



Scientific Committee on Consumer Safety

SCCS

OPINION ON

2,6-Diaminopyridine

COLIPA n° A136

The SCCS adopted this opinion at its 18th plenary meeting
of 26 February 2013

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCS

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

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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1. BACKGROUND

Submission I for 2,6-Diaminopyridine was submitted in December 2006 by a Japanese company through COLIPA¹.

The Scientific Committee on Consumer Safety (SCCS) adopted at its 14th plenary meeting on the 27th of March 2012 the opinion (SCCS/1450/11) with the following conclusion:

"The SCCS is of the opinion that the information submitted is insufficient to allow a final risk assessment to be carried out.

Before any further consideration, the following information must be submitted:

- data on the characterisation and quantification of the test materials using state of the art methods."

Submission II for 2,6-Diaminopyridine was submitted in October 2012 .

According to the applicant the submission II includes studies on the characterisation and quantification of the test material and the exposure evaluation was conducted based on the result.

2. TERMS OF REFERENCE

1. *Does the SCCS consider 2,6-Diaminopyridine safe for consumers when used as an ingredient in oxidative hair dye products with a maximum concentration of 0.15% on the scalp, taken into account the scientific data provided?*
2. *And/or does the SCCS recommend any further restrictions with regard to the use of 2,6-Diaminopyridine in any hair dye formulations?*

¹ COLIPA –COSMETICS EUROPE : European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2,6-Diaminopyridine (INCI name)

3.1.1.2. Chemical names

2,6-diaminopyridine
Pyridine-2,6-diamine

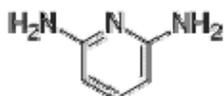
3.1.1.3. Trade names and abbreviations

2,6-Diaminopyridine

3.1.1.4. CAS / EC number

CAS: 141-86-6
EC: 205-507-2

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₅H₇N₃

3.1.2. Physical form

Slightly Brown powder

3.1.3. Molecular weight

Molecular weight: 109.13 g/mol

3.1.4. Purity, composition and substance codes

Lot No. A1426, A3221, A9554

Chemical characterisation of 2,6-Diaminopyridine was performed by IR and NMR spectroscopy

Nitrogen content A1426=96.42%, A3221=97.76%, A9554= 97.5%

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Content of 2,6-diaminopyridine in Lot No. A1426, A3221 and A9554 determined by HPLC (detection 240 nm) using a 99.9% pure reference standard of 2,6-diaminopyridine were as described below:

Lot No. A1426: 100.45%
 Lot No. A3221: 99.98%
 Lot No. A9554: 99.41%

Lot A3221

Content: 99.7% was assayed by potentiometric titration using perchloric acid, single experiment

3.1.5. Impurities / accompanying contaminants

Impurities (in all 3 batches)

2-aminopyridine: < 20 ppm (determined by GC-MS, using reference standard)
 4-aminopyridine: < 40 ppm (determined by GC-MS, using reference standard)
 Unknown (7-16 GC peaks): < 1500 ppm (determined by GC, and calculated as concentration relative to 2,6-diaminopyridine)

Heavy Metals

Fe: < 20 ppm
 Pb: < 20 ppm
 As: < 2 ppm

3.1.6. Solubility

1g was dissolved in 10 ml water, 10 ml 95% ethanol and 1 ml DMSO.

Comment

Water solubility has not been determined by EC Method A.6.

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: calculated Log P_{ow} at 25°C and various pH is described

Comment

Log P_{ow} has not been determined by EC Method A.8

3.1.8. Additional physical and chemical specifications

Melting point: 122.1-122.6 °C
 Boiling point: 285 °C
 Flash point: 155 °C
 Vapour pressure: /
 Density: /
 Viscosity: /
 pKa: /
 Refractive index: /
 pH: /
 UV_Vis spectrum (200-800 nm): λ_{max} 245±2 nm and 309±2 nm

3.1.9. Homogeneity and Stability

0.3 mg/ml and 3 mg/ml solutions of 2,6-diaminopyridine solutions in water were shown to be stable (variation <5%) when stored at room temperature for 8 days.

General Comments to physico-chemical characterisation

- Water solubility has not been determined by EC Method A.6.
- Log Po/w has not been determined by EC Method A.8.
- Stability of 2,6-diaminopyridine has not been demonstrated in typical hair dye formulations.

3.2. Function and uses

2,6-diaminopyridine is used in oxidative hair colouring products at a maximum concentration of 0.3%, which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use, corresponds to a concentration of 0.15% upon application.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: OECD 423 (2001)
 Species/strain: rat, Sprague-Dawley [CrI:CD (SD), SPF]
 Group size: 9 female rats (3 animals per step)
 Test substance: 2,6-diaminopyridine
 Batch: A3221
 Purity: 99.7%
 Vehicle: distilled water
 Dose levels: 50 (step 2 and 3) and 300 mg/kg bw (step 1)
 Administration: oral (gavage)
 GLP: in compliance
 Study period: 14 March – 13 April 2006

A single dose acute oral toxicity test was performed in female rats using the acute toxic class method. The test substance dissolved in water was administered at 300 mg/kg bw to 3 animals (step 1). At step 2 and 3, 50 mg/kg bw was given. Animals were observed for 15 days. Body weight of animals was determined just before the administration and on days 2, 4, 8, 11 and 15 during the observation period. All study animals were autopsied after death.

