EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

2,6-Diamino-3-((pyridin-3-yl)azo)pyridine

COLIPA n° B111

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 12th plenary meeting

Of 20 September 2011

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.).

Scientific Committee members

Contact
European Commission
Health & Consumers
Directorate D: Health Systems and Products
Unit D5 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-SCCS-Secretariat@ec.europa.eu

ISSN 1831-4767 ISBN 978-92-79-30720-1

Doi:10.2772/98638 ND-AQ-11-013-EN-N

ACKNOWLEDGMENTS

Prof. J. Angerer (rapporteur)
Dr. C. Chambers
Dr. W. Lilienblum
Dr. E. Nielsen
Prof. T. Platzek (chairman)
Dr. S.C. Rastogi
Dr. C. Rousselle
Prof. T. Sanner
Dr. J. van Benthem
Prof. M.P. Vinardell
Dr. I.R. White

External experts

Dr. Mona-Lise Binderup National Food Institute, Denmark

Keywords: SCCS, scientific opinion, hair dye, 2,6-diamino-3-((pyridine-3-yl)azo)pyridine, B111, CAS 28365-08-4, Directive 76/768/EEC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 2,6-diamino-3-((pyridine-3-yl)azo)pyridine, 20 September 2011

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.

Revised opinions carry the date of revision.

TABLE OF CONTENTS

1. BACKGROUND

Submission I for 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine was submitted in March 2003 by COLIPA [1, 2].

Submission II for 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine was submitted by COLIPA in July 2005. According to this submission the substance is used in hair colouring formulations as:

a) a non-reactive hair colouring agent ("direct dye") in non-oxidative hair dye formulations at a maximum on-head concentration of 0.25%. It is common practice to apply 35 to 50 g of the product over a period of 30 minutes followed by rinse off with water and shampoo. The application may be repeated at weekly intervals.

b) a non-reactive hair colouring agent ("direct dye") in oxidative hair dye formulations at a maximum on-head concentration of 0.25%. The colorant component and a developer (hydrogen peroxide) are mixed in ratios between 1:1 to 1:3. It is common practice to apply up to 100 g of the finished mixed product for a period of 30 minutes followed by rinse off with water and shampoo. The application may be repeated at monthly intervals.

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine safe for use as a non-oxidative hair dye with an on-head concentration of maximum 0.25 % taken into account the scientific data provided?*

2. *Does the SCCS consider 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine safe for use as an oxidative hair dye with an on-head concentration of maximum 0.25 % taken into account the scientific data provided.*

3. *Does the SCCS recommend any further restrictions with regard to the use of 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine in any non-oxidative or oxidative hair dye formulations?*

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

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2,6-Diamino-3-((pyridine-3-yl)azo)pyridine

3.1.1.2. Chemical names

2,6-Pyridinediamine, 3-(3-pyridinylazo) (CA INDEX NAME, 9CI)
3-[(E)-pyridinyldiazenyl]-2,6-pyridinediamine (IUPAC)
3-((2,6-Diamino-3-pyridyl)azo)pyridine
2,6-diamino-3,3´-azodipyridine

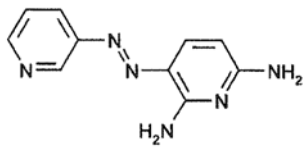
3.1.1.3 Trade names and abbreviations

Azogelb
DA 100491
A005288
COLIPA B111

3.1.1.4 CAS /EC number

CAS: 28365-08-4
EC: 421-430-9

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_{10}H_{10}N_6$

3.1.2 Physical form

Yellow powder

3.1.3 Molecular weight

Molecular weight: 214.23 g/mol

3.1.4 Purity, composition and substance codes

batch	GST 4-11029	L6/186
Purity (HPLC)		

batch	GST 4-11029	L6/186
210 nm	99.9 %	99.9 %
254 nm	100.0 %	100.0 %
434 nm	100.0 %	99.9 %
Purity (NMR)	100.0 %	99.8 %
loss on drying	0.010 %	0.015 %
water content	0.017 %	0.037 %
sulphate ash	0.009 %	0.059 %

The identity and content of the test substances, Batch GST 4-11019 and L6/186 were analysed by means of NMR. For further purity tests HPLC- UV(DAD) was used. Two possible minor components, 3-aminopyridine and 2,6-diaminopyridine, were searched for in these HPLC examinations. Furthermore IR and UV spectra were recorded and the contents of water, loss on drying and sulphated ash were determined.

The reference substances for B111 (R0075; 99.9%), which was registered in accordance with SOP- F01 has been supplied by the applicant.

Identity:
1H-NMR spectra of the test substance were performed in DMSO-d6/MeOD (1:1).
Purity was determined by comparing the integrated signals of the test substance with the spectra of the internal standards, dimethylaminobenzoic acid ethyl ester (DBEE) and thymole (THYM). 1H-NMR spectra confirmed the identity of test substance. The content of B111 was quantified as >99.8%.

Purity and impurities were determined by HPLC at λ=210, 254 and 434 nm. Purity was >99.9%.

UV spectra were recorded from 200-800 nm in ethanol. IR spectra were performed with potassium bromide pellets within the range of 4000-400 cm^{-1}. UV and IR spectra confirmed the proposed structure.

