



Scientific Committee on Consumer Safety

SCCS

OPINION ON

Hydroxyethoxy aminopyrazolopyridine HCl

COLIPA n° A161

The SCCS adopted this opinion at its 5th plenary meeting
of 27 March 2014

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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This opinion has been subject to a commenting period of six 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.

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1. BACKGROUND

Submission I for the new hair dye substance Hydroxyethoxy aminopyrazolopyridine HCl (CAS 1079221-49-0) A161 was received in August 2013 by Cosmetic Europe.

The present submission I includes a complete dossier according to the hair dye strategy and the applicant asks for the safety evaluation of Hydroxyethoxy aminopyrazolopyridine HCl (A161), when used in hair dye formulations at a concentration up to 2.0% in the finished cosmetic product.

2. TERMS OF REFERENCE

1. *Does the SCCS consider Hydroxyethoxy aminopyrazolopyridine HCl safe for use in oxidative hair dye formulations with a concentration on-head of maximum 2.0% taken into account the scientific data provided?*
2. *And/or does the SCCS recommend any restrictions with regard to the use of Hydroxyethoxy aminopyrazolopyridine HCl in oxidative hair dye formulations (e.g. max conc. in the finish cosmetic product, dilution ratio with hydrogen peroxide, warning)?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Hydroxyethoxy aminopyrazolopyridine HCl (INCI name)

3.1.1.2. Chemical names

2-[(3-aminopyrazolo[1,5-a]pyridin-2-yl)oxy]ethanol hydrochloride

3.1.1.3. Trade names and abbreviations

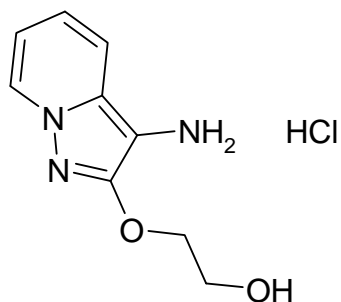
IMEXINE® FAM

3.1.1.4. CAS / EC number

CAS: 1079221-49-0

EC: 695-745-7

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₉ H₁₁ N₃ O₂, HCl

3.1.2. Physical form

Violet powder

3.1.3. Molecular weight

Molecular weight: 229.66

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3.1.4. Purity, composition and substance codes

Chemical structure of the batches R0056896C 001 P 001, R0056896C 001 P 003 and R0056896C 001 P 004 was established by elemental analysis and infra-red, mass and NMR spectrometry.

Contents of Hydroxyethoxy aminopyrazolopyridine HCl in these three batches determined by HPLC using a reference standard (*batch R0056896C 010 L 003, which was considered 99% pure*) were:

Batch R0056896C 001 P 001: 98.4±0.6%

Batch R0056896C 001 P 003: 99.7±0.5%

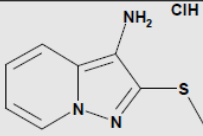
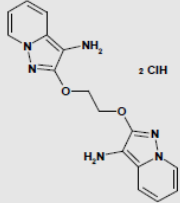
Batch R0056896C 001 P 004: 97.5±0.5%

Chloride content: 15.2-15.4% in all 3 batches (theoretical chloride content 15.4%)

3.1.5. Impurities / accompanying contaminants

Ash content of the batches R0056896C 001 P 001, R0056896C 001 P 003 and R0056896C 001 P 004 were <0.1%, <0.1% and 0.21% respectively.

Several impurities were demonstrated by HPLC in all 3 batches of Hydroxyethoxy aminopyrazolopyridine HCl. The two major impurities ([R0055535B](#) and [R0089617A](#)) were identified as described in the table below.

| | |
|---------------------------|--|
| R0055535B |  <p>Exact Mass =179.36 Molecular Formula =C8H9N3S · HCl</p> |
| R0069617A |  <p>Exact Mass =324.36 Molecular Formula =C16H16N6O2 · HCl</p> |

The content of the impurity R005553B in the three batches was 0.16-0.21%

The content of the impurity R0069617A in the three batches was 0.68-1.27%

All three batches of Hydroxyethoxy aminopyrazolopyridine HCl contained 0.12 - 0.17% isopropanol.

Water content of Hydroxyethoxy aminopyrazolopyridine HCl (batch R0056896C 001 P 001):
0.22 % w/w

Loss on drying of Hydroxyethoxy aminopyrazolopyridine HCl (batch R0056896C 001 P 004):
0.3 % w/w

Impurities of metals determined by ICP-MS:

Batch R0056896C 001 P 001 (mg/kg)

As, Cd, Hg, Pb, Pd: Each < 1

Ba, Co, Cr, Cu, Mn, Mo, Se, Ti, Vn, Zn: Each < 5

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Al, Fe, Ni, Sn : Each < 6

Batch R0056896C 001 P 003 (mg/kg)

As, Cd, Hg, Pb, Pd: Each < 1

Ba, Co, Cr, Cu, Mn, Mo, Se, Ti, Vn, Zn: Each < 5

Al, Fe, Ni, Sn: Each < 7

Batch R0056896C 001 P 004 (mg/kg)

As, Cd, Hg, Pb, Pd: Each < 1

Ba, Co, Cu, Mo, Se, Ti, Vn: Each < 5

Al, Sn: Each < 17

Cr: 118 (<5 when determined by X ray Fluorescence and ICP)

Mn: 10

Fe: 455

Ca: 149

Ni: 59 (<5 when determined by X ray Fluorescence and ICP)

Zn: 6

3.1.6. Solubility

Solubility for the batch R0056896C 001 P 003 at 23±2°C

Ultra Pure Water: Freely soluble (kinetic solubility between 500 and 1000g/L)

Absolute Ethanol: Very slightly soluble (kinetic solubility between 0.1 and 1g/L)

DMSO: Freely soluble (kinetic solubility between 500 and 1000g/L)

Corn Oil: Practically insoluble (kinetic solubility less than 0.1g/L)

SCCS Comment:

Water solubility was not determined by EU Method A.6

3.1.7. Partition coefficient (Log Pow)

Log P_{ow}: 0.34 (HPLC, pH=7.8)

3.1.8. Additional physical and chemical specifications

Melting point: 159-170°C

Boiling point: /

Flash point: /

Vapour pressure: /

Density: /

Viscosity: /

pKa: 5.05 (25°C and ionic strength 0.15M)

Refractive index: /

pH: For batch R0056896C 001 P 003 at 1% (w/w) in water at 23±2°C: pH = 3.1±0.1

UV_Vis spectrum (200-800 nm): λ_{max} 307±1nm

3.1.9. Homogeneity and Stability

Concentrations of the test item R0056896C in test formulations (1 mg/ml, 10 mg/ml and 100 mg/l in deionized water) used in a 13-week subchronic toxicity study and in a prenatal developmental toxicity study did not vary more than 10% of the target concentrations. The test item R0056896C in the test formulations (1 mg/ml, 10 mg/ml and 100 mg/ml in deionized water) was demonstrated to be stable for up to 9 days (relative standard deviation <8%), when these were stored at ambient temperature.

