OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

2,5,6-TRIAMINO-4-PYRIMIDINOL SULFATE

COLIPA n° A143

adopted by the SCCNFP during the 24th plenary meeting
of 24-25 June 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is 2,5,6-Triamino-4-pyrimidinol sulfate safe for use in cosmetic products?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

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

2.1.2. Chemical names

Chemical name : 4-Hydroxy-2,5,6-triaminopyrimidine sulfate
Synonyms : 4-OH-2,5,6-triamino-pyrimidine (sulfate)
2,4,5-Triamino-6-hydroxypyrimidine-sulfate

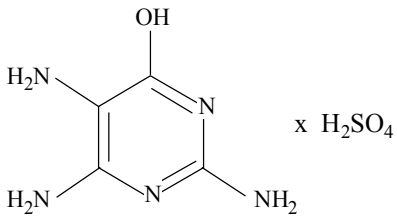
2.1.3. Trade names and abbreviations

Trade name : TRAP
COLIPA No. : A-143

2.1.4. CAS No. / EINECS No.

CAS No. : /
EINECS No. : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. formula : $C_4H_7N_5O \times H_2SO_4$
Mol. weight : 239.21

2.1.7. Purity, composition, and substance codes

All analytical data relate to Aldrich Product No. X12,277-7

Purity		
titre as determined by HPLC (confirmed by a sulfur combustion method)	:	≥ 97.5%
water content (Karl Fischer)	:	≤ 0.5%
Potential impurities and reaction intermediates	:	not assessed or not reported
Solvent residues	:	not assessed or not reported
Other (heavy metals)	:	< 20 ppm

2.1.8. Physical properties

Appearance	:	off-white to yellow or beige, odourless powder
Melting point	:	/
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{OW}	:	/
Stability	:	Stable for years when pure; for a few hours in water or corn oil, as specifically assessed
Storage	:	At room temperature, protected from light

HPLC procedure and features provided. IR spectral characteristics also available for identification purposes (ref.: Aldrich 1(2), 830B).

2.1.9. Solubility

Water : 4.48 g/100 ml (according to sensitisation study 2)
Solubility in receptor fluid, not assessed or not reported.

General comments on analytical and physico-chemical characterisation

* The compound identification reported by the applicant is controversial and that requires clarification. According to CAS, the reported CAS Reg. No. 1603-02-7 corresponds to the empirical formula $C_4H_7N_5O_4S$ (MW 221) and to the chemical structure 4-pyrimidinol, 2,5,6-triamino, hydrogen sulfate (where phenol/hydroxy group is not free but that is esterified with a loss of a water molecule). In an Australian report of December 1999, (File No. NA/768), CAS No. 1603-02-7 is identified with empirical formula $C_4H_7N_5O_4S$ but a wrong MW 239 is assigned to the compound.

* The purity criteria (2.1.7) relates to the Sigma-Aldrich Product No. X12,277-7. However, a recent enquiry revealed that this product is not available through Sigma-Aldrich. On the other hand, a compound with empirical formula $C_4H_7N_5O$ x H_2SO_4, MW 239, (Sigma-Aldrich product No. 17376) was associated with CAS No. 35011-47-3, and the name 4-hydroxy-2,5,6-triaminopyrimidine sulfate salt (and 2,5,6-triamino-4-pyrimidol sulfate salt). A web search revealed that the compound with CAS No. 35011-47-3 (6-hydroxy-2,4,5-triaminopyrimidine

sulfate, a synonym of the compound available from Sigma-Aldrich), MW 257 is also available on the market.

* Purity of chemical reported with reference to Aldrich Product No. X12,277-7, batch unspecified. No official measure of purity consistency is available from the analysis of more than one batch.

* Inadequate analytical characterisation, resulting in a lack of information on impurities, presence of intermediates, and solvent residues, if any.

* The physico-chemical profile of the substance is insufficiently characterised.

* Batch number and/or chemical purity have not been stated in several toxicity study reports.

* Suggestion of pure chemical sensitivity to light and moisture. Stability of test substance solutions assessed for a few hours only.