Results

The animals administered with 300 mg/kg of the test item showed extreme salivation, tremor, clonic and tonic convulsions, and died within approximately 1 hour. Following the dose of 50 mg/kg bw salivation, paleness of skin and ptosis were observed. These changes were absent 4 hours after the administration; thereafter, any abnormalities were not observed from the day after administration to the day of autopsy. Body weight gain of 4/6 animals of the 50 mg/kg bw group was normal.

Conclusion

The LD₅₀ was between 50-300 mg/kg bw.

Ref.: 2

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: OECD 404 (2002)
 Species/strain: rabbit, Kbl:NZW (SPF)
 Group size: 4 females
 Test substance: 2,6-diaminopyridine
 Batch: A3221
 Purity: 99.7%
 Vehicle: distilled water
 Dose level: 3% (w/v) test substance in water
 Dose volume: 0.5 mL
 Observation: 1, 24, 48, and 72 hours after the removal of patches
 GLP: in compliance
 Study period: 4 – 7 April 2006

A skin irritation study using rabbits was performed to investigate the irritative and corrosive potential of a 3% 2,6-diaminopyridine in water solution. A 0.5 mL volume of 3% test substance in water solution was applied to a lint patch and this patch was affixed to the dorsal skin of 3 New Zealand white female rabbits for 4 hours. Skin responses and general condition of animals were observed at 1, 24, 48, and 72 hours after the removal of patches. Animal body weights were measured just before the application and 72 hours after the removal of patches (at the end of the observation period).

Results

No abnormality was observed in any of the 3 application sites at 1, 24, 48, and 72 hours after removal of patches. Therefore, a 3% 2,6-diaminopyridine in water solution was judged to be a non irritant.

Conclusion

From the above results, a 3% 2,6-diaminopyridine in water solution was judged to be a non irritant to the skin of the rabbits under the conditions in this study.

Ref.: 3

3.3.2.2. Mucous membrane irritation

Guideline: OECD 405 (2002)
 Species/strain: rabbit, Kbl:NZW (SPF)
 Group size: 6 females (irrigation group: 3 animals; non-irrigation group: 3 animals)
 Test substance: 2,6-diaminopyridine
 Batch: A3221
 Purity: 99.7%
 Vehicle: distilled water
 Dose level: 3% (w/v) test substance in water
 Dose volume: 0.1 mL
 Observation: 1, 24, 48, and 72 hours after application
 GLP: in compliance
 Study period: 4 – 7 April 2006

An eye irritation study using rabbits was performed to investigate the irritation and corrosive potential of a 3% 2,6-diaminopyridine in water solution. A 0.1 mL volume of 3% test substance in water solution was applied to eyes of 6 female New Zealand white rabbits. Three rabbits for a non irrigation group and irrigation group were used to investigate an irrigation effect. Eye responses and general condition of animals were observed at 1, 24, 48, and 72 hours after application.

Results

In non irrigation group, slight redness of conjunctivae was observed in only one eye at 1 hour after application. No abnormality was observed in the other eyes in this group during the observation period. There was no obvious difference between non irrigation group and irrigation group, because no irritable responses were observed at any eye in irrigation group.

Conclusion

From the above results, a 3% 2,6-diaminopyridine in water solution was judged by the study authors to be a non irritant or mild irritant to the eye of the rabbits under the conditions of this study. The study was unable to confirm an irrigation effect from the results due to a very mild irritation of a 3% 2,6-diaminopyridine in water solution.

Ref.: 4

Comment

A 3% 2,6-diaminopyridine in water solution was slightly irritant.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species/strain:	mouse, CBA/JNCRlj [SPF]
Group size:	20 females (4 animals per dose group)
Test substance:	2,6-diaminopyridine
Batch:	A3221
Purity:	99.7%
Vehicle:	acetone: olive oil (4:1)
Concentration:	0, 0.25, 0.50 and 1.0% w/v
Positive control:	α -hexylcinnamaldehyde, 25% v/v
GLP:	in compliance
Study period:	5 – 11 April 2006

The mouse local lymph node assay (LLNA) was conducted to evaluate the skin sensitizing potential of 2,6-diaminopyridine by using 20 female CBA/JNCRlj [SPF] mice. This study consisted of 5 groups, including 3 doses of 2,6-diaminopyridine, no-treatment, and 25% v/v α -hexylcinnamaldehyde as positive control. Each group consisted of 4 mice.

Results

The SI (stimulation index) values for 0.25% w/v, 0.5% w/v, and 1% w/v 2,6-diaminopyridine groups were 3.4, 3.4, and 5.3, respectively. The SI value of the positive control group was 18.6.

Conclusion

On the basis of these results, it was concluded that 2,6-diaminopyridine was a strong sensitizer under the conditions of the present study, and the concentration of the test substance which induced an SI value of 3 was estimated to be 0.25% w/v.