General Comments to physico-chemical characterisation

- The stability of Hydroxyethoxy aminopyrazolopyridine HCl in typical hair dye formulations was not reported

3.2. Function and uses

The ingredient IMEXINE[®] FAM will be used in oxidative hair colouring formulations at a maximum concentration of 4%, which after mixing typically in a ratio of 1:1 with hydrogen peroxide prior to use, corresponds to a concentration of 2% upon application (final, on-head concentration).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: /
Species/strain: rat / CrI:OFA (SD) Sprague-Dawley
Group size: 3 females
Test substance: IMEXINE[®] FAM
Batch: R0056896C 002 L 001
Purity: >95%
Vehicle: water
Dose level: 500 mg/kg bw
Administration: oral, gavage
GLP: in compliance
Study period: March 2006

At the Sponsor's request, the potential acute oral toxicity of the test substance was evaluated after a single oral dose (gavage) of 500 mg/kg bw in three female rats, inspired by the general requirements of OECD TG 420 (2001) and EU B1.

The test substance was administered in sterile water (10 ml/kg). Mortality was recorded twice daily. Animals were examined clinically on the day of treatment, 30 minutes, 2 hours, 4 hours and 6 hours after administration and thereafter at least once daily for 14 days. Body weights were recorded on day 1 just before treatment and on days 7, 14 and 15. All animals surviving to the end of 14-day observations period were sacrificed, subjected to gross necropsy and their organs (liver, spleen, kidneys, stomach, intestines, gonads/reproductive tract, lungs, heart and any other organs with obvious abnormalities) were examined macroscopically.

Results

No deaths occurred, no clinical signs of toxicity were observed, the body weight gain was not affected by the treatment, and there were no organ or tissue gross findings at necropsy.

Conclusion

Under the conditions of this study, the maximal non-lethal dose of the test substance by oral route in rats was higher than 500 mg/kg bw.

3.3.1.2. Acute dermal toxicity

Guideline: /
Species/strain: rat / Crl:OFA (SD) Sprague-Dawley
Group size: 3 females
Test substance: IMEXINE® FAM
Batch: R0056896C 002 L 001
Purity: >95%
Vehicle: water
Dose level: 500 mg/kg bw
Administration: dermal
GLP: in compliance
Study period: March 2006

At the Sponsor's request, the potential acute dermal toxicity of the test substance was evaluated after a single dermal dose of 500 mg/kg bw in three female rats, inspired by the general requirements of OECD TG 402 (1987) and EU B3 (1992).

The day before treatment, the dorsal region of each rat was carefully clipped on an area of approximately 50 cm². On the day of study, the test substance was moistened with sterile water (1ml/kg) in order to allow good contact between the test item and the skin. A single dose of 500 mg/kg bw was applied to the prepared area from each animal to expose at least 10% of the total body surface on a piece of absorbent gauze protected by a pad and covered with an adhesive tape for 24 hours. At the end of the exposure period, any remaining test item was removed by rinsing with sterile water and the application site was gently rinsed with water. Mortality was recorded twice daily. Animals were examined clinically on the day of treatment, 30 minutes, 2 hours, 4 hours and 6 hours after administration and thereafter at least once daily for 14 days. The skin examination was performed daily and evaluated according to the Draize scoring system. Body weights were recorded on day 1 just before treatment and on days 7, 14 and 15. All animals surviving to the end of 14-day observations period were sacrificed, subjected to gross necropsy and their organs (liver, spleen, kidneys, stomach, intestines, gonads/reproductive tract, lungs, heart, treated area and any other organs with obvious abnormalities) were examined macroscopically. All organs showing macroscopic signs of pathology were fixed in an appropriate fixative for possible histopathological examination.

Results

No deaths occurred, no clinical signs of toxicity were observed, the body weight gain was not affected by the treatment, and there were no organ or tissue gross findings at necropsy. On day 2, after removal of the dressings, a purple colouring of the application site was observed in all animals which did not persist to the end of the study. On day 6 and day 7, one animal revealed a very slight erythema (grade 1). No other dermal reactions were observed during the course of the study. At necropsy a redness was well perceptible on the application site of any animal only after exsanguination which, according to the study report authors could be due to an irritation and/or a colouration induced by the test substance.

Conclusion

Under the conditions of this study, the maximal non-lethal dose of the test substance by the dermal route in rats was higher than 500 mg/kg bw.

Ref.: 7

3.3.1.3. Acute inhalation toxicity

No data submitted.

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

No *in vivo* skin irritation study was submitted.

In vitro Primary Cutaneous Tolerance

Guideline: /
Test System: Episkin (0.38 cm²)
Test substance: R0056896C
Purity: 98.4%
Batch: R0056896C 001 P 001
Dose: 2% in water
Controls: Negative (PBS+)
Positive (allyl heptanoate)
Solvent (Water)
Batch (sodium dodecyl sulfate)
GLP: compliant
Date: 2013

96 samples of reconstructed human epidermis Episkin (0.38 cm²) were used to assess the *in vitro* primary cutaneous tolerance of R0056896C.

The test item R0056896C diluted at 2% in water was evaluated on 3 different batches of Episkin.

The test substance and controls were all tested in triplicate.

R0056896C was also evaluated on 3 different batches of dead epidermis because of its interaction with MTT. R0056896C and negative control (PBS+) were tested in triplicate.

Additional tissues were used for R0056896C (three alive and three dead) and negative control (one dead and one alive) in order to quantify the colouring due to the dye-chemical interactions with the tissue.

10 µL of R0056896C diluted at 2% and of different controls were applied onto the epidermis using a positive displacement pipette. After 42 hours +/- 1 hour treatment period, tissues were rinsed with PBS+.