2.2. Function and uses

2,5,6-Triamino-4-pyrimidinol sulfate will be incorporated into oxidation hair dye formulations at a maximum concentration of 5% (with hydrogen peroxide, 2.5%). Indications of common practice of application were not provided.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

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 no	:	/
Purity	:	/
Dose	:	2000 mg/kg bw by gavage
Observ. 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 LD_{50} of the test substance administered to rats by the oral route was >2000 mg/kg bw.

Ref : 1

2.3.2. Acute dermal toxicity

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 no	:	/
Purity	:	/
Dose	:	2000 mg/kg bw applied on the intact skin (semi-occlusive, 24 h)
Observ. 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 an 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 LD_{50} of the test substance administered to rats by the dermal route was > 2000 mg/kg bw.

Ref : 2

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

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 number	:	815 7329
Purity	:	97 %
Dose levels	:	0, 50, 200 and 1000 mg/kg bw 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, application volume 10 ml/kg bw. 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 4 weeks on all animals. At 4

weeks, 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 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 (deep-yellow) and 1000 mg/kg bw (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 group had abnormalities of the kidneys: tubular basophilia and brownish pigment intratubular or in the pelvis. The NOAEL is 200 mg/kg bw.

Ref.: 7

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

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 number	:	67346
Purity	:	100.4 %
Dose levels	:	0, 50, 200 and 1000 mg/kg bw 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, 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 pretest 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 (both sexes) and 200 mg/kg bw (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.

Ref.: 8

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD 404 (1981)
Species/strain	:	New Zealand albino rabbit
Group size	:	1 male, 2 females
Test substance	:	TRAP, a yellow solid
Batch no	:	/
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

2.4.2. Irritation (mucous membranes)

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 no	:	/
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

2.5. Sensitisation

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 % ammomium water solution
Batch no.	:	/
Purity	:	/
Concentration	:	- Intradermal induction : a 5% solution of the test article was injected intracutaneously 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.

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

Ref.: 5

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 no.	:	Not given
Purity	:	Not given
Concentration	:	- Intradermal induction : a 5% solution of the test article was injected intracutaneously 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 pretest 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.

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

Ref.: 6

2.6. Teratogenicity

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 number	:	not given
Purity	:	> 98%
Dose levels	:	0 and 1000 mg/kg bw 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. 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 in this study.

Ref.: 10

2.7. Toxicokinetics (incl. Percutaneous Absorption)

2.7.2. Percutaneous absorption, distribution and elimination *in vivo*

Guideline	:	EPA (1993)
Species/strain	:	Rats, male, female, Sprague Dawley, SPF-quality
Test substance	:	ring-labelled ^{14}C-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.

Comment
Though the *in vivo* study is performed *lege artis*, it is unsuitable for the intended safety calculation, since a 33-fold lower dosage (0.075%) is applied than claimed (2.5%).

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 2.5% formulation:
2 mg of a 2.5% formulation contain 50 µg TRAP resulting in an exposure to 50 µg/cm². This assumption would lead to an exposure of 0.6 mg/kg bw.

Ref.: 9

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test, Study 1

Guidelines	:	OECD 471
Species/strain	:	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2 uvrA
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	Trap.1
Batch no	:	batch not indicated by the sponsor
Purity	:	/
Concentrations	:	Experiment #1 and #2 *S. typhimurium* and *E. coli* With or without metabolic activation ~~Test #1 :~~ 10, 33.3, 100, 333.3, 1000, 4424.6 µg/plate
GLP	:	In compliance

The test substance has been investigated for gene mutation in *S. typhimurium* and *E. coli* using the direct plate incorporation method both with or without S9 mix. S9 mix was obtained from male Wistar rats injected i.p. with Arochlor™ 1254. Negative and positive controls were in accordance with the OECD guideline.

Results
Toxicity: A slight toxicity as evidenced by a reduction of the background lawn was noted for the strains TA 1535, TA 100 and WP2 uvrA, under both activation conditions in each experiment.

Revertant number, Test # 1 & Test # 2
- In the absence of metabolic activation, no dose related or biologically relevant increase in revertant numbers was observed.