Ref.: 1

3.1.5 Impurities / accompanying contaminants

Batch GST 4-11019 and L6/186 were analysed for impurities by HPLC-DAD.
Impurities of 3-aminopyridine and 2,6-diaminopyridine were determined by HPLC-DAD at 232 and 240 nm respectively against standard solutions of 3-aminopyridine and 2,6-diaminopyridine. 3-Aminopyridine and 2,6-diaminopyridine were not detectable in both samples and therefore were <0.002 % and <0.003 % respectively for both batches according to the specified LOQs.

batch	GST 4-11029	L6/186
3-aminopyridine	< 0.002 %	< 0.002 %
2,6-diaminiopyridine	< 0.003	< 0.003 %

Ref.: 1

3.1.6 Solubility

Solubility in

water	52.1 +/- 3.4 mg/l	(ref. 3)
methanol	20 < S < 60 g/l	
DMSO	> 50 g/l	
propyleneglycol	5 < S < 15 g/l	
decane	< 1 mg/l	

3.1.7	Partition coefficient (Log P_{ow})

Log Pow was determined in accordance with EEC guideline A.8 and with GLP.

Log Pow: 2.2
Pow= 166.6 ± 8.5

Ref.: 2

3.1.8	Additional physicochemical specifications

Melting point:	197.7 °C
Boiling point:	265 °C (decomposition)
Relative self ignition:	> 400 °C
Vapour pressure:	5.0×10^{-10} hPa (20 °C)
Relative density:	1.354 (20 °C)
Viscosity:	/
Surface tension:	71.6 mN/m (20 °C)
Flammability:	not very flammable
Oxidising properties:	not oxidising
pH:	6.3 – 6.4 (0.005% solution)
Refractive index:	/

3.1.9.	Stability

Stability and homogeneity of a suspension of B111, batch GST 4-11029 in 0.5% CMC (Na-carboxymethylcellulose) were determined by HPLC-UV. Preparations of 0.03 and 0.45% B111 were analysed for stability in double (0.35%) and single (0.45%) determinations immediately after preparation and after storing for 24 hours at room temperature. The preparations were stable and deviation was <5%.

For homogeneity 3 samples of each preparation (0.03 and 0.45% B111) were taken and analysed. Preparation C (0.45% B1111) was homogeneous within < 1% deviation from the mean, while the deviation of preparation A (0.03% B111) showed increased deviations up to 6.8% from mean.

Ref.: 19

Stability of B111 in cream formulations is stated as four years, for stability in aqueous and DMSO solutions low degradation within 7 days was stated as well. No further information or analytical data are provided for this statement.

Ref.: 20

Three preparations of B111 in 0.5% CMC (Na-carboxymethylcellulose) at concentrations of 0.06 mg, 2.4 mg and 9.0 mg were analysed in triplicate on days 2, 30 and 65 for homogeneity and stability by spectrophotometry. The preparations were diluted with DMSO prior to analysis. The concentration was calculated using a calibration curve. Maximum deviation from the target concentration was 7% for stability tests and 6% for homogeneity tests.

Ref.: 23

In a solution of B111 and 6% H_2O_2 (1:1 w/w), the test substance was shown to be stable for at least 45 minutes. Recovery was 98%.

General Comments on Physico-chemical characterisation

- It should be pointed out that for all toxicological investigations batches of B111 have been used which have been characterized with respect to identity and purity.

- The purity of the B111 batches used is 99.9% or higher so that the possible impurities, 3-aminopyridine and 2,6-diaminiopyridine, would result in an on scalp concentration of 0.0000075% (75 ng/ml) maximum.
- The stability and homogeneity of B111 in the solutions used for testing are characterized sufficiently.
- The applicants claim long term stability of B111 in products which are similar to marketed ones, but they did not report the data. Typical hair dye formulations have not been investigated for the stability of B111.
- The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies

3.2. Function and uses

a) Oxidative Hair Colorants
2,6-Diamino-3-((pyridin-3-yl)azo)pyridine is used as a non-reactive hair colouring agent ("direct dye") in oxidative hair dye formulations at a maximum on-head concentration of 0.25%.

b) Semi-permanent Hair Colorants
2,6-Diamino-3-((pyridine-3-yl)azo)pyridine is used as a non-reactive hair colouring agent ("direct dye") in semi-permanent hair dye formulations at a maximum on head concentration of 0.25%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: EC Guideline 92/69, method B.1., 31 July 1992
Species/strain: Rat, strain Sprague Dawley, Him:OFA, SPF Group size: 5 per sex and dose
Test substance: DA 100491
Batch: L6/186
Purity: > 98% (HPLC at 254 nm)
Dose: 346, 600, 1039, 1800 mg/kg bw
Observation period: gavage/oral
GLP: In compliance
Study date: 1993

The test substance was suspended in a 0.5% aqueous solution of carboxymethylcellulose. A single dose by gavage was administered at concentrations of 346, 600, 1039 and 1800 mg/kg bw. The two lower doses were given to 5 males and 5 females each, whereas the two higher doses were only given to 5 females. The animals were checked daily for clinical signs and mortality. Body weights were recorded at start and on days 7 and 14. Animals were observed for 14 days. All animals were submitted to a gross necropsy at the end of the observation period.

Results

Dosing (mg/kg bw)	Mortality
346 (male & female)	No deaths
600 (male & female)	3/5 on application day of each sex
1039 (female)	2/5 females first day of dosing
1800 (female)	All died on application day

The clinical signs noted at all doses were described as a general malaise including tremors, pale skin, dyspnoea, piloerection, excess salivation and lacrimation and possibly diarrhoea. These were considered vagotonic effects. The pinna and eyeballs were stained yellow in most animals at all doses. Urine was also discoloured. These effects lasted up to 6 days in the surviving animals.

