminopyrimidine sulfate (corresponding to 2.0% of the free base).
The information submitted is incomplete. Analytical data of only one batch was provided. The purity of the substances was only determined by HPLC. No data on stability in cosmetic formulations was provided. The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

General toxicity
TAP has a low acute oral toxicity. In a subchronic oral (gavage, 90 days) toxicity study in rats some histopathological effects in kidney, thymus, spleen and adrenals were observed at all dose levels (150, 300 and 1000 mg/kg bw). Therefore, a LOAEL of 150 mg/kg bw/day was established. In a teratogenicity study, the maternal NOAEL was considered to be 375 mg/kg bw/day. The test substance was neither embryotoxic nor teratogenic up to 1500 mg/kg bw/day.

Irritation, sensitisation
The substance was not irritant when applied to skin but irritant to the eye.
In the LLNA test, the highest concentration of the test substance (10%) tested is considered too low; other vehicles should have been used to obtain possible higher test concentrations. A possible sensitising potential cannot be excluded.

Dermal absorption
In an absorption study with pig skin using a cream formulation with and without hydrogen peroxide the maximum absorption values were 1.64 µg/cm² and 2.21 µg/cm², respectively. Since, deviating from the SCCP recommendations, only 8 replicates from 2 donors and 10

mg/cm² formulation were used, the highest value found without hydrogen peroxide may be used for the calculation of the margin of safety.

Mutagenicity
The test substance was non-mutagenic in bacteria and in the mouse lymphoma assay. It was clastogenic in Chinese Hamster V79 cells *in vitro*. Genotoxicity was not expressed *in vivo* (bone marrow micronucleus tests). The test item had no cytotoxic properties in the bone marrow but the bioavailability was confirmed by chemical analysis (HPLC) of the blood of the treated animals. Therefore, the negative *in vivo* test indicates that the substance has no relevant mutagenic potential in vivo.

Toxicokinetics
Using a 0.067% aqueous dilution of [^{14}C]-TAP sulphate hemihydrate the mean minimum absorption of TAP via the intestine was 28.2% for the female rats and 41.4% for the male rats. Only minor amounts of the applied dose were found in the carcass and gastrointestinal tract (SCCNFP, 2003). Therefore, the oral absorption rate is considered to be approximately 30%. This should be taken into consideration when calculating the Margin of Safety.
It should be noted however that the concentrations of TAP used in the available toxicokinetic studies are far below the intended in-use concentration of 3.4% (or 2.0% free base).

4. CONCLUSION

The SCCP is of the opinion that the use of tetraaminopyrimidine sulfate as an ingredient in oxidative and non-oxidative hair dye formulations with a maximum on-head concentration of 3.4% (2.0% free base) does not pose a risk to the health of the consumer.

A possible sensitising potential of tetraaminopyrimidine sulfate cannot be excluded.

Tetraaminopyrimidine sulphate itself has no mutagenic potential *in vivo*. 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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