EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

# OPINION ON

# Climbazole

COLIPA n° P64

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

The SCCP adopted this opinion at its 19th plenary of 21 January 2009

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 Products (SCCP), 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 Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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## 1. BACKGROUND

Cosmetic products marketed in the EU may only contain those preservatives which are listed in Annex VI of the Cosmetics Directive 76/768/EEC, "List of preservatives which cosmetic products may contain".

Climbazole, with the chemical name 1-(4-chlorophenoxy)-1-imidazol-1-yl-3,3-dimethyl-2-butanone, is currently regulated in the Cosmetics Directive as a preservative in Annex VI, entry 32, with a maximum authorized concentration of 0.5%.

The preamble of the Annex states that preservatives marked with the symbol (+) may also be added to cosmetic products in concentrations other than those laid down in the Annex for other specific purposes apparent from the presentation of the products.

By way of a Commission amendment to the Cosmetics Directive in 2007 (Commission Directive 2007/17/EC1) the symbol (+) was removed from the entry for Climbazole. This amendment removed the possibility to use Climbazole in higher concentration for other specific purposes.

Moreover, in the SCCP scientific opinion (SCCP/0918/05) on Climbazole, the SCCP recommended a complete re-evaluation of the safety of this compound.

Submission II concerned the use of Climbazole as an anti-dandruff active ingredient in hair care formulations up to a maximum concentration of 2.0 % in rinse-off products and as a preservative up to a maximum concentration of 0.5 % in cosmetic products.

The present submission III requests for an additive use of Climbazole as an anti-aging agent in leave-on products in a concentration up to 0.5%.

## 2. TERMS OF REFERENCE

1) *Does the SCCP consider with the scientific data provided that Climbazole is safe for the consumers, when used as a preservative in cosmetic products up to a maximum concentration of 0.5%?*

2) *Does the SCCP consider with the scientific data provided that Climbazole is safe for the consumers, when used for non-preservative purposes as an anti-dandruff active ingredient in hair care formulations up to a maximum concentration of 2.0% in rinse-off products?*

3) *Does the SCCP consider with the new scientific data provided that Climbazole is safe for the consumers, when used for non-preservative purposes as an anti-aging ingredient in leave-on products up to a maximum concentration of 0.5%, even though this application might already be covered by (1)?*

[1] OJ L 82, 23.3.2007, p. 27.

## 3. OPINION

In its opinion of September 2005 on Climbazole (SCCP/0918/05), the SCCP formulated a comprehensive list of shortcomings related to the submitted dossier. These comments dealt with the general aspect of the file (outdated format, inconsistent reference system, full references lacking) and with its contents. Some examples:

- The identification of the compound and the physico-chemical characteristics needed to be determined on recent batches.
- The skin and eye irritation data packages were incomplete.
- There was inadequate information on skin sensitisation.
- No scientifically acceptable *in vitro* dermal absorption study was presented.
- The mutagenicity/genotoxicity data package was considered incomplete.

As the comments covered different chapters of the opinion, the whole information package is gathered in this opinion. Studies discussed in SCCP/0918/05 are briefly mentioned and the newly introduced studies are discussed in detail.
As such, this opinion deals with the full toxicological profile of Climbazole without repeating the full content of the previous SCCP opinion on the preservative. The reference list contains all references from both submissions.

### 3.1 Chemical and physical specifications

#### 3.1.1 Chemical identity

##### 3.1.1.1 Primary names and/or INCI names

Climbazole (INCI name)

##### 3.1.1.2 Chemical names

1-(4-chlorophenoxy)-1-imidazol-1-yl-3,3-dimethyl-2- butanone (IUPAC)
1-(4-chlorophenoxy)-1-(imidazol-1-yl)-3,3 dimethylbutan-2-one
1-(p-Chlorophenoxy)-3,3-dimethyl-1-(1-imidazolyl)-2-butanone
1-(4-Chlorphenoxy)-1-(1-imidazolyl)-3,3-dimethyl-2-butanon
1-(4-Chlorphenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethyl-2-butanon
2-Butanone, 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethyl

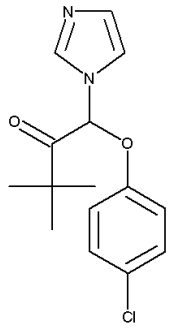
##### 3.1.1.3 Trade names and abbreviations

Trade name: Baypival®
Baysan®
Crinipan AD®
COLIPA number: P64

##### 3.1.1.4 CAS / EINECS number

CAS: 38083-17-9
EINECS: 253-775-4

3.1.1.5 Structural formula




3.1.1.6 Empirical formula

$C_{15}H_{17}ClN_2O_2$

## 3.1.2 Physical form

Solid (powder, crystals), white to pale brown

## 3.1.3 Molecular weight

292.76 g/mol

## 3.1.4 Purity, composition and substance codes

Crinipan AD®
Product N° 600306, Batch N°3: 98.3 % (HPLC method range 98-102%)

## 3.1.5 Impurities / accompanying contaminants

| | |
|---|---|
| 4-chlorophenol | not detected* |
| 2-chlorophenol | not detected* |
| 3-chlorophenol | not detected* |
| 2,4-chlorophenol | not detected* |
| 3,4-chlorophenol | not detected* |
| Sum Xylenes | < 10 ppm |
| Hexane | < 10 ppm |
| 2-ethyl-hexanol | < 10 ppm |
| Benzene | < 2 ppm |
| Toluene | < 26 ppm |
| Cyclohexane | < 10 ppm |
| Cyclohexane, methyl acetic acid ethyl ester mono chlorine | < 10 ppm |
| Octane | < 10 ppm |

The solvent traces of volatile substances were reported to be determined by gas chromatography within the equilibrated head-space above solid or liquid samples.