At post mortem, haemorrhaging with major vascular ruptures were seen in many organs, possible causes of the small spleen and small thymus. Enlarged spleens in surviving animals were considered to be due to reactive haematopoiesis. Large lymph nodes may be due to reactive antigen penetration through damaged intestinal walls. No indication for impaired blood coagulation was noted. Yellow/orange staining of the intestine and subcutaneous fat other fat was noted.
There are no differences between the sexes. The LD_{50} for 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine was calculated to be 789 mg/kg bw.

Ref.: 13

3.3.1.2. Acute dermal toxicity

Guideline: OECD 402 (1987)
Species/strain: Rat, strain CD(SD)BR
Group size: 5 per sex

Test substance:	DA 100491
Batch:	GST 4-11029
Purity:	99.7% (HPLC at 254 nm)
Dose:	2000 mg/kg bw
Observation period:	14 days
GLP:	In compliance
Study date:	1996

The dorsal surface was shaved (approximately 10% of the total body surface). The single dose of 2000 mg/kg bw was applied as a paste in water. The area was occluded for 24 h and then washed.

Results
No mortality occurred. Urine was discoloured from day 3 to 6. The treated skin was stained yellow for the full study period.
Body weights were within the normal range.
Post-mortem showed no significant abnormalities.

Conclusion
The LD_{50} for 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine was in excess of 2000 mg/kg bw following a single dermal application.

Ref.: 14

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 (1981)
Species/strain:	New Zealand albino rabbit
Group size:	3 females
Test substance:	DA100491
Batch:	L6/186
Purity:	> 98% (HPLC at 254 nm)
Dose:	0.5 g
Route:	Dermal
Application conditions:	single application, 4 h, semi-occlusive
GLP:	in compliance
Date	1992

A cellulose patch with 0.5 g DA100491 was placed on the shaved skin of three female rabbits and covered with semi-occlusive dressing for 4 h. Access to the application area was prevented by a plastic collar. After the 4-h application time, the area was wiped with a cellulose tissue. Skin reactions were evaluated 1, 24, 48, and 72 h after removing the patches.

Results
No mortality, no systemic symptoms and no skin reactions were recorded in any of the test animals.

Conclusion
The test substance was considered to be non-irritating to the skin of rabbits.

Ref.: 15

Comment
Neat DA100491 was not irritating to rabbit skin.

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405 (1987)
Species/strain:	rabbit, strain New Zealand albino
Group size:	3 female
Test substance:	DA100491
Batch:	L6/186
Purity:	> 98% (HPLC at 254 nm)
Dose level:	44-56 mg
Route:	ocular
Dosing schedule:	single administration
GLP:	in compliance
Date:	1992

The approximate equivalent of 0.1 ml of DA100491 (44 - 56 mg) was placed into the conjunctival sac of the right eye of each animal. Lids were then held together for about one second. The untreated left eye served as control. Ocular reactions were evaluated 1, 24, 48, and 72 h after instillation of the test article. The entire eye, especially cornea, iris and conjunctivae were examined, using an ophthalmoscope.

Results
There were no mortality and no clinical signs.
Minimal redness of the conjunctivae was observed in one animal 1 and 24 h. All other scores were zero at any time.

Conclusion
DA100491 was concluded to be non-irritating to the mucous membranes of rabbits.

Ref.: 16

Comment
Undiluted DA100491 does have minor irritant potential to rabbit eyes.

3.3.3. Skin sensitisation

Magnusson - Kligman

Guideline:	According to Directive 84/449/EEC, method B6 (1984);	
Species/strain;	Female guinea pigs, strain Dunkin-Hartley	
Group size:	20 animals for treatment and 20 for the negative (vehicle) control group	
Test substance:	DA100491	
Batch:	L6/186	
Purity:	> 98% (HPLC at 254 nm)	
Concentrations:	intradermal induction:	0.001% test substance in 1,2-propylene glycol
	dermal induction:	25% test substance in white petrolatum, occluded
	challenge:	25% test substance in white petrolatum, occluded
GLP:	in compliance	
Date:	1992	

The test group consisted of 20 female guinea pigs and another 20 female guinea pigs were used as negative control. Induction commenced with three intradermal injections of Freund's Complete Adjuvant and DA100491 (0.001%). One week later, the induction

process was completed with a single topical application of DA100491 (25% in white petrolatum) under an occlusive patch for 48 h.

The same test substance concentration was used for the first challenge exposure to the left flank of the animals two weeks after the last induction dose. The right flank was treated with the vehicle only. Control animals were treated with 1,2-propyłene glycol for intradermal induction and with white petrolatum for epicutaneous induction and for challenge. Evaluation of skin reactions was done 24 h after epicutaneous induction and 24 and 48 h after removal of the challenge patches.

Results
One animal of the control group and one animal of the test group died spontaneously on day 8 on day 19 of the study, respectively. As the treated skin sites were yellow stained by the test substance a histopathological examination was carried out. Acanthosis, parakeratosis and inflammation of the dermis were detected in 2/19 animals of the test substance group, i.e. 11% of the test substance treated animals. A peri-contemporaneous positive control used p-phenylenediamine.

Conclusion
The results obtained in this study indicate a sensitizing potential of DA100491 under the conditions of this study.

Ref.: 17

Comment
DA100491 was shown to be a moderate skin sensitizer under the conditions of this study.