Ref.: 5

Comment

An EC3 value of 0.09% was derived from the data. 2,6-diaminopyridine is an extreme sensitiser.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428 (2004)
Species/strain:	pig ear skin (6 females)
Membrane integrity:	tritiated water
Group size:	6 chambers
Method:	Franz-type horizontal static cells (1.77 cm ²)
Test substance:	2,6-diaminopyridine [¹⁴ C]-2,6-diaminopyridine
Batch:	A3221 3551167 (radiolabelled)
Purity:	99.7% 98.8% (HPLC) (radiolabelled)
Test item:	hair dye formulation containing 0.147% 2,6-diaminopyridine
Dose volume:	100 µL (84.7 µg/cm ² 2,6-diaminopyridine)
Receptor fluid:	phosphate buffered saline
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	8 – 24 February 2006

To clarify *in vitro* percutaneous penetration of 2,6-diaminopyridine, the radioactivity of receptor fluid and the mass balance were investigated using pig skin with Franz-type cells after the application of a typical oxidative hair dye formulation containing ¹⁴C-2, 6-diaminopyridine.

Results

The skin was wiped off using cotton after 30 minutes of exposure, and the radioactivity of the cotton was 97.70% of the applied dose. The radioactivity of the receptor fluid, donor chamber washing, terminal skin rinse (at 24 hours), filter paper (the moisture of the skin), corneum (tape strips), epidermal and dermal membranes, and flange skin was 0.24%, 0.14%, 0.06%, 0.01%, 0.68%, 0.74% and 0.01%, respectively, resulting in 99.57% of total recovery.

The percentage of applied dose in receptor fluid at 2, 4, 6, 8 and 24 hours after application of the hair dye formulation containing ¹⁴C-2,6-diaminopyridine was 0.02%, 0.08%, 0.12%, 0.15% and 0.24%, respectively. The permeability of ¹⁴C-2,6-diaminopyridine at 2, 4, 6, 8 and 24 hours after application was 0.01, 0.07, 0.10, 0.12 and 0.20 µg eq./cm², respectively.

Table 1: Percentage of applied dose in receptor fluid and skin after application of hair dye formulation containing 0.147% 2,6-diaminopyridine at 100 µL/1.77 cm² to pig skin for 30 minutes

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Sample	% of applied dose (Permeability, $\mu\text{g eq./cm}^2$)
Receptor fluid	0.24 \pm 0.05 (0.20 \pm 0.04)
Donor chamber	0.14 \pm 0.07
Cotton	97.70 \pm 1.11
Rince	0.06 \pm 0.04
Filter paper	0.01 \pm 0.01
Corneum	0.68 \pm 0.22
Epidermis and dermis	0.74 \pm 0.18 (0.62 \pm 0.15)
Flange	0.01 \pm 0.00
Total recovery	99.57 \pm 1.08
Absorption ratio	0.98 \pm 0.23 (0.82 \pm 0.18)

Each value represents the mean \pm S.D. of six pig skins.

Absorption ratio was calculated by the sum of percentage receptor fluid, epidermis and dermis.

Table 2: Individual percentage of applied dose in receptor fluid and skin after application of hair dye formulation containing 0.147% 2,6-diaminopyridine at 100 $\mu\text{L}/1.77\text{ cm}^2$ to pig skin for 30 minutes

Sample	Animal No.	% of applied dose (Permeability, $\mu\text{g eq./cm}^2$)					
		1	6	7	8	9	10
Receptor fluid		0.19 (0.16)	0.32 (0.26)	0.29 (0.24)	0.25 (0.20)	0.21 (0.18)	0.19 (0.16)
Donor chamber		0.15	0.02	0.11	0.24	0.18	0.11
Cotton		97.21	96.91	99.14	96.63	99.06	97.25
Rince		0.12	0.02	0.02	0.05	0.06	0.09
Filter paper		0.01	0.01	0.01	0.00	0.01	0.00
Corneum		0.64	0.39	0.68	0.62	0.64	1.08
Epidermis and dermis		0.84 (0.70)	0.94 (0.78)	0.80 (0.66)	0.82 (0.69)	0.62 (0.52)	0.43 (0.36)
Flange		0.01	0.01	0.02	0.01	0.01	0.01
Total recovery		99.17	98.62	101.07	98.62	100.79	99.16
Absorption ratio		1.03 (0.86)	1.26 (1.04)	1.09 (0.90)	1.07 (0.89)	0.83 (0.70)	0.62 (0.52)

Absorption ratio was calculated by the sum of percentage receptor fluid, epidermis and dermis.

After application of the hair dye formulation, the absorption ratio of 2, 6-iaminopyridine was calculated to be 0.98% by a sum of receptor fluid (0.24%) and epidermis and dermis (0.74%), corresponding to $0.82 \pm 0.18\ \mu\text{g eq./cm}^2$.

Ref.: 14

Comment

The authors justified the use of $56.5\ \mu\text{L}/\text{cm}^2$ instead of $10\ \mu\text{L}/\text{cm}^2$ based on the fact that the hair dye had to be washed out completely at 30 or 40 min after application.

The thickness of the membrane is not reported. Only 6 chambers were used.

Mean + 2 SD should be used to calculate MoS ($1.18\ \mu\text{g}/\text{cm}^2$).