MTT test:

At the end of the 42 hours, each epidermis unit was transferred to 12-well plates containing a dye solution (MTT) and incubated for 3 hours at 37°C. At the end of the incubation period, a biopsy of the epidermis was taken and for all tissues with the test item, the superficial epidermis layer (containing most of the remaining colour) was removed and discarded. The epidermis was separated from the collagen matrix. Epidermis and collagen matrix were transferred into a tube containing 500 µL of acidified isopropanol. Formazan was used to homogenise the solution. 2 x 200 µL of each extract were transferred onto a 96-well plate and the optical density was measured at 570 nm versus acidified isopropanol.

Determination of IL-1α concentrations in the culture medium:

IL-1α released in the culture medium was determined by a classic quantitative sandwich enzyme immunoassay technique. The optical density (optical density related to the IL-1α amount) was measured at 450 nm.

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Results

The mean viability value was 57.4% (> 50%) and the final release of Interleukin-1alpha was 30.6 pg/mL (< 50 pg/mL).

| VIABILITY LEVEL AND IL-1 α CONCENTRATIONS | Classification of the raw material under test |
|--|---|
| <p>Mean viability value \leq 50 %</p> <p>OR</p> <p>Mean viability value > 50 %</p> <p>and</p> <p>final [IL-1α RM release] \geq 50 pg/mL (two or three tests with a [IL-1α RM release] \geq 50 pg/mL)</p> | <p>Potentially Not well Tolerated raw material</p> |
| <p>Mean viability value > 50 %</p> <p>and</p> <p>final [IL-1α RM release] < 50 pg/mL (two or three tests with a [IL-1α RM release] < 50 pg/mL)</p> | <p>Potentially well Tolerated raw material</p> |

Conclusion

Accordingly, under the conditions of this study, R0056896C at 2% was considered to be well tolerated after dermal application.

Ref.: 3

SCCS comment

For viability, 4 of 9 individual values were below 50% viability. The mean viability value of 57.4% is therefore borderline.

3.3.2.2. Mucous membrane irritation

Acute Eye Irritation in Rabbits (diluted material)

Guideline: OECD 405
 Species: New Zealand white rabbits
 Group size: 3 females
 Test substance: R0056896C
 Batch: R0056896C 002 L 001
 Purity: >95% (HPLC)
 Dose: 10% in water; 0.1 mL
 GLP: compliant
 Date: 2006

0.1 mL of a 10% solution of R0056896C in sterile water was instilled into the left conjunctival sac of test animals. The eyes were not rinsed following instillation of the test item. The non-treated right eye served as control. The ocular reactions were assessed 1, 24, 48 and 72 hours after instillation.

Results

No ocular changes following instillation of R0056896C at a 10% solution in water were observed at any time point in any animal.

Conclusion

Under the conditions of this study, R0056896C was non-irritating to rabbit eyes when tested at 10% solution in sterile water.

Ref.: 8

Overall SCCS comment on irritation

As a 10% solution of hydroxyethoxy aminopyrazolopyridine HCl was non-irritating to the rabbit eye, it may be anticipated that this concentration is also not irritating to skin.

3.3.3. Skin sensitisation

Local Lymph Node Assay

Guideline: OECD 429
Species: mouse
Group: 48 female CBA/J
Test substance: R0056896C
Batch: R0056896C 002 L 003
Purity: >95%
Control: negative – (vehicle) propylene glycol
positive – alpha hexyl cinnamaldehyde 25% (v/v) in propylene glycol
Dose: Experiment 1: 0.1, 1 and 10% (w/v) in propylene glycol
Experiment 2: 0.256, 0.64, 1.6, 4 and 10% (w/v)
GLP: in compliance
Date: 2007

48 female CBA/J mice were used in the main study to assess the sensitising potential of R0056896C. This assessment was made through two independent experiments using 20 and 28 animals each.

In the first experiment, animals were separated in 5 groups (4 mice/group) consisting of:

- 3 treated groups receiving R0056896C at 0.1, 1 and 10% (w/v) in propylene glycol (PG). PG was selected in a previous solubility study showing that 10% (w/v) in PG was the maximum practicable concentration. This concentration was non-irritant in a preliminary test. The test substance was not soluble in acetone, olive oil, dimethylformamide or methylethylketone.
- A negative control group receiving the vehicle (PG) alone
- A positive control group receiving alpha-hexylcinnamaldehyde (HCA) at 25% (v/v) in PG

A similar second experiment was conducted at 0.256, 0.64, 1.6, 4 and 10% (w/v) R0056896C.

In each experiment, the test item, vehicle or HCA was applied over the ears (25 µL per ear) of respective animals for three consecutive days. After 2 days of resting (day 6), mice received a single intravenous injection of tritiated methyl thymidine (³H-TdR). Draining auricular lymph nodes were sampled, pooled per group, and the proliferation of lymphocytes was evaluated by measuring the incorporation of ³H-TdR. The values obtained

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were used to calculate stimulation indices (SI), and the EC₃ was estimated in each experiment (theoretical concentration resulting in a SI of 3).

Results

SI values of 34.65 and 7.86 were obtained with the positive control in the first and second experiments, respectively.

In the first experiment, the threshold value of 3 for positive results was exceeded at 10% but without any dose-effect response (SI values of 2.39, 1.76 and 14.16 at 0.1%, 1% and 10%, respectively). The EC₃ value was calculated to be 1.9%.

In the second experiment, the threshold value of 3 for positive results was exceeded at 10% with a dose-response effect (SI values of 0.5, 0.46, 1.0, 1.85 and 3.70 at 0.256%, 0.64%, 1.6%, 4% and 10% respectively). The EC₃ value was calculated to be 7.7%.

Conclusion

Under the conditions of this study, R0056896C induced delayed contact hypersensitivity. According to the EC₃ value calculated (7.7%), R0056896C was considered to have a moderate sensitising potential.

Ref.: 11

SCCS comment

The positive control gave widely different results in the two experiments, which were properly conducted. The test concentrations of hydroxyethoxy aminopyrazolopyridine HCl were advised by the sponsor. Despite only three dilutions being used in the first experiment, the results from this experiment should be used for indicating the sensitisation potential of hydroxyethoxy aminopyrazolopyridine HCl, which is an EC₃ 1.9% when tested in propylene glycol (vehicles affect stimulation indices). Therefore hydroxyethoxy aminopyrazolopyridine HCl is classified as a strong sensitizer.