Buehler

Guideline:	According to Directive 92/69/EEC, method B.6. (1992),	
Species/strain:	Hartley Crl:(HA)BR guinea pigs	
Group size:	20 female in test group, 10 female in negative control group	
Test substance:	DA100491	
Batch:	L6/186	
Purity:	> 98% (HPLC at 254 nm),	
Concentrations:	dermal induction:	25% test substance in white petrolatum, occluded (3 induction exposures)
	challenge:	15% test substance in white petrolatum, occluded
GLP:	In compliance	
Date	1993	

The test group consisted of 20 female guinea pigs, the control group of 10 female guinea pigs. A 25% formulation of DA100491in white petrolatum was prepared and applied to the clipped skin under occlusive dressing on days 0, 7 and 14 (induction phase) for 6 h each time. On day 28 (challenge exposure) a preparation of 15% DA100491in white petrolatum was used. The dressing was removed 6 h after the application. Twenty-four, 48 and 72 h after removal of the patches the skin reactions were scored. All animals were observed daily for signs of systemic toxicity. Body weights were recorded on days 0 and 30. As the treated skin sites were yellow stained by the test substance, a histopathological examination was carried out on all sites treated with the test substance.

Results
No mortality occurred. Body weights were not affected by the test compound. No animal was observed as reacting to the test substance.

Conclusion
The results obtained in this study indicate no sensitizing potential of DA100491.

Ref.: 18

Comment
DA100491 was not sensitizing in this Buehler test.

3.3.4.	Dermal / percutaneous absorption

Oxidative conditions

Guideline:	OECD 428 (2004)
Tissue:	Schweizer Landedelschwein, male porcine back and flank skin (frozen/thawed; thickness: 0.85±0.1mm)
Method:	Diffusion Teflon-chambers
Integrity	tritiated water
No. of chambers:	6 (five for the formulation containing the test item and one for the blank formulation)
Test substance:	Azogelb WR18805 tested at a concentration of 0.25 % in a typical oxidative hair dye formulation.
Batch:	GST4-11029
Purity:	100% (HPLC at 254 nm)
Dose:	100 mg/cm² of formulation
Receptor fluid	physiological phosphate buffer containing NaCl and antibiotics
Solubility in receptor fluid	0.28 mg/ml
Stability	9% decomposition after 7 days in receptor fluid
Analysis	HPLC
GLP:	In compliance
Date:	May 2005

400 mg of a cream formulation for oxidative hair dyes (100 mg/cm²) containing 0.25 mg/cm² WR1880 was applied once to the skin samples (4 cm²).

Sixty minutes after substance application, the test item was removed by washing the skin twice with 4 ml water, then once with 4 ml washing solution (shampoo-formulation with water), and again twice with water. The washing solutions were combined and the amount of dye was determined by HPLC.

Fractions of the receptor fluid were collected after 16, 24, 40, 48, 64 and 72 h, concentrated by solid phase extraction and analysed immediately. At termination of the experiment, the skin was heat-treated and the 'upper skin' (stratum corneum and upper stratum germinativum) was mechanically separated from the 'lower skin' (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the dye content was quantified by means of HPLC.

Results
The total balance (total recovery) of 102.56 ± 0.84% of the dose applied to the pig skin samples confirmed the validity of the experiment.
The majority of the applied dose of WR1880 remained on the skin surface (102.34 ± 0.87% of the applied dose).
After 72 h, 0.20 ± 0.08 µg/cm² WR1880 had penetrated into the receptor fluid, 0.06 ± 0.04 µg/cm² was found in the upper skin, and 0.29 ± 0.08 µg/cm² was recovered in the lower skin.

The amount of 0.59 ± 0.15 µg/cm² (receptor fluid + upper skin + lower skin) of WR1880 was considered biologically available.

Ref.: 20

Comment

Only 5 chambers were used and the dose was too high; therefore the amount of WR1880 considered available under oxidative condition should be mean+2SD; 0.89 µg/cm².

Non-oxidative conditions

Guideline:	OECD 428 (2004)
Tissue:	Schweizer Landedelschwein, male porcine back and flank skin (frozen/thawed; thickness: 0.97±0.05mm)
Method:	Diffusion Teflon-chambers
Integrity:	tritiated water
No. of chambers:	6 (five for the formulation containing the test item and one for the blank formulation)
Test substance:	Azogelb WR18805 tested at a concentration of 0.25 % in a typical non-oxidative hair dye formulation.
Batch:	GST4-11029
Purity:	100% (HPLC at 254 nm)
Dose:	100 mg/cm² of formulation
Receptor fluid:	physiological phosphate buffer containing NaCl and antibiotics
Solubility in receptor fluid:	0.28 mg/ml
Stability	9% decomposition after 7 days in receptor fluid
Analysis	HPLC
GLP:	In compliance
Date:	April/May 2005

400 mg of a cream formulation for oxidative hair dyes (100 mg/cm²) containing 0.25 mg/cm² WR1880 was applied once to the skin samples (4 cm²).

Sixty minutes after substance application, the test item was removed by washing the skin twice with 4 ml water, then once with 4 ml washing solution (shampoo-formulation with water), and again twice with water. The washing solutions were combined and the amount of dye was determined by HPLC.

Fractions of the receptor fluid were collected after 16, 24, 40, 48, 64 and 72 h, concentrated by solid phase extraction and analysed immediately. At termination of the experiment, the skin was heat-treated and the 'upper skin' (stratum corneum and upper stratum germinativum) was mechanically separated from the 'lower skin' (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the dye content was quantified by means of HPLC.

Results
The total balance (total recovery) of 106.92 ± 0.41% of the dose applied to the pig skin samples confirmed the validity of the experiment.
The majority of the applied dose of WR1880 remained on the skin surface (106.83 ± 0.37% of the applied dose).
After 72 h, 0.09 ± 0.08 µg/cm² WR1880 had penetrated into the receptor fluid, 0.03 ± 0.02 µg/cm² was found in the upper skin, and 0.09 ± 0.05 µg/cm² was recovered in the lower skin.