3.3.5. Repeated dose toxicity

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

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity
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Guideline:	OECD 408 (1998)
Species/strain:	rat, Sprague-Dawley [CrI:CD (SD), SPF]
Group size:	50 males and 50 females
	Toxicity study: 10 males and 10 females per dose group
	Recovery study (0, 30 mg/kg bw/d): 5 males and 5 females per dose group
Test substance:	2,6-diaminopyridine
Batch:	A3221
Purity:	99.7%
Vehicle:	distilled water
Dose levels:	0, 3, 10 and 30 mg/kg bw/d
Dose volume:	1 mL per 100 g bw
Route:	oral, gavage
Administration:	once daily for 92 consecutive days, 29 days recovery period
GLP:	in compliance
Study period:	13 September 2005 – 31 March 2006

A 90-day repeated oral dose toxicity study of 2,6-diaminopyridine in CrI:CD(SD) rats was conducted. Ten male and 10 female rats were assigned to each of four groups, a total of 80 rats were used in the study. 2,6-Diaminopyridine at doses of 0, 3, 10, and 30 mg/kg bw (water for injection was used as the vehicle) was orally administered to rats for each dose daily for 92 consecutive days. Animals were observed for general condition, body weight and food consumption were measured, and the functional observational battery (FOB) was conducted. In addition, urinalysis and ophthalmological examinations were conducted at week 13 of dosing. Examination of haematology, blood coagulation, blood chemistry, organ weights, and pathology were conducted at the end of the administration period. Five males and 5 females each in the satellite groups (control and 30 mg/kg bw/d groups) were observed for 29 days after cessation of the dosing of the test substance and were examined for recovery from toxic changes.

Results

No animals died during the administration or recovery period. As clinical signs, lacrimation, salivation, and ptosis of the eyelids were observed in both males and females given 10 mg/kg or more. The body weights in males in the 30 mg/kg bw/d group decreased during the administration period. The food consumption in both males and females in the 30 mg/kg bw/d group decreased in the early administration period. In the detailed observation on the functional observational battery (FOB), the number of animals showing salivation increased in both males and females in the 10 and 30 mg/kg bw/d groups. Decreased spontaneous motor activity was observed in males in the 10 and 30 mg/kg bw/d groups. The number of females sitting in the open field in the 30 mg/kg bw/d group increased. The number of rearing behaviour in females in the 30 mg/kg bw/d group decreased.

The haematocrit concentrations, haemoglobin concentrations, and red blood cell counts in males or females, or both in the 30 mg/kg bw/d group decreased, the MCV, MCH, and reticulocyte ratios in these animals increased. These findings suggested anaemia. In addition, the lymphocyte counts in males in the 30 mg/kg bw/d group increased. High reticulocyte counts in males in the 3 mg/kg bw/d group were noted but not judged to be test substance related because there were no changes in any of the other haematological parameters and no dose-response relationship. No toxic changes caused by the test substance were observed in the examinations of blood coagulation and clinical biochemistry. In urinalysis, the urine volumes in males in the 10 and 30 mg/kg bw/d groups increased, and the osmotic pressures in these animals decreased. The number of males having leukocytes in the urine sediments in the 10 and 30 mg/kg bw/d groups and the number of males having renal tubular epitheliocytes in the urine sediments in the 30 mg/kg bw/d group increased. The urine volumes in females in the 10 and 30 mg/kg bw/d groups increased, and the osmotic pressures in these animals decreased. The number of females

showing a positive reaction for urinary glucose and/or urinary protein in the 30 mg/kg bw/d group increased and the number of females showing a positive reaction for bilirubin in the 10 and 30 mg/kg bw/d groups increased. Furthermore, the number of females having renal tubular epitheliocytes in the urine sediments in the 10 and 30 mg/kg bw/d groups increased. In ophthalmology, no effects related to the administration of the test substance were observed. The kidney weights in both males and females in the 10 and 30 mg/kg bw/d groups increased, the liver weights in females in the 10 and 30 mg/kg bw/d groups increased, and the spleen weights in females in the 30 mg/kg bw/d group also increased.

In pathology, single cell necrosis of renal tubular epithelium and regeneration of epithelium in the proximal tubule (P3 segment) was observed in many male and female animals in the 10 and 30 mg/kg bw/d groups, and it was moderate in grade in 1 male in the 30 mg/kg bw/d group and in some females in the 10 and 30 mg/kg bw/d groups, more severe in females. Moderate or severe hyaline droplet in the proximal tubule was observed in males in the 10 and 30 mg/kg bw/d groups. In addition, brown pigment deposition in the proximal tubule was observed in females in the 30 mg/kg bw/d group. As treatment-related gross findings in the spleen, dark-coloration was observed in females in the 30 mg/kg bw/d group. Histopathologically, increased pigment deposition and increased extramedullary haematopoiesis were observed in both males and females in the 10 and 30 mg/kg bw/d groups; it was moderate in grade in females in the 30 mg/kg bw/d group. With these lesions of the spleen, congestion was observed in males in the 30 mg/kg bw/d group and females in the 10 and 30 mg/kg bw/d groups. A type of anaemia observed in the 30 mg/kg bw/d group was judged to be haemolytic anaemia. As histopathological hepatic findings, eosinophilic change of hepatocyte was observed in males in the 10 and 30 mg/kg bw/d groups and females in the 30 mg/kg bw/d group. Centrilobular hepatocellular hypertrophy was observed in 1 female in the 30 mg/kg bw/d group. In the recovery study, dark spleen was grossly observed in 3 females in the 30 mg/kg group, and increased pigment deposition was histopathologically observed in 4 females in the 30 mg/kg bw/d group. The treatment-related histopathological findings observed in the kidney, liver, and spleen in the toxicity study disappeared or the degree of pigment deposition of the spleen decreased in the recovery study. Therefore, treatment-related histopathological findings in the toxicity study were considered to be reversible changes. The kidney, erythrocyte, and liver were target organs for the test substance.

