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

CONCERNING

2,4,5,6-TETRAAMINOPYRIMIDINE

COLIPA n° A 53

adopted by the SCCNFP during the 25th plenary meeting
of 20 October 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 2,4,5,6-Tetraaminopyrimidine 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

2,4,5,6-Tetraaminopyrimidine

NB : the dossier is submitted for the free base, but the tests are performed with 2,4,5,6-Tetraaminopyrimidine sulfate hemihydrate/sesquihydrate or dihydrochloride.

2.1.2. Chemical names

Chemical name : 2,4,5,6-Tetraaminopyrimidine (IUPAC)
CAS name : Pyrimidinetetramine
Synonym : /

2.1.3. Trade names and abbreviations

Trade Name : TAP, Ro 1
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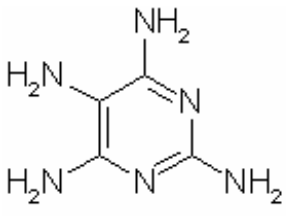
2.1.4. CAS No. / EINECS No.

CAS No. :	1004-74-6	(free base)	EINECS	:	/
	49647-58-7	(sulfate : x H_2SO_4)	EINECS	:	256-407-0
	5392-28-9	(sulfate : 1 H_2SO_4)	EINECS	:	226-393-0
	39944-62-2	(dihydrochloride)			

2.1.5. Structural formula



.H_2SO_4 + 0.5 H_2O/1.5 H_2O or 2HCl

2.1.6. Empirical formula

Emp. formula : $C_4H_8N_6$ (free base)
Mol. weight : 140.15 (free base)
238.21 (1 H_2SO_4)
213.05 (dihydrochloride)

2.1.7. Purity, composition, and substance codes

2,4,5,6-Tetraaminopyrimidine sulfate has been characterised by IR and UV spectroscopy (λ_{max} 202 nm and 274 nm).

Purity checked by HPLC, UV detection at 207 nm : 99.7 % (only one batch, batch number not identified)

Impurities : no information

2.1.8. Physical properties

Appearance	:	Pale yellow crystals, odourless
Melting point	:	> 218 °C with decomposition
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{OW}	:	/

2.1.9. Solubility

Slightly soluble in water (free base)
Soluble in water (salts)

2.1.10. Stability

No data

General comments on analytical and physico-chemical characterisation

The information provided on the compound is incomplete and confusing (See Annex 1)

2.2. Function and uses

2,4,5,6-Tetraaminopyrimidine(*) as a developer is intended for use in oxidation hair dye formulations up to 5%.

(*) to be defined as to its chemical nature

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Study 1 (screening)

Guideline	:	/
Species/strain	:	CF1 mice (Winkelmann)
Group size	:	10
Test substance	:	TAP (to pH8-10 adjusted with NaOH)
Batch No.	:	no data
Dose	:	1x5000 mg/kg oral (gavage); limit test
Observ. period	:	7 days
GLP	:	not in compliance

The acute oral toxicity of 2,4,5,6-tetraaminopyrimidine sulphate-hemihydrate was investigated in healthy adult male mice of CF1 strain. Ten animals were used in the test for the particular dose. The average body weight at the day of application was approx. 23 g. Aliquots of 40 ml/kg bw of the aqueous solution, adjusted to pH 8-10 by adding sodium hydroxide, were administered by gavage. The resulted oral dose was 5000 mg/kg bw. During a seven day observation period, mortalities and clinical-toxicological observations were recorded.

Results
During the study nearly all test animals were apathic. The acute lethal dose was calculated to be LD_{50} > 5000 mg/kg bw.

Ref. : 1

Study 2 (semi-final)

Guideline	:	/
Species/strain	:	CF1 male mice (Winkelmann)
Group size	:	10 (6 groups)
Test substance	:	TAP in water (Aqua dest.)
Batch No.	:	no data
Dose	:	1.99; 2.51; 3.16; 5.01; 6.31; 7.94 g/kg bw
Observ. period	:	7 days
GLP	:	not in compliance

The acute oral toxicity of 2,4,5,6-tetraaminopyrimidine sulphate-hemihydrate was investigated in healthy 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 solution were administered by gavage. The resulted oral doses were 1990, 2510, 3160, 5010, 6310 and 7940 mg/kg bw and the pH-value was found to be pH 2-3. During a seven day observation period, mortalities and clinical-toxicological observations were recorded.

Results
During the study nearly all test animals were apathic for a period. The acute lethal dose was calculated according to the method of Litchfield and Wilcoxon to be LD_{50} = 4700 mg/kg bw.
Ref. : 2

Study 3 (in a cream-formulation)

Guideline	:	OECD 401
Species/strain	:	Wistar rats (Winkelmann)
Group size	:	2x10 (5/5)
Test substance	:	TAP 1.6 % of the formulation
Batch No.	:	no data
Dose	:	Limit test (5.000 mg/kg bw of the formulation) oral, gavage
Observ. period	:	14 days
GLP	:	in compliance

The acute oral toxicity of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine, was investigated in healthy adult male and female rats of Wistar strain. Ten animals (five per sex) were used per group. The average body weight at the day of application was 183 g for the male and 151 g for the female rats.