The amount of 0.22 ± 0.09 µg/cm² (receptor fluid + upper skin + lower skin) of WR1880 was considered biologically available.

Ref.: 21

Comment
Only 5 chambers were used and the dose was too high; therefore the amount of WR1880 considered available under non-oxidative condition should be mean+2SD; 0.40 µg/cm².

3.3.5. Repeated dose toxicity

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

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline:	OECD guideline no, 408 (1981)
Species/strain:	Wistar rat, CRL strain:(WI) BR, SPF
Group size:	15 per sex and dose
Recovery group:	10 per sex, control and high dose
Test substance:	DA100491
Batch:	GST 4-11029
Purity:	>99%
Dose levels:	3, 12, 45 mg/kg bw/day
Vehicle:	Distilled water
Route:	Oral, gavage
Exposure period:	13 weeks, 7 days/week
GLP:	in compliance
Study date:	21 September 1994 – 18 January 1995

In a preliminary range finding, a NOEL of 15 mg/kg bw/day was found. The dose range was selected as a result of this study. Animals were dosed daily by gavage with a volume 10 ml/kg bw. After the 90-day period, a recovery group (high dose and controls) of 10 animals per sex were kept for further 4 weeks untreated to check for reversibility of possible test substance related effects.
Clinical observations and mortality were assessed daily. Body weight, food consumption were recorded weekly. Initially, followed by monthly checks of ophthalmic condition, blood biochemistry and haematology were performed. At 90 days, scheduled animals were killed and organ weights were determined. Organs and tissues from all animals in each group were preserved and the majority were examined histopathologically.

Results
All animals survived until the end of the study. Staining of bedding and discoloured urine were seen in all dosed groups. Initially, some animals from all dose groups showed diarrhoea. No other substance-related effects were observed in the low dose group.
At the higher doses, some alterations in group mean values for haematological and blood biochemical parameters were observed (e.g. percentage of neutrophils, lymphocytes, erythrocytes, levels of cholesterol, bilirubin). All parameters returned to normal values in the recovery period.
Dark colouration and enlargement of the thyroid glands were observed in the mid and/or high dose group. These alterations persisted until the end of the recovery period in the high dose group. Histological alterations were detected in the thyroid glands (cytoplasmatic pigment deposits in F-cells, proliferation) and spleens (haematopoiesis, pigment deposits). Pigment deposits in thyroid F-cells and spleen were still found at the end of the recovery period. These pigment deposits were considered to indicate only the presence of either the test substance or a metabolite and not a pathological finding. The relative organ weights of kidney and liver increased in the mid and high dose groups, and absolute kidney and liver weights increased in males of the high dose group. After recovery, the relative liver weights in high dose females remained increased. In high dose males, lowered absolute and relative testis weights were observed.
Since these observed haematological, blood biochemical, histopathological and organ weight changes did not have a consistent pattern, the study authors interpreted them as adaptive or reactive changes to test substance exposure at 3 mg/kg bw/d. However, they considered the changes adverse effects when dosed at 12 and 45 mg/kg bw/d.

Conclusion
The NOAEL for 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine in this 90 day study was 3 mg/kg bw/d.

Ref.: 23

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 (1983)	
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	
Replicates:	triplicates in 2 individual experiments	
Test substance:	DA100491 [2,6-diamino-3-((pyridine-3-yl)azo)pyridine]	
Batch:	GST 4-11029	
Purity:	99.7 (HPLC), 99.75 (DSC)	
Vehicle:	DMSO	
Concentrations:	experiment 1:	1, 10, 100, 1000 and 5000 µg/plate without and with S9-mix
	experiment 2:	30, 100, 300, 1000 and 3000 µg/plate without and with S9-mix
Treatment:	direct plate incorporation with 48 h incubation without and with S9-mix	
GLP:	In compliance	
Study date:	27 March 1996 – 19 April 1996	

DA100491 was tested in bacterial tester strains of *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538, in two independent experiments both in the presence and in the absence of a metabolic activation system. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. In both experiments, the direct plate incorporation method with 48 h exposure was used.
Toxicity was detected as significant reductions in the number of spontaneous revertant colonies or on the basis of the absence of a normal the bacterial background lawn. Negative and positive controls were in accordance with the OECD guideline.

Results
In the absence of S9-mix toxic effects were observed at concentrations ≥ 1000 µg/plate with TA1535 and TA100 and at ≥ 3000 µg/plate with TA1537 and TA98. TA1538 showed toxic effect at ≥ 3000 µg/plate in the first and at ≥ 1000 µg/plate in the second experiment. In the presence of S9-mix toxic effects were observed at concentrations ≥ 3000 µg/plate with all strains used.
A biologically relevant increase in the number of revertant colonies sufficient to be considered as indicative of any mutagenic activity of DA100491 was not observed for any of the strains used.