3.3.4. Dermal / percutaneous absorption

In Vitro Percutaneous Absorption Study using Human Dermatomed Skin

| | |
|-------------------------|---|
| Guideline: | OECD 428 |
| Membrane | 12 membranes from 5 human donors (frozen); 400 µm |
| Chambers: | static glass diffusion cells; 2.54 cm ² membrane area |
| Membrane integrity: | electrical resistance >10 kΩ |
| Test substance: | R0056896C |
| Batch: | R0056896C 001 P 001 |
| Purity: | >95% |
| Radiolabel: | CFQ41061[¹⁴ C]-R0056896C |
| Radiolabel purity: | 97.6% |
| Dose: | 2% R0056896C in oxidative hair dye formulation; 20mg/cm ² applied (400 µg/cm ² R0056896C) |
| Receptor: | phosphate buffered saline |
| Solubility in receptor: | / |
| Stability in receptor: | / |
| Detection: | Liquid scintillation counting |
| GLP: | in compliance |
| Date: | 2011 |

A typical oxidative hair dye formulation containing a nominal 4% R0056896C was mixed with peroxide developer (1:1, w/w) resulting in a concentration of 2% R0056896C. 20 mg/cm² of R0056896C (nominal 400 µg/cm² of R0056896C) was applied to the skin surface for 30 minutes. The skin surface was then washed with water followed by 2% sodium

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dodecyl sulphate in water. Between each set of washes the washing fluid was aspirated three times. The skin surface was washed further with water (10 x 1270 µL), after which the skin surface was dried with cotton wool swabs. Following decontamination, the cells were returned to the water bath for the remainder of the 24-hour run time. Following this, a 24 hour mass balance procedure was performed. The skin surface was washed with sponges soaked in 3% Teepol®L in water and further sponges pre-wetted with water. The *stratum corneum* was removed by a tape stripping process removing up to a maximum of 20 strips from each skin membrane. The flange skin was cut away from the dermis and the epidermis on the remaining skin disc was separated from the dermis using a heat separation technique. The penetration process was monitored using [¹⁴C]-radiolabelled R0056896C, which was incorporated into the formulation, prior to application. The receptor fluid was degassed phosphate buffered saline, in order to ensure that adequate sink conditions were maintained for this particular test material. The distribution of R0056896C within the test system was measured and a 24-hour penetration profile was determined by collecting receptor fluid samples 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 hours following application. The samples were analysed by liquid scintillation counting.

Results

None of the 12 dosed cells were rejected. Mean recovery of the applied test material was very good at 98.7%, with individual cell values ranging from 95.1% to 103% (n=12).

| Test Compartment n = 12 | µg R0056896C per cm ² | | % of applied dose | |
|----------------------------|----------------------------------|-------|-------------------|-------|
| | Mean | SD | Mean | SD |
| Donor chamber | 0.060 | 0.030 | 0.015 | 0.008 |
| Skin wash at 0.5 hours | 391 | 10.8 | 98.1 | 2.70 |
| Skin wash at 24 hours | 2.11 | 0.848 | 0.529 | 0.213 |
| <i>Stratum corneum</i> | 0.030 | 0.022 | 0.007 | 0.005 |
| Remaining epidermis | 0.111 | 0.047 | 0.028 | 0.012 |
| Dermis | 0.039 | 0.057 | 0.010 | 0.014 |
| Flange | 0.038 | 0.024 | 0.009 | 0.006 |
| Receptor fluid | 0.272 | 0.115 | 0.068 | 0.029 |
| Total non-absorbed | 393 | 10.1 | 98.6 | 2.54 |
| Systemically available | 0.422 | 0.174 | 0.106 | 0.044 |
| Total recovered | 394 | 10.2 | 98.7 | 2.56 |

The mean total non-absorbed dose (donor chamber, skin wash, *stratum corneum* and flange skin) represented 98.6% (393 µg/cm²) of the applied dose.

The mean total systemically available dose of R0056896C (remaining epidermis, dermis and receptor fluid) was 0.106% of the R0056896C applied dose corresponding to 0.422 µg/cm².

Conclusion

The results obtained in this study indicate that R0056896C at a final concentration of 2% in a typical oxidative hair dye formulation penetrated through human dermatomed skin at 0.068 ± 0.029 % (0.272 ± 0.115 µg/cm²) of the applied dose, after 24 hours.

The mean total systemically available dose of R0056896C (remaining epidermis plus dermis and receptor fluid) was 0.422 ± 0.174 µg/cm² (corresponding to 0.106 ± 0.044% of the applied dose).

Ref.: 4

SCCS comment

This was a well-performed experiment. Accordingly, the amount of hydroxyethoxy aminopyrazolopyridine HCl, at a final concentration of 2% in a typical oxidizing formulation, which is considered systemically available (lower epidermis, dermis, receptor) is taken as the mean +1SD. This is 0.60 µg/cm² (0.15 % of the applied dose). These figures may be used in calculating the MOS.

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 toxicity

Guideline: OECD 408 (1998)
Species/strain: rat / Wistar
Group size: 40 rats/sex, 10/sex/group
Test substance: IMEXINE[®] FAM
Batch: R0056896C 001 P 001
Purity: >95%
Vehicle: water
Dose levels: 0, 10, 30, 100 mg/kg bw/day
Dose volume: 10 ml/kg bw
Route: oral
Administration: gavage
GLP: in compliance
Study period: December 2009 – March 2012

The dose levels for this 13-week study were selected based on the results of a preliminary 14-day oral (gavage) study performed at 100, 200 and 1000 mg/kg bw/day. Mortality and severe clinical signs of toxicity occurred at the highest dose level of 1000 mg/kg bw/day. The intermediate and low dose levels (200 and 100 mg/kg bw/day) affected body weight gains, clinical parameters, organ weights and induced macroscopic and microscopic changes (particularly kidneys nephrosis).

The dose levels selected for the main study were 10, 30 and 100 mg/kg bw/day; the control group received the vehicle (deionised water). The test substance or vehicle was administered once daily by gavage for 7 days/week for 13 weeks in a constant dosage volume of 10 ml/kg bw. During the treatment period, signs of mortality and morbidity were recorded twice daily on working days and once daily on holidays. During the treatment period, clinical examinations were made once weekly, body weights were recorded twice a week, food consumption was recorded weekly, ophthalmoscopy was performed prior to the initiation of dosing and at the end of the treatment period, neurotoxicological evaluation (FOB) was performed during the 11th week, and haematology, blood clinical chemistry and urinalysis determinations were performed on all surviving animals on the day of scheduled necropsy (week 13). At the end of treatment period, surviving animals were killed and subjected to a complete macroscopic examination, selected organs were weighed, and a wide range of organs/tissues were collected and preserved for microscopic examination.