The control formulation represented a basic cream used for usual hair dyeing processes, containing a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-352) consisted of the same basic cream supplemented by 1.6 % 2,4,5,6-tetraaminopyrimidine as a developer and one per cent 2,7-dihydroxynaphthalene (Ro 575) as a coupler.
Aliquots of 20 ml/kg bw of the aqueous dilution (25 % w/v) of the formulation were administered by gavage. The resulted acute toxicity (oral dose) was 5,000 mg/kg bw (limit-test) of the formulation.
During a two weeks observation period, mortalities and clinical-toxicological observations were recorded daily, and the body weight was noted weekly. After sacrificing the animals at the end of the study, all rats were examined in a necropsy.

Results
No rat died relating to the administered formulations. Therefore the acute lethal dose of both formulations was found to be LD_{50} > 5,000 mg/kg bw.
The following clinical symptoms were observed during the study : only slight piloerection shortly after the application. In the necropsy no substance related alterations were noted.
Ref. : 3

Study 4 (in a cream-formulation)

Guideline	:	OECD 401
Species/strain	:	Wistar rats (Winkelmann)
Group size	:	2x10 (5/5)
Test substance	:	TAP 4.2 % of a formulation
Batch No.	:	no data
Dose	:	5000 mg/kg-“Limit-test”, oral (gavage)

Observ. period : 24 hrs, 7 and 14 days
GLP : in compliance

The acute oral toxicity of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine, was investigated in healthy adult male and female rats of Wistar strain. Ten animals (five per sex) were used per group. The average body weight at the day of application was 188 g for the male and 148 g for the female rats.

The control formulation represented a basic cream used for usual hair dyeing processes, containing a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-351) consisted of the same basic cream supplemented by 4.2 % 2,4,5,6-tetraaminopyrimidine as a developer and 2.0 % 2-methylresorcinol (Ro 261) as a coupler.

Aliquots of 20 ml/kg bw of the aqueous dilution (25 % w/v) of the formulation were administered by gavage. The resulted oral dose was 5,000 mg/kg bw (limit-test).
During a two weeks observation period, mortalities and clinical-toxicological observations were recorded daily, and the body weight was noted weekly. After killing the animals at the end of the study, all rats were examined in a necropsy.

Results
No rat died related to the administered formulations. Therefore the acute lethal dose of both formulations was found to be LD_{50} > 5,000 mg/kg bw.
The following clinical symptoms were observed during the study : only slight piloerection shortly after the application. In the necropsy no substance related alterations were noted.

Ref. : 4

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Guideline	:	/
Species/strain	:	Wistar rats (Han 67SPF)
Group size	:	10/10
Test substance	:	TAP (in the diet)
Batch No.	:	no data
Dose	:	0, 50, 500, 5000 ppm, from week 6-13 : 10,000 ppm (= 8 weeks) TAP sulfate hemihydrate in the diet
Exposure period	:	90 days
GLP	:	not in compliance

Male and female rats of Wistar strain (Han 67 SPF) were used. The average body weight of the animals at the beginning of the administration was 161 g for male respectively 143 g for female rats. 20 rats (10 per sex) were used per dose and control group. Additionally a group of 20 animals (10 per sex) was used for preliminary chemical and haematological analysis.

Doses of 50 ppm, 500 ppm and 5,000 ppm of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were fed in the diet for 12 weeks. The highest dose of 5,000 ppm was increased after five weeks of dosing to 10,000 ppm. Such treatment is corresponding to daily dosages of 0, 3, 30 and 300/600 mg/kg bw.

During the study mortality, signs of intoxication, body weight, food and water consumption, clinical, haematological and biochemical parameters were recorded. At the end of the study, the animals were sacrificed and subjected to pathological investigations.

Results
The test substance 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was well tolerated by rats after feeding of up to 5,000 ppm/10,000 ppm during the 12 week-treatment. The body weight gain as well as the water consumption were generally comparable to the control groups. Yellowish coloured urine was seen in the high dose group, beginning after one week of application. The organ weights were in the normal range. The histopathological evaluation of the tissues revealed no lesions in the test group examined.

According to the results of the study, none of the doses led to cumulative toxic effects of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate. Therefore, the No-Observed-Effect-Level (NOEL) for rats has to be calculated as 600 mg/kg bw/d (8 w).

Ref. : 24

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 and corrosivity

2.4.1. Irritation (skin)

2.4.1.1. Acute dermal irritation in rabbits

Guideline	:	/
Species/strain	:	New Zealand White rabbits
Group size	:	6
Test substance	:	TAP as 10 % g/v suspension (in Aqua dest.)
Batch No.	:	no data
Dose	:	500 µl of a 10 % suspension in water, applied 24 hrs
GLP	:	not in compliance

The acute dermal irritation properties of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were investigated in healthy adult male albino rabbits of New Zealand strain. Six animals were used in the test. Each animal served as its own control. Approximately 24 hours before treatment, the dorsal fur was shaved.