Conclusion

The results from this study clearly demonstrated the 90-day repeated oral dose toxic effects of 2,6-diaminopyridine at 10 and 30 mg/kg bw/d on the Crl:CD(SD) rats. From these results, the no observed adverse effect level was judged to be 3 mg/kg bw/d in both males and females. The animals recovered from most toxic changes after the 4-week substance withdrawal. The erythrocytes, kidney, spleen, and liver were target organs for 2,6-diaminopyridine induced toxicity.

Ref.: 6

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline: OECD 471 (1997)
 Species/Strain: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537; *E. coli* WP2 uvrA

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Replicates: Triplicate plates in two independent studies.
 Treatment: preincubation method
 Test substance: 2,6-diaminopyridine
 Batch: A3221
 Purity: 99.7%
 Vehicle: Distilled water
 Concentration: 0, 313, 625, 1250, 2500, 5000 µg/plate without and with S9-mix
 Positive control: without S9-mix:
 AF-2 (2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide)
 SA (sodium azide)
 9AA (9-aminoacridine)
 With S9-mix
 2AA (2-aminoanthracene)
 GLP: in compliance
 Study period: 27 February – 16 March 2006

2,6-diaminopyridine was investigated for the induction of gene mutations in bacteria with and without phenobarbital/5,6-benzoflavone induced rat liver S9-mix. A preliminary toxicity study with 8 concentrations from 1.5 to 5000 µg/plate was performed. The concentrations in the main tests were based on the results from the initial toxicity study, where no growth inhibition was observed.

Results

No toxicity, measured as growth inhibition of tester strains, was observed in any strains used with or without S9-mix. No precipitation derived from the test item was observed at any concentration tested with or without S9-mix.

The number of revertants did not increase more than twice that of the negative control in any strains used with or without S9-mix in the duplicate mutagenicity tests.

Conclusion

Based on the results in this study and under the experimental conditions performed it is concluded the 2,6-diaminopyridine did not induce gene mutations in bacteria.

Ref.: 7

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

Guideline: OECD 476 (1997)
 Species/strain: mouse lymphoma cell line (L5178Y *tk*^{+/-})
 Replicates: One single culture in two independent experiments
 Test substance: 2,6-diaminopyridine
 Batch: A3221
 Purity: 99.7%
 Vehicle: Sterile water
 Treatment: Experiment I: 3 h treatment without and with S9-mix; expression period 48 h and selection period of 12-14 days
 Experiment II: 24 h treatment without S9-mix; expression period 48 h and selection period of 12-14 days
 Concentrations: short term without S9-mix
 18.3, 30.5, 50.9, 84.8, 141, 236, 393, 655, 1091 µg/mL
 short term with S9-mix
 0.855, 1.42, 2.37, 3.96, 6.60, 11.0, 18.3, 30.5, 50.9, 84.8 µg/mL
 continuous treatment, 24h
 6.60, 11.0, 18.3, 30.5, 50.9, 84.8, 141, 236, 393, 655 µg/mL
 Positive control: Methyl methanesulfonate (MMS): without S9-mix
 Cyclophosphamide with S9-mix
 GLP: in compliance

Study period: 20 February – 28 March 2006

A136 was tested for the induction of gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of phenobarbital/5,6-benzoflavone induced rat liver S9-mix. Test concentrations were based on the results of a pre-test on toxicity with exposure up to the prescribed maximum concentration of 10 mM ($\approx 1091 \mu\text{g/ml}$) measuring relative suspension growth. Toxicity was measured in the main experiments as percentage relative total growth (RTG) of the treated cultures relative to the total growth of the solvent control cultures. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. Negative and positive controls were in accordance with the OECD guideline.

Results

No precipitation of the test substance was observed at any concentrations or treatments. In the first experiment without S9-mix toxicity was not below 10% at any tested concentrations, and (all) 9 concentrations were evaluated. In the other experiments severe toxicity (RTG below 10%) was observed at the highest tested concentration(s) and 8 (with S9-mix and 3 h exposure) or 9 (24 h without S9-mix) concentrations were evaluated. In all experiments a clear and concentration related increase in mutant frequency was observed. There was no significant increase in small colonies in any of the experiments, indicating a mutagenic but not a clastogenic effect of the test substance.

Conclusion

Under the experimental conditions performed A136 induced gene mutations in mammalian cells in this *in vitro* assay.

Ref.: 8

***In vitro* Mammalian Chromosome Aberration Test**

Guideline:	OECD 473 (1997)
Species/strain:	cultured Chinese Hamster Lung cells (CHL/IU)
Replicates:	Two cultures in two independent tests
Test item:	2,6-diaminopyridine
Batch:	A3221
Purity:	99.7%
Vehicle:	water
Concentrations:	short term (6 h) without S9-mix 0.28, 0.55, 1.1 mg/mL short term (6 h) with S9-mix 0.14, 0.28, 1.1 mg/mL continuous treatment, 24h 0.069, 0.14, 0.28 mg/mL
Performance:	6 h or 24 h treatment without S9-mix; harvest time 24 h after the start of treatment 6 h treatment with S9-mix; harvest time 24 h after start of treatment
Positive controls:	mitomycin C cyclophosphamide
GLP:	in compliance
Study period:	24 February – 19 April 2006

A136 was tested for the induction of chromosomal aberrations in Chinese hamster female lung cells both with and without phenobarbital/5,6-benzoflavone induced rat liver S9-mix. A preliminary toxicity test was performed in order to determine the concentration range for the cytogenetic test. Based on the results from this pre-test the highest concentration selected for evaluation was 1.1 mg/mL ($\approx 10 \text{ mM}$).