- In the presence of metabolic activation : a dose related and biologically relevant increase in revertant numbers was observed, in the TA 1538 tester strain in both experiment. A trend for positivity was also noted for TA 1537 in experiment # 2 in the presence of activation.
- In the absence of metabolic activation : a decrease in revertant numbers was observed for several *S. typhimurium* tester strains at the highest concentrations. Cytotoxicity could have prevented the expression of revertant.
- For *E. coli*, no statistically or biologically relevant increase of mutant frequencies have been observed as compared with the controls in any test or conditions.
- Positive controls showed the expected response.

The test is not acceptable for evaluation since the identity, the batch and purity of the compound were not given. Based on the reversion rate, and under the conditions of the 2 assays performed, it is concluded that the test agent in the presence of S9 mix, shows clear evidence of mutagenic activity in the frameshift tester strain TA 1538.

Ref. : 11

Bacterial Reverse Mutation Test, Study 2

Guideline	:	OECD 471; OECD 472
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, TA 1538 *E. coli* WP2 uvrA
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	FO-Trap.2 : one part of FO-Trap.2 was mixed with one part of oxycreme (6%). The mixture was incubated for 30 min. After this incubation period the remaining hydrogen peroxide was decomposed with the addition of catalase. The dilution were made with bidestilled water.
Batch no	:	batch not indicated by the sponsor
Purity	:	/
Concentrations	:	Experiment # 1 and # 2 *S. typhimurium* and *E. coli* with or without metabolic activation ~~Test #1 :~~ 10, 100, 333.3, 1000, & 2500 µg/plate : The top dose has been selected according to the instructions of the sponsor
GLP	:	In compliance

The test substance has been investigated for gene mutation in *S. typhimurium* and *E. coli* using the direct plate incorporation method both with or without S9 mix. S9 mix was obtained from male Wistar rats injected i.p. with Arochlor^{TM} 1254. Negative and positive controls were in accordance with the OECD guideline.

Results
Toxicity: No toxicity as evidenced by a reduction of the background lawn was noted for any tester strains under both activation conditions in each experiment.

Revertant number, Test # 1 & Test # 2
- In the absence of metabolic activation, no dose related or biologically relevant increase in revertant numbers was observed.

- In the presence of metabolic activation : no dose related or biologically relevant increase in revertant numbers was observed, in any tester strains in both experiments. No trend for positivity was noted.
- For *E. coli*, no statistically or biologically relevant increase of mutant frequencies has been observed as compared to the controls in any test or conditions.
- Positive controls showed the expected response.

The test is not acceptable for evaluation since the identity, batch and purity of the compound were not given. The test seems to be performed on a completed reaction product rather than on TRAP. Based on the reversion rate, and under the conditions of the 2 assays performed, it is concluded that the test agent COLIPA FO-Trap.2 does not show evidence of mutagenic activity in any tester strains under the conditions of the 2 independent experiments performed.

Ref. : 12

***In Vitro* Mammalian Cell Gene Mutation Test**

OECD guideline	:	OECD 476 (1984)
Species/strain	:	V79 cell line / HPRT Locus
Replicates	:	2 independent tests with and without metabolic activation
Test substance	:	4-hydroxy-2, 5, 6,-triamino-pyrimidine sulfate
Batch no	:	reported to be in the sponsor file but not found.
Concentrations	:	Experiment # 1: without metabolic activation 1.0, 3.0, 10.0, 30.0, 100.0, 60.0* & 100.0 * μg/ml with metabolic activation 10, 100, 300, 1000 & 2500 μg/ ml Experiment # 2: without metabolic activation 1.0, 3.0, 10.0, 20.0, 30.0 & 40.0 μg/ ml with metabolic activation 100, 300, 1000, 2000 & 2500 μg/ ml * Excessive toxicity preventing analysis.
Treatment time	:	4 hours
Purity	:	Stated to be ≥ 97.5 % in the sponsor file (HPLC method).
GLP	:	In compliance

The test substance has been investigated for gene mutation in the HPRT locus of V79 Chinese hamster cell lines, both with or without S9 mix. S9 mix was obtained from male Wistar rats injected i.p. with Arochlor^{TM} 1254. Negative and positive controls were in accordance with the OECD guideline.