An aliquot of 500 µl of a 10 % dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was applied to the intact shaved back skin of each animal. After 24 hours occlusive contact the patch was removed. Animals were examined for signs of erythema and oedema formation. The skin reactions were observed 24, 48 and 72 hours after termination of the exposure and the effects were scored according to the scheme of Draize.

Results
Under the conditions of the study, the tested 10 % dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was neither irritating nor corrosive when applied to the intact rabbit skin under occlusive conditions. Only slight redness was seen in two animals shortly after removal of the patch.

Ref. : 5

2.4.1.2. Acute dermal irritation of formulation I (cream) in rabbits

Guideline	:	OECD 404
Species/strain	:	New Zealand White rabbits (Erkrath)
Group size	:	5 (male) rabbits
Test substance	:	TAP 1.6 % in formulation (Ma-S-352)
Batch No.	:	no data
Dose	:	500 µl undiluted preparation on 3x3 cm patch
GLP	:	in compliance (reading 1, 24, 48 hrs and 3 7 days after patch removal)

The acute dermal irritation properties of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine, were investigated in healthy adult male albino rabbits of New Zealand

strain. Five animals were used in the test. Approximately 24 hours before treatment, the lateral and dorsal fur was shaved. Each animal served as its own control.
The control formulation represented a basic cream used for usual hair dyeing processes, containing a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-352) consisted of the same basic cream supplemented by 1.6 % 2,4,5,6-tetraaminopyrimidine as a developer and 1.0 % 2,7-dihydroxynaphthalene (Ro 575) as a coupler. Shortly before application, both formulations were mixed with an equal amount of 6.0% aqueous hydrogen peroxide.

Aliquots of 500 µl of the formulations were applied to the intact shaved skin of each animal, one formulation per flank. After four hours occlusive contact the patches were removed and residuals were washed off. Animals were examined for signs of erythema and oedema formation. The skin reactions were assessed 1, 24, 48 and 72 hours as well as three and seven days after patch removal. The effects were scored according to the scheme of Draize.

Results
Under the conditions of the study, the tested formulations were neither irritating nor corrosive when applied to the intact rabbit skin under occlusive conditions. Only slight redness was seen in a few animals up to 48 hours after removal of the patch. It can be concluded that the test compound was not irritating.

Ref. : 7

2.4.1.3. Acute dermal irritation of formulation II in rabbits

Guideline	:	OECD 404
Species/strain	:	New Zealand White (Erkrath)
Group size	:	5 male rabbits
Test substance	:	TAP 4.2 % in a formulation
Batch No.	:	no data
Dose	:	500 µl undiluted preparation on 3x3 cm patch
GLP	:	in compliance

The acute dermal irritation properties of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine, were investigated in healthy adult male albino rabbits of New Zealand strain. Five animals were used in the test. Approximately 24 hours before treatment, the lateral and dorsal fur was shaved. Each animal served as its own control.

The control formulation represented a basic cream used for usual hair dyeing processes, containing a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-351) consisted of the same basic cream supplemented by 4.2 % 2,4,5,6-tetraaminopyrimidine as a developer and 2.0 % 2-methylresorcinol (Ro 261) as a coupler. Shortly before application, both formulations were mixed with an equal amount of six per cent aqueous hydrogen peroxide.
Aliquots of 500 µl of the formulations were applied to the intact shaved skin of each animal, one formulation per flank. After four hours occlusive contact the patches were removed and residuals were washed off. Animals were examined for signs of erythema and oedema formation. The skin reactions were assessed 1, 24, 48 and 72 hours as well as three and seven days after patch removal. The effects were scored according to the scheme of Draize.

Results
Under the conditions of the study, the tested formulations were neither irritating nor corrosive when applied to the intact rabbit skin under occlusive conditions. Only slight redness was seen in two animals of the group which was treated with the basic cream shortly after removal of the patch. It can be concluded that the test substance was not irritating.

Ref. : 8

2.4.1.4. Dermal irritation in *rabbits* after repeated application

Guideline	:	/
Species/strain	:	New Zealand White rabbits
Group size	:	2 animals
Test substance	:	TAP as a 10 % solution/suspension in water (Aqua dest.)
Batch No.	:	no data
Dose	:	repeated application (60x)
GLP	:	not in compliance

The dermal irritation properties after repeated contact of the diluted 2,4,5,6-tetraamino-pyrimidine sulphate hemihydrate were investigated in healthy adult male rabbits of New Zealand strain using the method described by Burghardt (Berufsdermatosen 18, 179-88 (1970)). Two animals were used in the test. Approximately 24 hours before treatment, the dorsal fur was shaved. Each animal served as its own control.

One to two drops of a 10 per cent aqueous dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were applied to a small skin area of each animal. This treatment was repeated every 30 seconds during a 30 minutes time period to the same area of the back skin according to the method of Burckhardt. Each application was gently massaged into the skin.
Animals were examined continuously for signs of erythema and oedema and any observed effects were scored.

Results
During and after the application period no primary skin irritation could be observed. From these test results it can be concluded, that the occasional repeated contact with 2,4,5,6-tetraamino-pyrimidine sulphate hemihydrate in a concentration of up to 10 per cent did not cause any skin irritation.