Results

No precipitation was observed at any concentration tested. No toxicity was observed after short term treatment without S9-mix. After short term treatment with S9-mix and continuous treatment for 24 h a dose dependant decrease in cell growth was observed. However, an unexpected but reproducible increase in cell growth was observed at the highest concentration with S9-mix, resulting in a U-shaped concentration-response curve. This was the reason for the concentration selection with S9-mix (omitting the most toxic concentration of 0.55 µg/mL). Although, there was a statistical significant increase in structural chromosomal aberrations after short term treatment with S9-mix at all evaluated concentrations (percentage of cells with aberrations excluding gaps: 24%, 51% and 9% at 0.14, 0.28 and 1.1 mg/mL respectively, compared to 1% in the negative control and 44% in the positive control), the trend test was negative. No statistical significant increase in cells with chromosomal aberrations was observed without S9-mix after short term treatment. After continuous treatment without S9-mix a statistically significant increase in structural chromosomal aberrations was observed at the highest tested concentration. No increase in numerical aberrations was observed.

Conclusion

Under the experimental conditions performed A136 was clastogenic in mammalian cells.

Ref.: 9

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline:	OECD 474 (1997)
Species/strain:	ICR mice [Crj:CD-1 (ICR), SPF]
Group size:	5 male mice per dose group
Test substance:	2,6-diaminopyridine
Batch:	A3221
Purity:	99.7%
Vehicle:	0.5% methylcellulose solution
Dose level:	0, 7.5, 15, 30 mg/kg bw/d 50 mg/kg bw/d (cyclophosphamide)
Route:	oral
Administration:	2 dosings at an interval of 24h Positive control: 1 dosing
Positive control:	cyclophosphamide monohydrate
GLP:	in compliance
Study period:	2 June – 30 July 2004

The clastogenic/aneugenic activity of A136 was measured in the micronucleus assay in mice after oral administration. Preliminary toxicity tests were carried out to obtain the maximum tolerated dose (MTD), and a main micronucleus test was carried out based on the results of preliminary toxicity tests.

The first preliminary toxicity test was planned to administer orally to male and female mice twice at 24-hour intervals, at doses of 250, 500, 1000 and 2000 mg/kg bw/day of 2,6-diaminopyridine. However, the second dosing was not carried out, since all the mice in the groups of 500 mg/kg bw/day and more died shortly after the first dosing, and mice in the group of 250 mg/kg bw/day (males and females) died within 24 hours after the first dosing. Since the MTD was not obtained from the first preliminary toxicity test, the second preliminary toxicity test was carried out at five doses of 7.81, 15.6, 31.3, 62.5 and 125 mg/kg bw/day. As no difference was observed in the time of death between males and females in the first preliminary toxicity test, it was judged that there was no marked difference in toxicity between the two sexes. Therefore, only male mice were used in the

second preliminary toxicity test. As a result, death was observed in the groups of 62.5 mg/kg bw/day and more; therefore, the MTD was set at 31.3 mg/kg bw/day. Based on preliminary toxicity tests, the main micronucleus test was carried out using male mice by 2 oral administrations at 24-hour intervals with dose levels of 7.5, 15 and 30 mg/kg bw/day of 2,6-diaminopyridine. Smear specimens of bone marrow cells were prepared 24 hours after the last dosing, and microscopic observation was carried out.

Results

In the main test toxicity was observed as a decrease in locomotor activity at the highest dose tested. No other sign of toxicity was observed at any tested doses, although the substance was tested up to MTD.

2,6-diaminopyridine did not induce a statistical significant or biological relevant increase of micronucleated polychromatic erythrocytes (MNPCEs). The proportion of polychromatic erythrocytes in erythrocytes in any 2,6-diaminopyridine dosed group or the positive control group was not significantly different from that in the negative control group. Thus, suppression of bone marrow cell proliferation by administration of the test item was not observed. However, there was some toxicity at the highest tested dose, indicating systemic availability. In addition in the 90 days study in rats, there was a decrease in red blood cell counts at 30 mg/kg bw/day. This effect may also indicate that the substance was systemically available. The frequency of MNPCEs was significantly increased at a level of 1% in the positive control group (cyclophosphamide monohydrate 50 mg/kg bw).

Conclusion

It was concluded that 2,6-diaminopyridine has neither a clastogenic nor an aneugenic potential in bone marrow cells of mice under these experimental conditions.

Ref.: 10

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*

Guideline:	OECD 486 (1997)
Species/strain:	rat, Crj: CD (SD) IGS [SPF]
Group size:	Four male rats for each of the 8 test groups
Test substance:	2,6-diaminopyridine
Batch:	A3221
Purity:	99.7%
Vehicle:	water for injection
Dose level:	0, 120, 240 mg/kg bw
Dosing volume:	1 mL per 100 g bw
Route:	oral
Administration:	2 dosings at an interval of 24h
Control:	dimethylnitrosamine (DMN), 5 mg/kg bw, 2-hour treatment group 2-acetylaminofluorene (2AAF), 100 mg/kg bw, 16-hour treatment group
Treatment:	2 and 16h treatment
GLP:	in compliance
Study period:	10 December 2003 – 19 February 2004

An *in vivo/in vitro* unscheduled DNA synthesis assay with rat hepatocytes was conducted to examine the DNA damaging potential of 2,6-diaminopyridine.