Conclusion
Under the experimental conditions used DA100491 was not genotoxic (mutagenic) in this gene mutation test in bacteria

Ref.: 24

***In Vitro* Mammalian Chromosomal Aberration Test**

Guideline:	OECD 473 (1983)
Cells:	V79 cells
Replicates:	duplicates in a single experiment
Test substance:	DA100491
Batch:	4-11029
Purity:	> 99%
Vehicle:	DMSO
Concentrations:	3, 10 and 30 µg/ml without S9-mix 30, 100 and 300 µg/ml with S9-mix
Treatment:	18 h or 28 h (lowest concentration only) treatment without S9-mix; harvest time 18 h or 28 h (lowest concentration only) after the start of treatment 4 h treatment with S9-mix; harvest time 18 h or 28 h (lowest concentration only) after start of treatment
GLP:	in compliance
Study date:	25 May 1994 – 23 November 1994

DA100491 has been investigated for the induction of chromosomal aberrations in V79 cells in the absence and presence of metabolic activation. Liver S9-fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were chosen on the basis of a pre-test for toxicity, evaluating cell number and cell morphology 4 h and 18-20 h after start of treatment, or on the results of a XTT assay, measuring cell viability.
In the main test, cells were treated for 18 or 28 h without S9-mix or for 4 h with S9-mix and harvested 18 h or 28 h after the start of treatment. Approximately 2.5 h before harvest, each culture was treated with colcemid (0.2 µg/ml culture medium) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations and the mitotic index. Reduction in the mitotic index was taken as a measure for cytotoxicity. Negative and positive controls were in accordance with the OECD guideline.

Results
In the XTT test, cytotoxicity was observed starting at concentrations of 30 µg/ml (without S9-mix) and 100 µg/ml (with S9-mix). In the presence of S9-mix, the mitotic index was reduced after the highest concentration used. In the absence of S9-mix the mitotic index was either not reduced or no or not enough scorable cells could be found.
At the 18 h fixation time, both in the absence and presence of S9-mix, treatment with DA100491 resulted in concentration-dependent increases in the number of cells with chromosomal aberrations compared to concurrent negative control cultures. A biologically relevant increase in the rate of polyploidy metaphases compared to concurrent negative control cultures was not found.

Conclusion
Under the experimental conditions used, DA100491 was genotoxic (clastogenic) in the chromosome aberration test in V79 cells both in the absence and the presence of S9 metabolic activation.

Ref.: 25

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* unscheduled DNA synthesis (UDS) test**

Guideline:	OECD draft guideline 486 (1991)
Species/strain:	male Wistar HanIbm: WIST (SPF) rats
Group size:	5 rats per dose

Test substance:	DA 100491
Batch no:	4-11029
Purity:	> 99 %
Vehicle:	0.5% carboxymethylcellulose
Dose level:	0, 20 and 200 mg/kg bw
Route:	oral gavage
Sacrifice times:	2 h (high dose only) and 16 h after dosing
GLP:	in compliance
Date:	19 May 1994 – 30 August 1994

DA 100491 was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on the results of a pre-experiment on acute toxicity with doses up to 790 mg/kg bw. Rats were treated orally and examined for acute toxic symptoms at intervals of 1 h and 24 h after start of treatment. In the main experiment mice were exposed orally to 0, 20 and 200 mg/kg bw. Hepatocytes for UDS analysis were collected approximately 2 h and 16 h after administration of DA 100491 by liver perfusion with 0.05% collagenase. The quality of the performed perfusion was determined by the trypan blue dye exclusion method.
At least 90 minutes after plating the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 12-14 days. The number of silver grains above the nucleus and the number of grains above one nuclear-sized cytoplasm adjacent to the nucleus were counted. These and the net grain count (nuclear grain count – cytoplasm grain count) were reported. Unscheduled DNA synthesis was determined in 50 randomly selected hepatocytes on 2 replicate slides per rat from at least 3 treated rats. Appropriate reference positive controls were included.

Results
In the pre-experiment on toxicity, rats died when treated with doses above 200 mg/kg bw. Rats treated with 200 mg/kg bw expressed toxic reactions like pilo-erection already 1 h after treatment and additionally 24 h after treatment reduction of spontaneous activity, eyelid closure, disturbance of the locomotorsystem and apathy. All treated rats had yellow coloured urine. The coloured urine and the clinical symptoms confirmed bioavailability of DA 100491 after oral administration.
On the basis of these results 200 mg/kg bw was chosen as the top dose. In the main experiment 2 of the 5 rats of the 16 h preparation interval treated with the top dose died. The surviving animals showed behavioural toxic reactions like reduced spontaneous activity. The viability of the hepatocytes was not substantially affected by the *in vivo* treatment with DA 100491.
A biological increase in mean net nuclear grain count as compared to the untreated control was not found in hepatocytes of any treated animal both for the 2 h and the 16 h treatment time.

Conclusion
Under the experimental conditions reported DA 100491 did not induce DNA-damage leading to unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the in vivo UDS test.

Ref.: 27

In vivo Mammalian Erythrocytes Micronucleus Test

Guideline:	OECD 474 (1983)
Species/strain:	NMRI mice
Group size:	5 rats/sex/group
Test substance:	DA 100491
Batch no:	4-11029

Purity:	> 99 %
Vehicle:	0.5% carboxymethylcellulose
Dose level:	0, 80, 300 and 800 mg/kg bw
Route:	orally
Sacrifice times:	24 h and 48 h (high dose only) after treatment
GLP:	in compliance
Date:	7 June 1994 – 30 August 1994

DA 100491 was investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the results of a pre-experiment on acute toxicity. Mice were treated orally with 800 and 1000 mg/kg bw and examined for acute toxic symptoms and survival at 1, 6, 24 and 48 h after treatment.
In the main experiment mice were exposed orally to 0, 80, 300 and 800 mg/kg bw. Bone marrow cells were collected 24 h or 48 h (high dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment on acute toxicity 1 of the mice treated with 1000 mg/kg bw died. The remaining mice and those treated with 800 mg/kg bw showed reduction of spontaneous activity, eyelid closure, apathy and tremor. For the main experiment 800 mg/kg bw was estimated to be suitable.
In the main experiment one of the 10 females treated with 800 mg/kg bw died. After treatment with the test item the ratio PCE/NCE was not substantially decreased as compared to the mean ratio of the vehicle control, thus indicating that DA 100491 did not exert any cytotoxic effects in the bone marrow. However, the clinical signs observed in the pre-experiment after treatment indicated the systemic distribution of DA 100491 and thus its bioavailability.
Biologically relevant increases in the number of polychromatic erythrocytes with micronuclei compared to the concurrent vehicle controls were not found at any dose tested, neither 24 nor 48 h after treatment and neither for males nor for females.