Results
Solubility: The test agent was completely soluble in the test medium up to 5.500 g/ml. However, during the exposure period (4 hours), precipitation of the test substance occurred starting at a final concentration of 300 μg/ml.
Osmolarity: An Osmolarity measurement of post treatment medium was performed. No significant changes in these parameters were found.
Plating efficiency, test # 1 & 2 with or without S9 mix:

A dose-related decrease in plating efficiency and culture growth has been observed at : 10 µg/ml without S9 and at 1000 µg/ml with S9.

Mutant frequencies: In the absence or presence of activation, no dose related significant increase in mutant colony numbers was found.

No biologically relevant significant increase in mutant colony numbers over the concurrent solvent controls was observed after treatment with 4-hydroxy-2, 5, 6,-triamino-pyrimidine sulfate in either test in the presence or absence of activation. Therefore, the test substance does not demonstrate mutagenic potential on the HGPRT gene of V79 cells.

Ref. : 13

***In vitro* mammalian chromosomal aberration test**

Guideline	:	OECD 473			
Species/strain	:	Chinese Hamster V79 Cells			
Replicates	:	Duplicate cultures but no independent repeat experiment			
Test substance	:	4-hydroxy-2, 5, 6,-triamino-pyrimidine sulfate dissolved in culture medium			
Batch no	:	reported to be in the sponsor file but not found.			
Purity	:	Stated to be ≥ 97.5 % in the sponsor file (HPLC method).			
Concentrations	:	Test without S9	:	3, 30, 50 µg/ml	18 hours
				50 µg/ml	28 hours
		Test with S9	:	100, 600, 1000 µg/ml	18 hours
				600 µg/ml	28 hours
GLP	:	In compliance			

The test substance has been investigated for gene mutation in the HPRT locus of V79 Chinese hamster cell lines, both with or without S9 mix. S9 mix was obtained from male Wistar rats injected i.p. with Arochlor™ 1254. Negative and positive controls were in accordance with the OECD guideline.

Exposure: The test material has been added to exponentially growing cultures for :
- 18 and 28 hours without metabolic activation;
- 4 hours with metabolic activation. Cultures were prolonged for 18 and 28 hours before harvest.

Results
Toxicity: Relevant toxic effects as evidenced by a decrease in plating efficiency (PE) was observed in the absence or in the presence of S9 mix in from 10 and 300 µg/ml respectively. Mitotic index was reduced after treatment, without S9 mix, with the top dose at both fixation intervals. Mitotic index was reduced after treatment, without S9 mix, with the top dose at 18 hours fixation interval but not 28 hours.

Structural chromosome aberrations
- Without S9 mix : 18 hours fixation interval

A statistically and biologically significant increase in the aberration rate was observed as compared to the corresponding solvent control for the top dose selected (50 µg/ml) (Top dose : 18.5 % aberrant cells with fragments,; 21 % exchanges ; negative control : 1.5 %)

- Without S9 mix : 28 hours fixation interval

A statistically and biologically significant increase in the aberration rate was observed as compared to the corresponding solvent control for the top dose selected (50 µg/ml) (Top dose : 7.5 % aberrant cells with fragments, exchanges, etc.; negative control: 1.0 %)

- With S9 mix : 18 hours fixation interval

A statistically and biologically significant increase in the aberration rate was observed as compared to the corresponding solvent control for the top dose selected (1000 µg/ml) (Top dose : 7 % aberrant cells with fragment, exchanges, etc. ; negative control : 1.5 %)

- With S9 mix : 28 hours fixation interval

A statistically and biologically significant increase in the aberration rate was observed as compared to the corresponding solvent control for the top dose selected (600 µg/ml) (Top dose : 3 % aberrant cells with fragment, exchanges, etc. ; negative control : 1.5 %)

- Positive controls EMS : 18 hours fixation interval

A statistically and biologically significant increase in the aberration rate was observed as compared to the corresponding solvent control for the top dose selected (200 µg/ml : 13 % aberrant cells ; 7 % exchanges).