As a result of a dose-finding study, 2 deaths in the highest dose group of 480 mg/kg bw were observed until 16 hours after administration. No death was observed in the dose groups of 240 mg/kg bw or less. Therefore, a single oral dose was used to compare a 240 mg/kg dose, which was considered to be near to the maximum tolerated dose (MTD), with a 120 mg/kg bw dose (as a low dose) in this assay.

Hepatocytes were isolated by the collagenase perfusion method at 2 or 16 hours after administration, and cultured. Thereafter, the nuclear grain count and cytoplasm grain count were determined.

Results

The mean net nuclear grain (NNG) counts of the 2,6-diaminopyridine treatment groups were comparable to those of the negative control group. The incidence of cells in repair (UDS positive) in the treatment groups was also comparable to that of the negative control group, showing no remarkable trend of increase.

In two positive control groups, i.e., in a group treated with dimethylnitrosamine (DMN: 5 mg/kg dose) for 2 hours and in a group treated with 2-acetylaminofluorene (2AAF: 100 mg/kg dose) for 16 hours, the hepatocytes of all rats showed large counts of NNG and high DNA repair cell appearance frequencies, indicating a positive reaction.

Conclusion

It was concluded that 2,6-diaminopyridine did not have DNA damaging potential under the test conditions used.

Ref.: 11

3.3.7. Carcinogenicity

In vitro Syrian Hamster Cells (SHE) Transformation Assay

Guideline:	/
Species/strain:	Syrian Hamster Embryo (SHE) cells
Test substance:	2,6-diaminopyridine
Batch:	A3221
Purity:	99.7%
Vehicle:	DMSO
Concentrations:	200, 400, 500, 550, 575, 600, 625 µg/mL
Positive control:	benzo[a]pyrene, 5 µg/mL
GLP:	in compliance
Study period:	2 July 2004 – 5 March 2005

Cryopreserved cell stocks of both target and feeder SHE cells were prepared from embryo cells derived from time-pregnant Syrian golden hamster at 13 to 13.5 days of gestation. The cells were kept in liquid nitrogen. The SHE cells were cultured in LeBoeuf's modification of Dulbecco's Modified Medium (DMEM) supplemented with 20% fetal bovine serum (FBS) and 4 mM L-glutamine. The sodium bicarbonate content of the medium was formulated to maintain a pH of 6.65 – 6.75. The cells were cultured at 37±1 °C in humidified air containing 10±0.5% CO₂. Sufficient target SHE cells expected to generate 25 – 45 colonies/dish were seeded in 2 ml of culture dishes containing 2 ml of culture medium and approximately 4x10⁴ feeder SHE cells (previously X-irradiated sufficiently to prevent replication) that were seeded 24 hours earlier. 45 dishes were prepared for each concentration of 2,6-diaminopyridine and control groups. The cultures were incubated for a period of 7 days.

While the positive control, benzo(a)pyrene, induced a significant number of morphologically altered colonies, none of the concentrations of 2,6-diaminopyridine caused a significant number of morphologically transformed colonies.

It was concluded the 2,6-diaminopyridine did not induce transformation of SHE cells.

Ref.: 12

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline: OECD 414 (2001)
 Species/strain: rat, Sprague-Dawley [CrI:CD(SD), SPF]
 Group size: 25 females per dose group
 Test substance: 2,6-diaminopyridine
 Batch: A3221
 Purity: 99.7%
 Vehicle: water for injection
 Dose levels: 0, 3, 10, 30 mg/kg bw/d
 Dose volume: 1 mL per 100 g bw
 Route: oral (gavage)
 Administration: daily during days 5 to 19 of gestation
 GLP: in compliance
 Study period: 26 December 2005 – 8 May 2006

A teratogenicity study with 2,6-diaminopyridine in rats was performed. 2,6-Diaminopyridine at doses of 3, 10, and 30 mg/kg bw (water for injection was used as the vehicle) was orally administered daily to 25 female successfully copulated CrI:CD(SD) rats for each dose during days 5 to 19 of gestation. During the gestation period, the general conditions of the dams were observed, and their body weights and food consumption were measured. Caesarean section and necropsy were performed on day 20 of gestation. The mortality, growth, and morphological abnormalities of the foetuses were recorded.

Results

None of the animals died or aborted during the gestation period. As clinical signs, lacrimation, salivation, and ptosis of the eyelids were observed in the 10 and 30 mg/kg bw/d groups. The test substance at 10 mg/kg bw/d caused a decrease in food consumption, and at 30 mg/kg bw/d decreases in body weight gain and food consumption. The gross post-mortem examinations revealed dark spleen in the 30 mg/kg bw/d group. There were no effects related to the administration of the test substance on the gravid uterus weights.