Conclusion
Under the experimental conditions used DA 100491 did not induce a biologically relevant increase in the number of erythrocytes with micronuclei of treated mice and, consequently, DA 100491 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 26

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Prenatal developmental study

Guideline:	OECD guideline 414 (1981)
Species/strain:	Rat, strain Wistar Crl: (WI) BR
Group size:	20-21 females (mated), 25 (control group)

Test substance:	DA 100491
Batch:	4-11029
Purity:	/
Vehicle:	0.5% aqueous sodium-carboxymethylcellulose
Dose levels:	3, 12, 45 mg/kg bw/day, by gavage
Dose volume:	10 ml/kg bw
Route:	oral, gavage
Administration:	Days 6 through 15 of pregnancy, inclusive
GLP statement:	In compliance
Study date:	1996

In a preliminary experiment, doses for the main study were selected as follows: 3, 12 and 45 mg/kg bw/d. In the main study, pregnant females were allocated to 4 groups 25 control, 21 low dose, 20 mid dose and 21 high dose animals. The dose groups received 2,6-diamino-3-((pyridine-3-yl)azo)pyridine suspended in a 0.5% aqueous sodium-carboxymethylcellulose preparation from day 6 - 15 of gestation. Control animals received the vehicle alone. On day 20 of gestation, the animals were sacrificed and a complete necropsy of the dams, including macroscopic evaluation of the organs was carried out. All foetuses were externally examined. Approximately 50 % of the foetuses of each dam were examined for visceral abnormalities; the remaining 50 % were checked for skeletal defects.

Results
No test substance-related effects were observed at the low and intermediate dose levels (3 and 12 mg/kg). At the highest tested dose (45 mg/kg bw/d) slight maternal toxicity was observed as indicated by decreased body weight gain and decreased food consumption. In addition, litter data indicated a slight adverse effect of the test substance at the high dose level exclusively. The mean number of viable foetuses per litter was decreased (not statistically significant) partly due to influences from the pre-implantation period (thus unrelated to test substance administration) but also in part due to a slightly increased post-implantation loss (not statistically significant). Further, mean uterus weights and mean foetal weights were slightly decreased (both not statistically significant) in the high dose group (45 mg/kg bw/d). Thus, despite the lack of statistical significance of the differences to control values in any individual parameter, the decreases recorded for foetal weights and foetal survival in conjunction are considered as indication of a slight adverse effect of the test substance in the high dose group.
No test substance-related changes were observed during external, visceral and skeletal examinations of the foetuses.

Based on results stated above, the NOAEL was determined to be 12 mg/kg bw/d for the dams and foetuses.

Ref.: 19

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-Diamino-3-((pyridine-3-yl)azo)pyridine)

Oxidative conditions

Absorption through the skin	**A (mean + 2SD)**	**=**	**0.89 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.52 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.01 mg/kg bw/d**
No observed adverse effect level (subchronic, oral, rat)	**NOAEL**	**=**	**3 mg/kg bw/d**
50% oral bioavailability*		**=**	**1.5 mg/kg bw/d**
MOS		**=**	**175**

Non-oxidative conditions

Absorption through the skin	**A (mean + 2 SD**	**=**	**0.40 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.23 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.004 mg/kg bw/d**
No observed adverse effect level (subchronic, oral, rat)	**NOAEL**	**=**	**3 mg/kg bw/d**
50% oral bioavailability*		**=**	**1.5 mg/kg bw/d**
MOS		**=**	**388**

* standard procedure according to the SCCS Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

3.3.14. Discussion

Physico-chemical specification

2,6-Diamino-3-((pyridin-3-yl)azo)pyridine is used as a non-reactive hair colouring agent ("direct dye") in oxidative hair dye formulations as well as in semi-permanent hair dye formulations

Typical hair dye formulations have not been investigated for the stability of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine.

The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the

respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

General toxicity
The acute oral toxicity of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine was calculated to be 789 mg/kg bw and the acute dermal toxicity was > 2000 mg/kg bw in rats. The NOAEL in a 90-day study was considered to be 3 mg/kg bw/d, based on adaptive or reactive haematological, blood biochemical, histopathological and organ weight changes to the test substance seen at the higher doses. In a prenatal development study, the NOAEL was determined to be 12 mg/kg bw/d for the dams and foetuses.

Irritation, sensitisation
The test substance (neat) was considered to be non-irritating to the skin of rabbits.
Undiluted 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine does have minor irritant potential to rabbit eyes.
2,6-Diamino-3-((pyridin-3-yl)azo)pyridine was shown to be a moderate skin sensitizer under the conditions of a Magnusson Kligman study. It was not sensitizing in a Buehler test.

Dermal absorption
Only 5 chambers were used and the dose was too high; therefore the amount of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine considered available under oxidative conditions should be mean+2SD; 0.89 µg/cm².
Only 5 chambers were used and the dose was too high; therefore the amount of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine considered available under non-oxidative conditions should be mean+2SD; 0.40 µg/cm².