Ref. : 9

2.4.1.5. Dermal irritation in *hairless mice* after repeated application

Guideline	:	/
Species/strain	:	Hairless mice, strain hrhr
Group size	:	5 male mice
Test substance	:	TAP in a 10 % dilution/suspension in water
Batch No.	:	no data
Dose	:	repeated topical application (1-2gtt.) 10x at 5 days
GLP	:	not in compliance

The dermal irritation properties after repeated contact of the diluted 2,4,5,6-tetraamino-pyrimidine sulphate hemihydrate were investigated in healthy adult male hairless mice of hr/hr strain. Five animals were used in the test. Each animal served as its own control.

One to two drops of a 10 per cent aqueous dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were applied to a small skin area of each animal. This treatment was repeated twice daily on five consecutive days to the same area of the back skin. Each application was gently massaged into the skin. Animals were examined continuously for signs of erythema and oedema and any observed effects were scored according to the scheme of Draize.

Results
During and after the application period no primary skin irritation could be observed. From these test results it can be concluded, that the occasional repeated contact with 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate in a concentration of up to 10 per cent did not cause any skin irritation.

Ref. : 10

2.4.1.6. Dermal irritation of a formulation in *hairless mice* after repeated application

Guideline	:	/
Species/strain	:	Hairless mice, strain hrhr
Group size	:	2x8
Test substance	:	1.6 % TAP in cream formulations
Batch No.	:	no data
Dose	:	repeated topical application (vol. not identified)
GLP	:	not in compliance

The dermal irritation properties of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine, after repeated contact, were investigated in healthy adult male hairless mice of hr/hr strain. 16 animals (eight per formulation) were used in the test. Each animal served as its own control.

The control formulation represented a basic cream used for usual hair dyeing processes, containing a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-352) consisted of the same basic cream supplemented by 1.6 % 2,4,5,6-tetraamino-pyrimidine as a developer and one per cent 2,7-dihydroxynaphthalene (Ro 575) as a coupler. Shortly before application, both formulations were mixed with an equal amount of six per cent aqueous hydrogen peroxide.

One to two drops of the formulations were applied to a small skin area of each animal. 30 minutes later the test articles were washed off. This treatment was repeated once daily on five consecutive days to the same area of the back skin. Animals were examined four respectively five hours after each application for signs of erythema and oedema and any observed effects were scored according to the scheme of Draize.

Results
After the first application no primary skin irritation could be observed for both formulations. After the second and third treatment, some animals showed a slight erythema, which increased after the fourth respectively the fifth application. Three days after the last treatment all mice recovered.
It can be concluded that the test substance showed the same mild irritation as the basic cream. The animals did not show any symptoms of systemic intoxication.

Ref. : 12

2.4.1.7. Acute dermal irritation in *humans*

Guideline	:	/
Species/strain	:	/
Group size	:	5 human volunteers
Test substance	:	TAP as 10 % suspension in water
Batch No.	:	no data
Dose	:	10 % suspension under occlusion
GLP	:	not in compliance

The acute dermal irritation properties of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were investigated in five adult human volunteers. Each test person served as its own control.

Aliquots of a 10 % dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were applied to the intact brachium skin of each test person. After eight hours occlusive contact the exposure was terminated by patch removing.
Volunteers were examined for signs of erythema and oedema formation. Any observable skin reaction was assessed and the effects were scored according to the scheme of Marzulli & Maibach.

Results
Under the conditions used in this study, the tested 10 % dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was neither irritating nor corrosive when applied to the intact human skin under occlusive conditions.

Ref. : 6

2.4.1.8. Dermal irritation in human volunteers after repeated application

Guideline	:	/
Species/strain	:	/
Group size	:	5 human volunteers
Test substance	:	TAP 10 % dilution/suspension in water
Batch No.	:	no data
Dose	:	for 30 minute intervals of 30 sec. 1-2 drops/applications
GLP	:	not in compliance

The dermal irritation properties of the diluted 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate, after repeated contact were investigated in five adult human volunteers using the method described by Burckhardt. Each test person served as its own control.

One to two drops of a 10 per cent aqueous dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were applied to a small skin area of the individual forearm. This treatment was repeated every 30 seconds during a 30 minutes time period to the same area of the back skin according to the method of Burckhardt. Each application was gently massaged into the skin. Test persons were examined continuously for signs of erythema and oedema and any observed effects were scored.

Results
During and after the application period no primary skin irritation could be observed. From these test results it can be concluded, that the occasional repeated contact with 2,4,5,6-

tetraaminopyrimidine sulphate hemihydrate in a concentration of up to 10 per cent did not cause any skin irritation.

Ref. : 11

2.4.2. Irritation (mucous membranes)

Eye irritation in rabbits

Guideline	:	/
Species/strain	:	New Zealand White rabbits (Erkrath)
Group size	:	6 rabbits
Test substance	:	5 % TAP solution/suspension in water
Batch No.	:	no data
Dose	:	100 µl in the conjuncival sac of one eye (1x)
GLP	:	not in compliance

The acute eye irritation properties of the diluted 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were investigated in six healthy adult male albino rabbits of New Zealand strain. An amount of 100 µl of the five per cent aqueous dilution of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate was instilled into the conjunctival sac of one eye of the test animals. The substance remained in permanent contact with the eyes. The other eye served as control.
The eye irritation reactions were scored 1, 6, 24, 48 and 72 hours after instillation of the test substance according to the method of Draize.