Placental weights as well as mean foetal weights of both male and female foetuses were significantly lower in the 30 mg/kg bw/d group than those in the control group. There were no effects related to the test substance on the number of corpora lutea graviditatis, number of implantations, number of live foetuses, sex ratios, or number of resorptions and dead foetuses. The placental examination and external and visceral examinations of the live foetuses did not reveal effects related to the test substance. The skeletal examinations did not reveal skeletal malformations or variations related to the test substance in any of the groups but showed incomplete ossification of the thoracic vertebrae in the 30 mg/kg bw/d group. Slight retardation of foetal growth was suggested.

Conclusion

From these results, the no observed adverse effect level (NOAEL) for maternal toxicity was determined to be 3 mg/kg bw/d based on lacrimation, salivation, ptosis of the eyelids, and a decrease in food consumption at 10 mg/kg bw/d. The NOAEL for developmental toxicity in foetuses was determined to be 10 mg/kg bw/d due to foetal growth retardation at 30 mg/kg bw/d (decreases in foetal weights and placental weights, and incomplete ossification of the thoracic vertebrae). Teratogenicity was not observed even at 30 mg/kg bw/d.

Ref.: 13

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

2,6-Diaminopyridine

(oxidative conditions)
(0.3% formulation, on head concentration 0.15%)

Absorption through the skin	A	=	1.18 µg/cm²
Skin Area surface	SAS	=	580 cm²
Dermal absorption per treatment	SAS x A x 0.001	=	0.6844 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.0114 mg/kg bw
No observed adverse effect level (90-day, oral, rat)	NOAEL	=	3 mg/kg bw/d
Bioavailability 50%*		=	1.5 mg/kg bw/d

Margin of Safety	adjusted NOAEL/SED = 132
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* standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

3.3.14. Discussion

Physico-chemical properties

2,6-diaminopyridine is used in oxidative hair colouring products at a maximum concentration of 0.3%, which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use, corresponds to a concentration of 0.15% upon application.

Water solubility of 2,6-diaminopyridine has not been determined by EC Method A.6. Log Po/w has not been determined by EC Method A.8.

Stability of 2,6-diaminopyridine has not been demonstrated in typical hair dye formulations.

General toxicity

In a single dose acute oral toxicity test in female rats using the acute toxic class method the LD₅₀ of 2,6-diaminopyridine was between 50-300 mg/kg bw.

From the results of a 90-day repeated dose oral toxicity study in rats the no observed adverse effect level of 2,6-diaminopyridine was judged to be 3 mg/kg bw/d in both males and females. The erythrocytes, kidney, spleen, and liver were target organs of 2,6-diaminopyridine induced toxicity.

From the results of a developmental toxicity study in rats, the no observed adverse effect level (NOAEL) for maternal toxicity was determined to be 3 mg/kg bw/d based on lacrimation, salivation, ptosis of the eyelids, and a decrease in food consumption at 10 mg/kg bw/d. The NOAEL for developmental toxicity in foetuses was determined to be 10 mg/kg bw/d due to foetal growth retardation at 30 mg/kg bw/d (decreases in foetal weights and placental weights, and incomplete ossification of the thoracic vertebrae). Teratogenicity was not observed even at 30 mg/kg bw/d.

No additional study on reproductive toxicity was provided.

Irritation / sensitisation

A 3% 2,6-diaminopyridine in water solution was non irritant to the skin of the rabbits and slightly irritant to the eye of the rabbits under the conditions in the study.

2,6-diaminopyridine is an extreme sensitiser.

Dermal absorption

The dermal absorption of 2,6-diaminopyridine was investigated after application of a typical oxidative hair dye formulation.

The thickness of the membrane employed was not reported and only 6 chambers were used. The mean + 2 SD (1.18 µg/cm²) was used to calculate MoS.

Mutagenicity / genotoxicity

Overall, 2,6-diaminopyridine was evaluated for the three endpoints of genotoxicity: gene mutations, structural chromosomal aberrations and aneuploidy. The test substance did not induce gene mutations in bacteria, but was mutagenic and clastogenic in mammalian cells. The genotoxic effects observed *in vitro* were not confirmed in two *in vivo* tests: a mouse micronucleus assay on bone marrow, and a rat UDS assay on liver cells. Consequently, there is no need for additional tests.

Carcinogenicity

2,6-diaminopyridine did not induce transformation in the *in vitro* Syrian hamster transformation assay. No *in vivo* carcinogenicity studies have been submitted.

4. CONCLUSION

The SCCS is of the opinion that the use of 2,6-diaminopyridine as oxidative hair dye with a maximum on head of 0.15% does not pose a risk to the health of the consumer, apart from its sensitising potential.

2,6-Diaminopyridine is an extreme sensitiser.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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Data Base Literature Search

Literatures in relation to safety of 2,6-diaminopyridine were researched.

STN International (STN) is the online scientific and technical information service, and provides a complete collection of in-depth databases in science and technology.

STN was used with CAS registry number (automatically including all listed synonyms) to access information related to safety of 2,6-diaminopyridine in the following online databases:

RTECS, HSDB, MEDLINE, EMBASE, BIOSIS, ESBIODATABASE, FSTA, LIFESCI, CSNB, HEALSAFE, ULIDAT, SCISEARCH, NTIS, TOXCENTER

In addition, TOXNET, one of the National Library of Medicine (NLM) databases and the National Toxicology Program (NTP) database were searched.

List of references not included in the safety assessment

The following studies were not included in the safety assessment due to missing information on the specification of the material (identity/quality), inadequate reporting and/or limitations in test performance (not GLP, not standard guideline).

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Submission II, 2012

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