Mutagenicity
The genotoxicity of 2,6-diamino-3-((pyridine-3-yl)azo)pyridine is investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. It did not induce gene mutation in bacteria. However, a gene mutation test using cultured mammalian cells was not performed. Treatment with 2,6-diamino-3-((pyridine-3-yl)azo)pyridine resulted in an increase in cells with chromosome aberrations in mammalian cells *in vitro*.
In mice exposure to 2,6-diamino-3-((pyridine-3-yl)azo)pyridine did not result in an increase in erythrocytes with micronuclei. Likewise 2,6-diamino-3-((pyridine-3-yl)azo)pyridine exposure did not induce unscheduled DNA synthesis in rats. As the positive *in vitro* finding can not be confirmed in *in vivo* tests, 2,6-diamino-3-((pyridine-3-yl)azo)pyridine can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of 2,6-diamino-3-((pyridine-3-yl)azo)pyridine with a maximum on-head concentration of 0.25% in oxidative and non-oxidative hair dye formulations does not pose a risk to the health of the consumer.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Tenberken-Pötzsch, B.; identity and purity test of Azogelb; *WELLA AG;* 2002
2. Schneider, S.; Determination of the partition coefficient (1-Octanol/Water) of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridin in accordance with EEC-Guideline A.8.; *CASSELLA;* 1996
3. Schneider, S.; Determination of the water solubility of 2,6-Diamino-3-((pyridin-3-yl) azo)pyridin in accordance with EEC-Guideline A.6.; *CASSELLA;* 1996
4. Schneider, S.; Determination of the melting point of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridin in accordance with EEC-Guideline A.1.; *CASSELLA;* 1996
5. Schneider, S.; Determination of the boiling point of 2,6-Diamino-3-((pyridin-3-yl) azo)pyridin in accordance with EEC-Guideline A.2.; *CASSELLA;* 1996
6. Schneider, S.; Determination of the relative density of 2,6-Diamino-3-((pyridin-3-yl) azo)pyridin in accordance with EEC-Guideline A.3.; *CASSELLA;* 1996
7. Hoffmann, H.; Vapour pressure; *HOECHST AG;* 1996
8. Schneider, S.; Determination of the surface tension of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridin (in aqueous solutions) in accordance with EEC-Guideline A.5.; *CASSELLA,* 1996
9. Wendt, W.; Determination of the flammability of 2,6-Diamino-3-((pyridine-3-yl)azo)pyridin in accordance with EEC-Guideline A.10.; *CASSELLA;* 1996
10. Wendt, W.; Determination of the explosive properties of 2,6-Diamino-3-((pyridine-3-yl)azo)pyridin in accordance with EEC-Guideline A. 14.; *CASSELLA;* 1996
11. Wendt, W.; Determination of the auto-flammability of 2,6-Diammo-3-((pyridin-3-yl)azo)pyridin in accordance with EEC-Guideline A.16.; *CASSELLA;* 1996
12. Wendt, W.; Determination of the oxidizing properties of 2,6-Diamino-3-((pyridin-3-yl)azo)pyridin in accordance with EEC-Guideline A.17.; *CASSELLA;* 1996
13. Klein, W.; Acute oral toxicity of "DA 100491" in rats; *SEIBERSDORF;* 1993
14. Haynes, G.; DA 100491 Acute dermal toxicity study in the rat; *RTC;* 1996
15. Klein, W.; Acute dermal irritation/corrosion study with "DA 100491"; *SEIBERSDORF;* 1992
16. Klein, W.; Acute eye irritation/corrosion study with "DA 100491"; *SEIBERSDORF;* 1992
17. Kocsis, F.; Skin sensitisation study with "DA 100491" (Guinea Pig Maximisation Test); *SEIBERSDORF;* 1993
18. Kocsis, F.; Skin sensitisation study with "DA 100491", Bühler Test; *SEIBERSDORF;* 1993
19. Fend, C; Teratogenicity study with "DA 100491" in rats; *SEIBERSDORF;* 1996
20. Beck, H.; Cutaneous absorption of 0.25% 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine (=WR188Ö5) in a typical hair dye formulation with hydrogen peroxide through pig skin in vitro; *COSMITAL SA;* 2005
21. Beck, H.; Cutaneous absorption of 0.25% 2,6-Diamino-3-((pyridin-3-yl)azo)pyridine (=WR18805) in a typical hair dye formulation through pig skin in vitro; *COSMITAL SA;* 2005
22. ADME: Permeability - Study of 2,6-Diamino-3-((Pyridin-3-yl)Azo)Pyridine-; *CEREP;* 2005
23. Klein, W.; 90-Day oral toxicity study with "DA 100491" in rats; *SEIBERSDORF;* 1995
24. Aeby, P.; Assessment of the potential mutagenicity of 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine in the AMES reversion assay with Salmonella Typhimurium; *COSMITAL SA;* 1996
25. Völkner, W.; Chromosome aberration assay in Chinese hamster V79 cells in vitro with DA 100491; *CCR;* 1994
26. Fautz, R.; Micronucleus assay in bone marrow cells of the mouse with DA 100491; RCC *CCR; 1994*
27. Fautz, R.; In vivo / in vitro unscheduled DNA synthesis in rat hepatocytes with DA 100491; *RCC-CCR;* 1994
28. König, P.; Data base search for references for B111 - 2,6-Diamino-3-((pyridine-3-yl)azo)pyridine: 28365-08-4; *WELLA AG;* 2005