The microscopic examination was performed for the collected tissues/organs from the control and high dose rats killed at the end of the dosing period, for the liver and kidneys from the low and intermediate dose groups, as well as for any gross anomaly.

Results

The chemical analysis of the test suspensions administered during the study showed that achieved concentrations were close to the intended values.

No deaths were observed during the treatment period. Transient and isolated clinical signs of toxicity were noted, but were not considered by the study report authors to be treatment-related. Mean body weight and overall body weight gain, and mean food consumption in the treated groups were similar to the control group. No ocular changes were noted in the ophthalmoscopic examinations. In the FOB statistically lower values for ambulation and rearing and higher freezing times were observed in the male intermediate dose group (30 mg/kg bw/day), but in the absence of a dose-related trend these findings were not considered to be treatment-related by the study report authors.

The haematological examination revealed a statistically significantly lower red blood cell count, haemoglobin concentration and haematocrit (-11%, -10.6% and -11.9%, respectively) in high-dose males; the same differences were observed in high-dose females although of less magnitude and only statistically significant for haematocrit. Other isolated changes of low magnitude and/or not dose-related were recorded, but not considered by the study authors to be of toxicological significance.

Platelet counts were statistically significantly higher in both intermediate and high-dose males (+38% and +34%, respectively) and females (+33% and +65%, respectively). Prothrombin times were statistically significantly lower in intermediate and high-dose males (-6.9% and -7.5%, respectively) and non-statistically significantly lower in intermediate and high-dose females (-4.2% and -8.3%, respectively). These changes were considered by the study report authors to be of very small magnitude (<10%) and therefore, not considered to be treatment-related.

The clinical chemistry examination revealed dose-related, higher values of total cholesterol in both males (+24%, +38% and +43%, respectively) and females (+14%, +15% and +32%, respectively). The changes were considered by the study report authors to be small and did not reach statistical significance and were therefore, considered to be treatment-related, but not adverse. Other isolated changes and/or not dose-related were recorded, but not considered by the study authors to be treatment-related.

The urinalysis revealed higher urine volume in high-dose males (+38%) and females (+65% - statistically significant), and protein, bilirubin, urobilinogen (males only) and occult blood in the urine exceeded normal thresholds in both high-dose males and females.

Statistically significantly increased absolute and relative liver weights were observed in intermediate and high-dose males (relative to body: +19% and +29%, respectively); similar effects were also noted in females, but was only statistically significant in high-dose females. Statistically significantly higher relative kidney weights were observed in high-dose females (+13%) and statistically significantly higher absolute relative spleen weights were observed in high-dose males (relative to body: +30%).

No gross findings were observed at necropsy. Histopathological changes were observed in the liver and kidneys of high-dose animals. The main findings were degenerative changes in the hepatocytes and epithelial tubular cells of the kidneys with incidences being similar in the control and high-dose groups except for the following findings which occurred at a slightly higher incidence in the high-dose group compared to the control group: Kidney bilateral or unilateral slight diffuse vacuolar or swelling cell or tubular degeneration (both sexes), bilateral and unilateral tubular proteinaceous material in the kidneys (both sexes), and slight swelling cell degeneration or vacuolar degeneration in the liver (both sexes).

Conclusion

On the basis of the adverse kidney changes observed at 100 mg/kg bw/day in this study, the NOAEL was established at 30 mg/kg bw/day for both male and female Wistar rats.

Ref.: 12

SCCS comment

As the platelet counts were statistically significantly higher in both intermediate and high-dose females (+33% and +65%, respectively) and relative liver weights were statistically significantly increased in intermediate and high-dose males (relative to body weight: +19% and +29%, respectively), the SCCS considers the intermediate dose level as a LOAEL. The NOAEL is consequently 10 mg/kg bw/day.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity in vitro

Bacterial Reverse Mutation Test

Guideline: OECD 471 (1997)
Test system: *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and TA102
Replicates: triplicates per test concentration
Test substance: R0056896C (IMEXINE® FAM)
Solvent: purified water
Batch: R0056896C 001 P 001
Purity: > 95% pure
Concentrations: Experiment I: In the absence and presence of S9-mix 1.6, 8, 40, 200, 1000 and 5000 µg/mL:
Experiment II: In the absence and presence of S9-mix: 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL
Treatment: the standard plate incorporation and pre-incubation assay with and without Aroclor 1254 induced S9-mix
GLP: In compliance
Study period: October 2009 - January 20, 2010

The test item IMEXINE® FAM was evaluated in two independent experiments in the absence and presence of metabolic activation (S9-mix). The experiments were conducted according to the direct plating incorporation method. The second test with S9-mix was performed according to the pre-incubation method. Toxicity was evaluated for 6 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies. Appropriate positive and negative controls were used.

Results

All solvent and positive controls gave counts of revertants within expected ranges. IMEXINE® FAM did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system S-9mix.

Conclusion

Under the test conditions used IMEXINE® FAM did not induce base pair and frame shift mutations both with and without metabolic activation and is considered not to be mutagenic in the *Salmonella typhimurium* assay.

Ref.: 2

In vitro Gene Mutation Assay (*hprt*-locus)

| | |
|-----------------|---|
| Guideline: | OECD 476 (1997) |
| Species/strain: | Mouse lymphoma cell line L5178Y |
| Replicates: | Duplicate cultures per concentration, two independent experiments |
| Test substance: | IMEXINE® FAM |
| Solvent: | purified water |
| Batch No.: | R0056896C 001 P 001 |
| Purity: | > 95% pure |
| Concentrations: | Experiment 1: In the absence of S9 mix: 10, 20, 60, 80, 100, 135 and 200 µg/mL; In the presence of S9 mix 200, 400, 800, 1150, 1300, 2000 µg/mL; Experiment 2: In the absence of S9 mix: 10, 20, 40, 80, 100, 125 and 150 µg/mL; In the presence of S9 mix: 200, 400, 800, 1000, 1200, 1400 µg/mL; |
| Treatment | 3h treatment in the absence and presence S9-mix (Aroclor 1254) |
| GLP: | In compliance |
| Study period: | October 2009 – February 2010 |

Mouse lymphoma cell line L5178Y was used to evaluate genotoxicity of IMEXINE® FAM in *hprt* locus for 6-thioguanine (6-TG) resistance. Cells were treated for 3h in the absence and presence of metabolic activation S9-mix. The IMEXINE® FAM was tested in both experiments at a broad range of concentrations. A pre-test for toxicity was performed to determine the concentration range for the mutagenicity experiments measuring the plating efficiency using 6 concentrations between 71.78 to 2297 µg/ml. Complete/extreme toxicity was observed at the highest five concentrations tested in the absence of S9-mix (143.6 to 2297 µg/mL), and at the highest concentration tested in the presence of S9-mix (2297 µg/mL). Thus concentrations for mutation experiment between 10-200 µg/ml in the absence of S9-mix and 100-2000 µg/ml in the presence of S9-mix were selected. Appropriate positive and negative controls were included.