Results
Instillation of diluted 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate into the rabbits eye did not affect the cornea and the iris. The slight irritation of the conjunctivae persisted only for six hours.

Ref. : 13

Eye irritation of hair dye formulations in rabbits

Guideline	:	/
Species/strain	:	New Zealand White rabbits
Group size	:	2x5 rabbits
Test substance	:	TAP 1.6 % in a cream-formulation, with or without coupler
Batch No.	:	no data
Dose	:	application of an aliquot in the conjunctival sac. Rinsing after 10 sec.
GLP	:	not in compliance

The acute eye irritation properties of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine, were investigated in five healthy adult male albino rabbits of New Zealand strain.

The control formulation represented a basic cream used for usual hair dyeing processes, containing a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-352) consisted of the same basic cream supplemented by 1.6 % 2,4,5,6-tetraaminopyrimidine as a developer and one per cent 2,7-dihydroxynaphthalene (Ro 575) as a

coupler. Shortly before instillation, both formulations were mixed with an equal amount of six per cent aqueous hydrogen peroxide.

An aliquot of 100 µl of these representative formulations was instilled into the conjunctival sac of one eye of the test animals. The substance remained for 10 seconds in contact with the eyes, before rinsing with 60 ml tap water. The other eye served as control.

The eye irritation reactions were scored 1, 6, 24, 48, 72 and 144 hours after instillation of the test substance according to the method of Draize. The cornea was investigated additionally on potential injuries using fluorescein after 24, 72 and 144 hours.

Results
Instillation of both formulations into the eye did not affect the iris. The cornea was slightly affected in some animals only at the 24-hour inspection, independently on the test article. Additionally, the conjunctivae were found to be slightly irritated.

It can be concluded that the test substance was not irritating.

Ref. : 14

2.5. Sensitisation

2.5.1 Skin sensitisation in guinea pigs

Guideline	:	/
Species/strain	:	Pirbright White Guinea pigs
Group size	:	20 test/10 control animals
Test substance	:	TAP
Batch No.	:	/
Concentration	:	5 % solution (Aqua dest.) + Freund’s adjuvant (1/1)
GLP	:	not in compliance

The dermal sensitisation properties of 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were investigated in healthy adult male guinea pigs of Pirbright White strain. Twenty animals were used in the induction-group and additional 10 animals served as controls.
A dose of 0.1 ml of an aqueous solution, consisting of 5 parts of the test substance, 7.5 parts of dimethylformamide (adjusted to pH 9.0-9.5 by ammonium hydroxide) and diluted by the addition of Freund's Adjuvant, was intradermally injected every 2-3 days for a total number of ten.

The challenge was carried out fourteen days later by exposing 0.1 ml of a five per cent ethanolic solution (24 hours, occlusively) to the animal flanks.
Animals were examined 24 hours later and were re-challenged in the same way to be examined a second time after further 24 and 48 hours for signs of erythema and oedema, all responses were scored.

Results
During the induction period, all animals showed irritation effects and typical reactions to Freund's Adjuvant. No animal showed any relevant skin alteration after the two above mentioned challenges. Thus 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate did not cause sensitisation.

Ref. : 15

2.5.2 Skin sensitisation of formulation I in guinea pigs

Guideline	:	OECD n° 406
Species/strain	:	Pirbright White Guinea pigs
Group size	:	20 female animals (2 test), (1 control group, 10 animals)
Test substance	:	TAP
Batch No.	:	/
Concentration	:	1.6 % in the formulation-cream, with and without coupler
GLP	:	in compliance

The dermal sensitisation properties of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were investigated in healthy female albino guinea pigs of Pirbright White strain. 40 animals (20 per group) were used in the test, an additional group of 10 animals served as control.

The control formulation represented a basic cream used for usual hair dyeing processes, containing only a fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-352) consisted of the same basic cream supplemented by 1.6 % 2,4,5,6-tetraaminopyrimidine as a developer and one per cent 2,7-dihydroxynaphthalene as a coupler. Shortly before application, both formulations were mixed with an equal amount of six per cent aqueous hydrogen peroxide.

The test procedure for the investigation of the skin sensitisation was carried out as described by Magnusson and Kligman and according to the competent OECD guideline (n° 406).
The first - intradermal - induction was performed using a one per cent aqueous solution.
The second - topical - induction was performed one week later with a five per cent dilution of the test substance in vaseline under occlusive conditions (48 hours). Controls received the vehicle used in test animals only.
The first challenge was carried out fourteen days later by exposing 0.1 ml of a one per cent aqueous solution (open conditions) to the animal flanks. A second challenge was performed one week later by exposing 30 µ1 of a one per cent aqueous solution (occlusively, 24 hours) to the opposite animal flanks.
Animals were examined 24, 48 and 72 hours after removal of the Finn Chambers for signs of erythema and oedema all the responses were scored.