Polyploidy

No biologically relevant increases in the number of polyploidy cells were noted as compared to the control values.

The test substance does clearly demonstrate clastogenic potential on Chinese hamster V79 cells.

Ref.: 14

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (1983)
Species	:	NMRI mice
Group sizes	:	5 male and 5 female
Test substance	:	4-hydroxy-2, 5, 6,-triamino-pyrimidine sulfate dissolved in bidistilled water
Batch no	:	reported to be in the sponsor file but not found.
Purity	:	Stated to be ≥ 97.5 % in the sponsor file (HPLC method).
Dose levels	:	The test was administered by 1 single oral dose of : 100, 333 and 1000 mg/kg bw for the 24 h sacrifice time 1000 mg/kg bw for the 48 h sacrifice time.
GLP	:	In compliance

The test substance has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. A preliminary range finding study in which ~~observable~~ clinical toxic effects were seen at doses of 1000, 1500 and 2000 mg/bw.

The substance was administered by a single intragastric gavage and the animals were sacrificed 24 and 48 hours after administration. Negative and positive controls were in accordance with the OECD guideline.

Number of cells scored
A total of at least 1000 erythrocytes were examined from each animal ; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.

Results
NCE: the mean number of NCE (mature differentiated cells) was not significantly increased after treatment as compared with controls; this reflects the lack of cytotoxicity of the test agent.
PCE 24 h sampling time: no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were observed for any dose levels.
PCE 48 h sampling time: no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were observed.

Under the conditions of the test it can be concluded that Colipa A143 at doses at which some signs of clinical toxicity were recorded, does not induce statistically significant increase in the frequency of micronucleated PCE. Therefore, Colipa A143 is not clastogenic and/or aneugenic in this mouse bone marrow micronucleus test.

Ref.: 15

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

The 2,5,6-triamino-4-pyrimidinol sulfate identity indicated in the dossier is controversial and requires clarification. Purity was officially reported for Aldrich Product No. X12,277-7, unspecified batch. No official measure of purity consistency is available from the analysis of more than one batch. The analytical characterisation of the chemical is inadequate. Batch number and/or chemical purity were not stated in several toxicity study reports. The physico-chemical profile of the substance is insufficiently characterised. Stability of test substance solutions was assessed for a few hours only.

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.

In the repeated dose oral toxicity in rats study abnormalities of the kidneys were noted in the dose group 1000 mg/kg bw: tubular basophilia and brownish pigment intratubular or in the pelvis. The NOAEL is 200 mg/kg bw. 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 (both sexes) and 200 mg/kg bw (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. In the teratogenicity study the limit dose 1000 mg/kg bw 2,5,6-Triamino-4-pyrimidinol sulfate exhibited no maternal and embryo/foetotoxicity.

Percutaneous absorption : though the *in vivo* study is performed *lege artis*, it is unsuitable for the intended safety calculation, since a 33-fold lower dosage (0.075%) is applied than claimed (2.5%). 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 2.5% formulation. 2 mg of a 2.5% formulation contain 50 µg TRAP resulting in an exposure to 50 µg/cm². This assumption would lead to an exposure of 0.6 mg/kg bw.

2,5,6-Triamino-4-pyrimidinol sulfate has been tested *in vitro* for the induction of mutations in bacteria and mammalian cells and for chromosome aberrations and for the in vivo induction of micronuclei. The *in vitro* tests indicate that the chemical induces gene mutations and chromosome aberrations. The *in vivo* micronucleus test in mice gave negative results.

In general, the data provided are insufficient to conclude on the potential mutagenic/genotoxic activity of this chemical.

2.13. References

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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

3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required :

* proper identification of the compound or confirmation of the identity reported in the dossier;

* proper analytical and physico-chemical data (e.g., characterisation of the purity and impurities of all the batches used, related health hazards of impurities, basic physico-chemical parameters, extended experimental data on stability);

* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

4. Other considerations

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5. Minority opinions

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