Results

Mutant frequencies in solvent negative controls fell within normal ranges, and treatment with positive controls yielded distinct increases in mutant frequency except one from two concentrations of NQO in the first experiment. No marked changes in osmolality or pH were observed in any of the concentrations used in the range finder or main experiments. The highest concentrations selected in the first experiment were 200 µg/mL in the absence of S9-mix and 2000 µg/mL in the presence of S9-mix, which gave 14% and 18% relative survival, respectively. The highest concentrations selected for genotoxicity in the second experiment were 150 µg/mL in the absence of S9-mix and 1400 µg/mL in the presence of S9-mix, which gave 15% relative survival and 14% relative survival, respectively. There were no statistically significant increases in mutant frequencies in the presence or absence of S9 mix.

Conclusion

Under the conditions of this study, IMEXINE® FAM was considered not to be mutagenic in the mouse lymphoma cells (*hprt* locus), either in the absence or presence of metabolic activation S9-mix.

Ref.: 14

SCCS comment

Only 3h treatment was conducted that might not be sufficient for inducing genotoxicity.

In Vitro Micronucleus Test in Cultured Human Lymphocytes

| | |
|-----------------|---|
| Guideline: | Draft OECD 487 (2004) |
| Species/strain: | Human peripheral blood lymphocytes from 2 male donors |
| Replicates: | Duplicate cultures in two independent experiments; 4 cultures for the solvent control, cultured human peripheral blood lymphocytes from two healthy, non-smoking female volunteers. |
| Test substance: | IMEXINE® FAM |
| Solvent: | purified water |
| Batch.: | R0056896C 001 P 001 |
| Purity: | > 95% pure |
| Concentrations: | 70, 80, 90, 100 µg/mL in 3h-treatment in the absence of S9-mix 500, 800, 1000 µg/mL in 3h-treatment in the presence of S9-mix 10, 30 and 50 µg/mL in 24h-treatment in the absence of S9-mix |
| Treatment: | phytohaemagglutinin (PHA) for 48 hours and then a 3h-treatment in the absence or presence of S9-mix, (Aroclor 1254), harvest time 21 h after the treatment; 24h-treatment without S9-mix |
| GLP: | In compliance |
| Study period: | October 2009-February 2010 |

The IMEXINE® FAM has been investigated for induction of chromosomal damage in the absence and presence of metabolic activation (S9-mix) in peripheral blood lymphocytes pooled from two male donors. Test concentrations were based on the results of a preliminary toxicity assay on cell growth inhibition relative to growth inhibition in the solvent control in order to determine the cytotoxicity of IMEXINE® FAM. Lymphocytes were exposed to 3-4 concentrations, ranging from 10 up to 1000 µg/ml. The highest concentration and test condition were selected on the basis of cytotoxicity criteria. Top concentrations were selected to yield approximately 55+/-5% cytotoxicity (reduction in replication index, RI). Cytochalasin B was added after the 3h treatments or before the 24h treatments to block cells at metaphase of mitosis. Lymphocytes were stained and examined microscopically for determining the replication index (RI) and the proportion of micronucleated binucleate cells (MNBN). Two thousand binucleate cells per concentration (one thousand from each replicate) were analysed blind. Cells were harvested 72 hours after the beginning of stimulation. Negative and positive controls were included under each treatment condition in accordance with the draft OECD guideline.

Results

Treatments in the absence of S9-mix (3+21 hour) and continuous (24+0 hour) treatment of cultures with R0056896C in the absence of S9-mix resulted in frequencies of MNBN cells that were similar to and not significantly higher than those observed in the concurrent vehicle controls for the majority of concentrations analysed. The single exception to this was noted at the highest concentration analysed post 24+0 hour treatment (50.00 µg/mL, inducing 57% cytotoxicity) where a statistically significant ($p \leq 0.01$) increase was noted. However, this increase over the concurrent vehicle control was small with the MNBN cell frequency of all R0056896C treated cultures (all concentrations, both treatment regimes) falling within historical vehicle control (normal) values. As such, the statistically significant increase observed was not considered of biological importance.

Treatment of cultures with R0056896C in the presence of S9-mix resulted in frequencies of MNBN cells that were statistically significantly ($p \leq 0.05$, $p \leq 0.001$, respectively) higher than those observed in concurrent vehicle controls for all three concentrations analysed. Concentrations of 500, 800 and 1000 µg/mL (inducing 5%, 27% and 46% cytotoxicity

Revision of the opinion on Hydroxyethoxy aminopyrazolopyridine HCl (A161)

respectively), induced dose dependent increase in mean MNBN cell frequencies (0.55%, 0.55% and 0.95% MNBN cells) compared to the concurrent vehicle control. The MNBN cell frequency of both cultures at 500 µg/mL and 800 µg/mL were within normal historical values. Both replicate cultures at 1000 µg/mL (2000 binucleate cells scored per replicate) demonstrated MNBN cell frequencies that exceeded normal values. As such, these data fulfilled the protocol criteria for a positive response. However, as the observed increase was small and occurred at a high concentration (1000 µg/mL) inducing relatively high cytotoxicity (46%), with lower concentrations analysed inducing intermediate and low toxicity (27% and 5%) showing MNBN cell frequencies within normal values, this observation (in consideration of recent IWGTP guidance on interpretation of *in vitro* cytogenetic assay data) may be considered of questionable biological importance.

Conclusion

Under the conditions of the study, IMEXINE® FAM did not induce cells with micronuclei in cultured human peripheral blood lymphocytes.

SCCS comment

The SCCS does not agree with the conclusion of the study author. Both 24 h-treatment without S9-mix as well as treatment with S9-mix demonstrated statistically significant dose dependent increase in MNBN cell frequencies that exceeded historical values. Consequently, SCCS considers this test as positive.