Results
After the induction, all animals showed typical reactions to Freund's Complete Adjuvant. The substance provoked irritation in both induction periods. The macroscopic examinations did not show evidence of any pathological lesion of delayed hypersensitivity in the 40 guinea-pigs of the treated groups. Furthermore no cutaneous abnormality was noted in the control group.
All treated animals failed to show any evidence for any substance-related systemic toxicity.
From the test results obtained under the experimental conditions employed, the test substance did not cause sensitisation.

Ref. : 16

2.5.3 Skin sensitisation of formulation II in guinea pigs (Magnusson-Kligman-Test)

Guideline	:	OECD 406
Species/strain	:	Pirbright White Guinea pigs
Group size	:	2 x 20 female animals (open and closed application) + 10 controls

Test substance : TAP
Batch No. : no data
Concentration : see description
GLP : in compliance

The dermal sensitisation properties of two formulations, one of them containing 2,4,5,6-tetraaminopyrimidine sulphate hemihydrate were investigated in healthy female albino guinea pigs of Pirbright White strain. 40 animals (20 per group) were used in the test, an additional group of 10 animals served as control.

The control formulation represented a basic cream used for usual hair dyeing processes, containing only fatty alcohol, anionic surfactants and inorganic salts. The test formulation (coded as Ma-S-351) consisted of the same basic cream supplemented by 4.2 % 2,4,5,6-tetraaminopyrimidine as a developer and two per cent 2-methylresorcinol (Ro 261) as a coupler. Shortly before application, both formulations were mixed with an equal amount of six per cent aqueous hydrogen peroxide.
The test procedure for the investigation of the skin sensitisation was carried out as described by Magnusson and Kligman and according to the competent OECD guideline (n° 406).
The first - intradermal - induction was performed using a one per cent aqueous solution.
The second - topical - induction was performed one week later with a five per cent dilution of the test substance in vaseline under occlusive conditions (48 hours). Controls received the vehicle used in test animals only.
The first challenge was carried out fourteen days later by exposing 0.1 ml of a one per cent aqueous solution (open conditions) to the animal flanks. A second challenge was performed one week later by exposing 30 µl of a one per cent aqueous solution (occlusively, 24 hours) to the opposite animal flanks.

Animals were examined 24, 48 and 72 hours after removal of the Finn Chambers for signs of erythema and oedema all the responses were scored.

Results
After the induction, all animals showed typical reactions to Freund's Complete Adjuvant. The substance provoked irritation in both induction periods. The macroscopic examinations did not show evidence of any pathological lesion of delayed hypersensitivity in the 40 guinea-pigs of the treated groups. Furthermore no cutaneous abnormality was noted in the control group. All treated animals failed to show any evidence for substance-related systemic toxicity.
From the test results obtained under the experimental conditions employed, the test substance did not cause sensitisation.

Ref. : 17

2.6. Teratogenicity

Guideline : /
Species/strain : Wistar 67 Han SPF
Group size : 20-26
Test substance : TAP
Batch No. : 1965/50
Dose levels : 0, 250, 500, 1000 mg/kg bw
Treatment period : day 6-19 of gestation
GLP : not in compliance

The study was performed with 94 pregnant rats of Wistar strain (MuRa 67 Han SPF). Prior to the treatment, females weighted between 190 g and 220 g. After acclimatisation the females were paired overnight with sexually mature males. One male rat was paired with two to three female rats. The test animal groups as well as the control group consisted of 20 - 26 dams each.

2,4,5,6-tetraaminopyrimidine dihydrochloride was dissolved in water and dose levels of 0, 250, 500, and 1,000 mg/kg body weight were administered daily (day 6 - 19 of pregnancy) by the means of a stomach tube. The time elapsed from conception was determined by examination of vaginal smear for spermatozoa as day 0 of pregnancy. The mortality and the body weight gain were observed daily.
The dams were sacrificed on day 20 of gestation and the foetuses removed by caesarean section. The number of alive and dead foetuses, their distribution and site in the uterus, early and late resorptions, implantations and number of *corpora lutea* were determined. The weight of the foetuses, gravid uteri, uteri without foetuses, placenta and the sex of foetuses was recorded. The foetuses were examined for skeletal malformations, variations and retardations of the normal organogenesis after staining with alizarin red S.

Results
The oral administration of up to 500 mg/kg bw/d 2,4,5,6-tetraaminopyrimidine dihydrochloride to dams from day 6 to 19 of gestation did not show indications of maternal toxicity and revealed no adverse effects to the foetal development. Only in the high dose group the mean implantation loss was slightly increased, but there was no evidence of treatment-related foetal lesions or malformations. Therefore 2,4,5,6-tetraaminopyrimidine dihydrochloride is neither embryo-lethal, embryo-toxic nor teratogenic.
With respect to the test results, the NOAEL for embryo-toxicity was considered to be 500 mg/kg bw/day.