Ref.: 16

3.3.6.2 Mutagenicity/Genotoxicity in vivo**Bone Marrow Micronucleus Test by the oral Route in Rats****Study Design**

| | |
|------------------|---|
| Guideline: | OECD 474 (1997) |
| Species/strain: | Wistar rats |
| Group size: | 6/sex/dose level |
| Test substance: | R0056896C. (IMEXINE® FAM) |
| Batch No.: | R0056896C 010 L 001 |
| Purity: | > 97% pure |
| Dose levels: | 0, 375, 750 or 1500 mg/kg bw (24h) and 1250 mg/kg bw (48h), |
| Route: | oral, gavage |
| Vehicle: | deionized water in first experiment and sterile water in second |
| Sacrifice times: | 24 and 48 h (high dose only) |
| GLP: | In compliance |
| Study period: | January 2009 - June 2010 |

Wistar rats (6/sex/dose level) received a single oral (gavage) dose of IMEXINE® FAM at 0, 375, 750 or 1500 mg/kg in deionized water (10 mL/kg). These dose levels were selected on the basis of the results of a preliminary test where clinical signs were observed at 1500 mg/kg and higher, and 2000 mg/kg was associated with the death of 1 male and 2 females. Due to the deaths of 4 males and 4 females in the high dose group, a further experiment was performed at 1500 mg/kg. For each animal, smears were prepared from the femoral bone marrow. The nucleated cells were separated from the erythrocytes using the method of Romagna. The smears were stained with MGG/Giemsa and scored blind for the incidence of micro-nucleated polychromatic erythrocytes (MN-PCE, 2000 PCE counted) and for the polychromatic/total erythrocyte ratio (PCE/total erythrocyte ratio, 2000 erythrocytes counted). Negative and positive controls were included under each treatment conditions in accordance with the OECD guideline.

Results

When compared to controls, the incidence of micro-nucleated polychromatic erythrocytes was statistically significantly increased in animals given the positive control.

Treatment with IMEXINE[®] FAM was associated with the death of 4 males and 4 females at 1500 mg/kg in the main experiment and of one male and two females in the repeat experiment. Clinical signs were observed at 375, 1000 and 1500 mg/kg.

After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs in the vehicle control indicating that IMEXINE[®] FAM did not exert any cytotoxic effects in the bone marrow. Death and clinical signs, as well as the renal elimination of IMEXINE[®] FAM shown by urine discolouration, were considered to be evidence of systemic exposure following oral administration of IMEXINE[®] FAM.

In comparison to the corresponding vehicle controls there was no increase in frequency of cells with micronuclei at any preparation interval and dose level after administration of IMEXINE[®] FAM.

Conclusion

Under the conditions of the study, IMEXINE[®] FAM did not induce DNA damage leading to micronucleus formation in the bone marrow of rats treated orally up to the lethal dose of 1500 mg/kg and can be considered not mutagenic *in vivo*.

Ref.: 10

3.3.7. Carcinogenicity

No data submitted.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted.

3.3.8.2. Teratogenicity

| | |
|-----------------|-----------------------------|
| Guideline: | OECD 414 (2001) |
| Species/strain: | rat / Wistar |
| Group size: | 25 mated females |
| Test substance: | IMEXINE [®] FAM |
| Batch No.: | R0056896C 001 P 001 |
| Purity: | >95% |
| Vehicle: | water |
| Dose level: | 0, 10, 30, 100 mg/kg bw/day |
| Dose volume: | 10 ml/kg bw |
| Route: | oral |
| Administration: | gavage |
| GLP: | in compliance |
| Study period: | December 2009 – May 2012 |

The dose levels for this prenatal developmental toxicity study were selected based on the results of a preliminary prenatal developmental oral (gavage) study performed at 50, 100 and 200 mg/kg bw/day. Maternal toxicity (body weight loss / lower body weight gain from day 6 to day 9 associated with lower food consumption, slightly lower body weight gain

from gestation day 12 to day 20 and lower corrected body weight gain) and foetal toxicity (lower body weight) occurred at all dose levels.

The dose levels selected for the main study were 10, 30 and 100 mg/kg bw/day; the control group received the vehicle (deionised water). The test substance or vehicle was administered once daily by gavage from implantation until one day prior to the scheduled caesarean section, i.e. from gestation day 6 to day 19) in a constant dosage volume of 10 ml/kg bw. During the treatment period, mortality and clinical symptoms were recorded twice daily on working days and once daily on holidays. The observations included changes in skin and fur, eyes and mucous membranes, effects on the respiratory, circulatory, autonomic and central nervous systems, and on motor activity and behavioural patterns. Body weights were recorded on gestation days 0, 3, 6, 9, 12, 15, 18 and 20 and body weight change was calculated from these results. Food consumption was recorded on the same days except for day 0. The dams were euthanized on gestation day 20, necropsied and examined for structural abnormalities or pathological changes. The uteri and ovaries were removed, pregnancy status ascertained and the following data were recorded: weight of the unopened gravid uterus (including the cervix), number and location of live and dead foetuses, number and location of early and late resorptions, number of corpora lutea in each ovary. At necropsy, placentas and foetuses were weighed individually, and foetuses were sexed and examined macroscopically for external findings. One-half of the foetuses of each litter were examined for soft tissue alterations, and remaining foetuses were examined for skeletal alterations.

Results

The chemical analysis of the test suspensions administered during the study showed that achieved concentrations were close to the intended values.

No mortality and no clinical signs of toxicity were observed during the treatment period. Body weight gains were lower from gestation day 6 to 9 at all dose levels (only statistically significant at 30 and 100 mg/kg bw/day) and showed a dose-response relationship. Body weight gains were slightly lower from gestation day 9 at all dose levels (only statistically significant at 30 mg/kg bw/day from gestation day 18 to day 20). Overall body weight gains (from gestation day 6 to 20) were slightly lower at all dose levels (only statistically significant at 30 and 100 mg/kg bw/day) but did not show a dose-response relationship. Lower food consumption (statistically significant) was observed from gestation day 6 to 15 at 100 mg/kg bw/day and from gestation day 6 to 20 at 10 and 30 mg/kg bw/day. Corrected maternal body weight gain was statistically significantly lower in the treated groups and showed a dose-response relationship. No changes in gestation parameters or macroscopic lesions in dams were observed. A slightly lower mean foetal body weight was observed at 100 mg/kg bw/day. The external examination of the foetuses revealed no malformations or variations. No soft tissue or skeletal malformations were recorded. Soft tissue variations were observed in the kidneys (unilateral renal pelvis dilatation) in all groups including the control group, but were not considered to be treatment-related by the study report authors. A higher foetal incidence of short supernumerary ribs were observed at 30 and 100 mg/kg bw/day, but was not dose-related and therefore not considered to be treatment-related by the study report authors. Higher foetal and litter incidences of sternabrae not or incompletely ossified were observed at 30 and 100 mg/kg bw/day and showed a dose-response relationship; this finding was not considered to be adverse by the study report authors as it was associated with maternal toxicity and concerned mainly 5th and 6th sternabrae which is a common finding. The foetal ossification level revealed no differences between foetuses from treated and control dams.