Ref. : 25

2.7. Toxicokinetics (including Percutaneous Absorption)

2.7.1. Percutaneous Absorption *in vitro*

Percutaneous penetration / dermal absorption of a hair dye formulation in rats

Guideline	:	/
Tissue	:	(in vivo) Wistar rats (SPF-Cpb) 5/5
Method	:	radioactive labelled TAP in a cream preparation (without developer) radioactive purity > 91 %
Test substance	:	TAP (0.226 %) in formulation + non labelled TAP; total 0.451 %
Batch no	:	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 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 $C0_2$-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 cm^2. The diluted (1:1) formulation was exposed to the intact, clipped skin of rats under semi-occlusive conditions without rinsing. Finally 200 mg of the formulation were applied per animal, which resulted in a test substance dose of 0.045 mg/cm^2.

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. : 21

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

Guideline	:	/
Tissue	:	(in vivo) Wistar rats (SPF-Cpb) 8/8
Method	:	radioactive labelled TAP
Test substance	:	TAP 0.12 % after combination with H_2O_2 (6 %)
Batch no	:	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_20_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 semiocclusive exposure for 30 minutes to a clipped skin area of 8 cm^2. Finally 400 mg of the formulation were applied per animal, which resulted in a test substance dose of 0.058 mg/cm^2.

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 $\mu g/cm^2$, male rats) and 0.27 % (0.153 $\mu g/cm^2$, 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. : 22

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 peroral 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. : 22

Percutaneous absorption of a complete hair dye formulation in rats

Guideline	:	/
Tissue	:	(in vivo)
Method	:	radioactive labelled TAP with and without coupler
Test substance	:	0.24 %
Batch no	:	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 cm^2 (form. I) or 10 cm^2 (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/cm^2.
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/cm^2, males) and 0.30 % (0.29 µg/cm^2, females) corresponding to the described test conditions with the coupler. In the absence of the coupler 0.64 (0.62 µg/cm^2, male rats) respectively 0.35 % (0.33 µg/cm^2, 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. : 23

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 were measured in specific organs after 48 hours, with the highest level in the stomach and the large intestine (0.18 % and 0.11 %).

Ref. : 23

Remark
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 %.

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, TA 1538
Replicates	:	Triplicate plates, no independent repeat
Test substance	:	Ro 1 in DMSO
Batch no	:	no data
Purity	:	no data
Concentrations	:	Without metabolic activation a) 2.7, 27.0, 270.0, 2700.0 μg/plate b) 100, 1000, 2000, 3000-5000 μg/plate (TA 1538 & TA 98) c) 6.7, 67.5, 675, 6750 μg/plate (TA 1535 pH 8.5) d) 3.2, 32.4, 324, 3240 μg/plate (TA 1535 pH 9.5) With metabolic activation (rat liver) a) 2.7, 27.0, 270.0, 2700.0 μg/plate b) 10, 100, 1000, 2000, 3000, 4000, 5000 μg/plate c) 6.7, 67.5, 675, 6750 μg/plate (TA 1535 pH 8.5) d) 3.2, 32.4, 324, 3240 μg/plate (TA 1535 pH 9.5)
GLP	:	not in compliance

COLIPA A 053 has been investigated for gene mutation in *Salmonella typhimurium* using the standard plate incorporation method both with or without S9 mix. No confirmatory assay has been performed.
S9 mix from rats injected i.p. with Aroclor^{TM} 1254 was used. In some assay, the test agent has been tested in 2 pH conditions (pH 8.5; pH 9.5).

Results
Dose range finding assay

* No data are presented
* Test # 1 In the absence or the presence of activation : no relevant increase in revertant numbers was observed under any conditions.

Conclusions
The test is unsuitable for genotoxicity and/or mutagenicity evaluation. (test substance, purity, batch not characterised; no dose range finding data, no repeat experiment).

Ref. : 18

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

Guideline	:	OECD 473
Species/strain	:	Chinese Hamster V79 Cells
Replicates	:	Duplicate cultures, No repeat experiment
Test substance	:	2,4,5,6-tetraaminopyrimidine sulfate
Batch no	:	3933/10
Purity	:	> 98 %; certificate of analysis
Concentrations	:	0.6 – 17.0 µg/ml with and without metabolic activation.
GLP	:	in compliance

After 48 hours in cultures, cells were exposed as followed :

Fixation interval	*Exposure period*	*Concentrations in µg/ml*	
7 hrs	4 hrs	17	Without S9 mix
18 hrs	4 hrs	0.6, 6.0, 17.0	Without S9 mix
18 hrs	4 hrs	0.6, 6.0, 13.0, 17.0	With S9 mix
28 hrs	4 hrs	13.0, 17.0	Without S9 mix
28 hrs	4 hrs	17.0	With S9 mix

Results
Toxicity
In the pre-experiment, no relevant toxic effect was observed in the absence or in the presence of S9 mix as evidenced by either the plating efficiency (PE)(cloning forming ability) or Mitotic Index (MI). The top dose was chosen on the basis of solubility.

Structural chromosome aberrations
With or Without S9 mix : no statistically and biologically increase of the frequency of cells displaying structural chromosome aberrations was observed at any dose and/or fixation time interval.
Polyploidy
No relevant increase of the number of polyploid cells was noted.

Conclusions
The assay is acceptable for evaluation. 2,4,5,6-tetraaminopyrimidinesulfate is considered negative for clastogenic and or aneugenic potential in Chinese hamster V79 cell line in the absence or in the presence of activation under the conditions of the tests.

Ref. : 19

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	/
Species	:	CD-1 mice
Group sizes	:	5 males and 5 females
Material	:	Ro 1 in methylcellulose
Batch no	:	no data
Purity	:	no data
Dose levels	:	Maximum Tolerated Dose (MTD) Two preliminary dose-range finding assays were conducted. According to clinical signs and toxic reactions of the mice, the top dose has been chosen to be 10,000 mg/kg bw Ro 1 in methylcellulose was administered by 2 single oral gavage 24 hours apart. 100, 5000 and 10,000 mg/kg bw. Sacrifice was performed 6 hours after the last dosing.
GLP	:	not in compliance

Ro 1 in methylcellulose has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. Dose levels were determined by 2 preliminary range finding studies. The substance was administered by 2 single intragastric gavage 24 hours apart and the groups of animals sacrificed 6 hours after the last administration.

Number of cells scored : a total of at least 2000 erythrocytes were examined from each animal ; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.

Results
NCE/PCE ratio : the mean ratio of NCE/PCE was comparable with the concurrent control value (individual data not presented).
µ-PCE, 6 h sampling time : no significant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were observed for any dose levels.

Conclusions
The study is unsuitable for genotoxicity/clastogenicity/aneugenicity evaluation due to the protocol followed, the absence of test agent characterisation, lack of batch and purity description and the absence of proof of reaching the target cells.

Ref. : 20

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

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 (LD 50) 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 radiolabelled 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, 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.

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25. Potokar, M., Sudkamp, R. & Pittermann, W. (1980) 2,4,5,6-tetraaminopyrimidindihydrochlorid - Prüfung auf embryotoxische Wirkung an der Ratte. Henkel KGaA, Düsseldorf, Report No. R 9400601

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; 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);

* percutaneous absorption study in accordance with the Notes of Guidance.

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

4. Other considerations

/

5. Minority opinions

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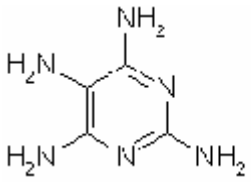
Annex 1

1. Nomenclature/Identification of 2,4,5,6-Tetraaminopyrimidine and its salts



2,4,5,6-Tetraaminopyrimidine
$C_4H_8N_6$
CAS : 1004-74-6
EINECS : /

According to ECB/JRC

Pyrimidinetetrayltetraamine sulfate
$C_4H_8N_6 .H_2SO_4$ (free base with 1 H_2SO_4)
CAS : 5392-28-9
EINECS : 226-393-0

Pyrimidinetetraamine sulfate
$C_4H_8N_6 .xH_2SO_4$ (free base with x H_2SO_4)
CAS : 49647-58-7
EINECS : 256-407-0

According to CTFA

2,4,5,6-Tetraaminopyrimidine sulfate
$C_4H_8N_6 .xH_2SO_4$ (free base with x H_2SO_4)
CAS : 5392-28-9
EINECS : 226-393-0
Synonym : Pyrimidinetetrayltetraamine sulfate

According to Aldrich-Sigma (Laboratory Chemical Supplier)

2,4,5,6-Tetraaminopyrimidine sulfate salt, and
2,4,5,6-Tetraaminopyrimidine sulfate salt hydrate
CAS : 5392-28-9

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2,4,5,6-Tetraaminopyrimidine
CAS : 49647-58-7 (as sulfate), 39944-62-2 (as chloride)
Synonym : 2,4,5,6-Tetraaminopyrimidine *(H_2)*sulfate hemihydrate *($C_4H_8N_6 .H_2SO_4.0.5 H_2O$)*

Molecular weight
140.15 (as free base)
236.21 (as sulfate) - corresponding to empirical formula $C_4H_8N_6 .O_4S$
211.05 (as dichloride) - corresponding to empirical formula $C_4H_8N_6 .Cl_2$

Conclusion
An ambiguous identification of the compounds is presented.

2. Chemical and physical properties

Odourless pale yellow crystals : it is not identified whether these properties describe the free base or its salts.
The Log P_{ow} of the test substances is not reported.

3. Purity

Several batches of 2,4,5,6-Tetraaminopyrimidine and its salts have been used for the safety evaluation studies. Purity by HPLC of only one batch (batch no. not identified) of 2,4,5,6-tetraaminopyrimidine *(H_2)*sulfate *(hemihydrate)* has been reported. The UV-detection is performed only at 207 nm. The UV detection should also have been performed at 274 nm. The HPLC chromatogram showing 99.7% peak area of the test compound is not convincing. A better chromatography with UV detection at relevant wavelengths is required. The purity is based on the peak area count, absolute purity of the test compound is not reported.

No information on impurity of other organic compounds (reagents and intermediates), residual solvent content, metal content and ash content is provided.

3. Solubility

Quantitative information on the solubility of the test substances is not given. No information is provided on the solubility of 2,4,5,6-tetraaminopyrimidine in the receptor fluid used for percutaneous absorption.

4. Stability

No data on stability of the compound is provided.