Conclusion

On the basis of the results obtained in the present study, and specifically the effects on maternal body weight, the Lowest Observed Effect level (LOEL) for maternal toxicity was established at 10 mg/kg/day and the NOAEL (No Observed Adverse Effect Level) for foetal and developmental toxicity was established at 30 mg/kg/day.

SCCS comment

As the corrected maternal body weight gain was statistically significantly lower in all the treated groups (-28%, -42% and -56%, respectively) and showed a dose-response relationship, and as the magnitude of the decrease was considerable also at the lowest dose level (-28%), the SCCS considers the lowest dose level of 10 mg/kg bw/day as a LOAEL. A NOAEL for maternal toxicity could not be established based on this study.

3.3.9. Toxicokinetics

No data submitted.

3.3.10. Photo-induced toxicity

No data submitted.

3.3.11. Human data

No data submitted.

3.3.12. Special investigations

No data submitted.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(under oxidative conditions)

| | | |
|---|---------------------------|---------------------------------|
| Absorption through the skin | A | = 0.60 µg/cm² |
| Skin Area surface | SAS | = 580 cm² |
| Dermal absorption per treatment | SAS x A x 0.001 | = 0.35 mg |
| Typical body weight of human | | = 60 kg |
| Systemic exposure dose (SED) | SAS x A x 0.001/60 | = 0.0058 mg/kg bw |
| Lowest observed adverse effect level (maternal toxicity in prenatal oral developmental toxicity study) | LOAEL | = 10 mg/kg bw/d |
| Assessment factor of 3 for LOAEL to NOAEL | | = 3.33 mg/kg bw/d |
| Bioavailability 50%* | | = 1.67 mg/kg bw/d |

| | |
|-------------------------|---------------------------------|
| Margin of Safety | adjusted NOAEL/SED = 287 |
|-------------------------|---------------------------------|

* 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

The ingredient hydroxyethoxy aminopyrazolopyridine HCl will be used in oxidative hair colouring formulations at a maximum concentration of 4%, which after mixing typically in a ratio of 1:1 with hydrogen peroxide prior to use, corresponds to a concentration of 2% upon application (final, on-head concentration).

EC No. of hydroxyethoxy aminopyrazolopyridine HCl was not reported. The stability of hydroxyethoxy aminopyrazolopyridine HCl in typical hair dye formulations was not reported.

General toxicity

The maximal non-lethal dose in rats by the oral and the dermal routes was higher than 500 mg/kg bw.

In a 90-day oral (gavage) study in rats, the NOAEL was 10 mg/kg bw/day based on statistically significantly higher platelet counts in females and statistically significantly increased relative liver weights in males at the higher dose levels (30 and 100 mg/kg bw/day).

In a prenatal developmental oral (gavage) toxicity study in rats, the LOAEL was 10 mg/kg bw/day for maternal toxicity and the NOAEL was 30 mg/kg bw/day for foetal and developmental toxicity. The LOAEL of 10 mg/kg bw/day for maternal toxicity in the prenatal developmental toxicity study is used for the calculation of the MoS.

No two-generation reproduction study was submitted.

Irritation / sensitisation

Under the conditions of an *in vitro* Primary Cutaneous Tolerance study, hydroxyethoxy aminopyrazolopyridine HCl at 2% was considered to be well tolerated after dermal application. It was non-irritating to rabbit eyes when tested at 10% solution in sterile water. As 10% hydroxyethoxy aminopyrazolopyridine HCl was non-irritating to the rabbit eye, it may be anticipated that this concentration is not irritating to skin.

Based on the results from a local lymph node assay hydroxyethoxy aminopyrazolopyridine HCl is classified as a strong sensitizer (EC3 1.9% when tested in propylene glycol).

Dermal absorption

The amount of hydroxyethoxy aminopyrazolopyridine HCl, at a final concentration of 2% in a typical oxidizing formulation, which is considered systemically available (lower epidermis, dermis, receptor) is $0.42 \mu\text{g}/\text{cm}^2$. The mean +1 SD ($0.422 \pm 0.174 \mu\text{g}/\text{cm}^2$) = $0.60 \mu\text{g}/\text{cm}^2$ ($0.11 \pm 0.04\%$ = 0.15 % of the applied dose) is used for the calculation of the MoS.

Mutagenicity / genotoxicity

Hydroxyethoxy aminopyrazolopyridine HCl was tested for the three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. Both bacterial reverse mutation as well as *in vitro* mammalian gene mutation assays were negative for induction of mutants by hydroxyethoxy aminopyrazolopyridine HCl, however, in the latter test only a 3h-treatment was investigated. In *in vitro* micronucleus test, hydroxyethoxy aminopyrazolopyridine HCl induced statistically significant increase in chromosomal aberrations in human lymphocytes in the presence of metabolic activation.

According to SCCS the *in vitro* micronucleus test is positive. However, hydroxyethoxy aminopyrazolopyridine HCl did not induce cytogenetic damage leading to micronucleus formation in the bone marrow of rats treated orally up to the lethal dose of 1500 mg/kg and can be considered not mutagenic *in vivo*.

To conclude on genotoxic potential of hydroxyethoxy aminopyrazolopyridine HCl, it can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity

No data submitted.

4. CONCLUSION

The SCCS is of the opinion that, apart from its sensitising potential, hydroxyethoxy aminopyrazolopyridine HCl is safe for use in oxidative hair dye formulations with a concentration on-head of maximum 2.0%.

Hydroxyethoxy aminopyrazolopyridine HCl is a strong sensitizer.

5. MINORITY OPINION

Not applicable.

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