* by HPLC, detection limit not specified.

### 3.1.6 Solubility (at 21°C, % as w/w)

| | |
|---|---|
| Water solubility: | immiscible (about 5.5 ppm) |
| Isopropanol: | miscible |
| Cyclohexanone: | miscible |
| Benzyl alcohol: | 55% |
| Denaturated 96% vol. ethanol: | 50% |
| Phenoxyethanol: | 45% |
| 2-(2-ethoxy-ethoxy) ethanol: | 30% |
| Parfum oils: | up to 50% |
| DMSO*: | miscible |
| Polyethylene glycol 200 and 400*: | miscible |

(*determined within recent mutagenicity studies)

### 3.1.7 Partition coefficient (Log $P_{ow}$)

Log $K_{ow}$ (KOWWIN estimated): 3.76

(Software: EPIWIN v2.2, Copyright (c) William Meylan, 1994-1996, Methods were developed by Howard and Meylan)

Comment
The $P_{ow}$ strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log $P_{ow}$, without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the percutaneous absorption studies.

### 3.1.8 Additional physical and chemical specifications

| | |
|---|---|
| Melting point: | 96.8°C |
| Bulk density: | 0.76 g/ml |
| Loss on drying: | 0.09% (air oven, 2 g, 50°C, constant weight, high vacuum) |
| Organoleptic properties: | Colour: white to pale brown<br>Odour: characteristic |
| Boiling point (°C)*: | 411.96 (Adapted Stein & Brown method) |
| Vapour pressure (mm Hg, 25°C)*: | 2.1E-007 (Modified Grain Method) |

* Boiling point and vapour pressure estimations according to Software: EPIWIN v2.2 (Copyright (c) William Meylan, 1994-1996, Methods were developed by Howard and Meylan)

UV visible absorption spectrum
The absorption spectrum of Climbazole was recorded by absorption measurement in the ultraviolet (UV) and visible (VIS) range according to ISO Standard 4735. The respective spectrum is contained in the data package. Two peaks were seen at 276 nm and 283 nm, respectively.

Transmission spectrum
The transmission spectrum was recorded according to a validated internal method and is included in the submission.

Particle size
The particle size of Climbazole was determined by laser diffraction. The respective particle size spectrum is included in the data file.

Photodegradation
The effect of pH on the photodegradation kinetics of 15.8 µg/ml aqueous solutions of Climbazole was determined by means of a high-performance liquid chromatographic method for the quantification of Climbazole after irradiation of aqueous solutions using a RP-18 column with an acetonitrile - 0.05M sodiumperchlorate mobile phase. The detector was set at a wavelength of 220 nm. The calibration curve showed linear response in the interval 5 to 25 µg/ml.
Photodegradation appeared to follow first-order kinetics and was found pH-dependent. The degradation rate constant was calculated to be 10.5 x 10-$^{3}$, 9.9 x $10^{-3}$ and 16.5 x $10^{-3}$ $min^{-1}$ at pH 5, 7 and 9, respectively.

Comment
In section 3.1.6, the water solubility of Climbazole is reported to be very poor (about 5.5 µg/ml), whereas here 15.8 µg/ml aqueous solutions of Climbazole are described.

### 3.1.9 Stability

Shelf-life is indicated to be at least 2 years.

Degradation of Climbazole in aqueous diluted solutions
The thermodegradation of aqueous solutions (5.40 x $10^{-5}$ mol) of Climbazole at temperatures of 50°C, 70 °C and 90°C was examined using HPLC. The degradation followed first order kinetics at each temperature and the experiments revealed an activation energy $E_a$ of 5.4 kcal/mol.
The degradation rate constants and half-lives were as follows:

| Temperature (°C) | Degradation rate constant ($10^{-3}$) | Half-life (days) |
|---|---|---|
| 50 | 3.03 | 228.7 |
| 70 | 4.94 | 140.6 |
| 90 | 7.62 | 91.1 |

Comment
No stability test with marketed products has been performed.

## 3.2 Function and uses

Climbazole is currently regulated in the Cosmetics Directive as a preservative in Annex VI, with a maximum authorized concentration of 0.5%.

According to the submitted dossier, Climbazole is used as an anti-dandruff active agent in hair cosmetic preparations up to a maximum concentration of 2.0% in rinse-off products or up to a maximum concentration of 0.5% in leave-on products. In addition, it is used in leave-on face creams up to a maximum concentration of 0.5%.

## 3.3. Toxicological evaluation

### 3.3.1. Acute toxicity

#### 3.3.1.1. Acute oral toxicity

***Taken from SCCP/0981/05***

$LD_{50}$-oral-rat = 400 mg/kg bw (334 - 481 mg/kg bw).
$LD_{50}$-oral-mouse = 664 mg/kg bw (572 - 797 mg/kg bw).
$LD_{50}$-oral-rabbit = ± 250 mg/kg bw.
$LD_{50}$-oral-dog = 250 - 500 mg/kg bw.

#### 3.3.1.2. Acute dermal toxicity

***Taken from SCCP/0981/05***

A single publication mentions a $LD_{50}$-dermal-rat of > 5000 mg/kg bw.

#### 3.3.1.3. Acute inhalation toxicity

No data submitted

### 3.3.2 Irritation and corrosivity

#### 3.3.2.1. Skin irritation

***Taken from SCCP/0981/05:***

Only compatibility studies in human volunteers with Climbazole-containing end products (shampoos and lotions) are available, but information on the skin irritative properties of Climbazole as a compound, is lacking.

***From submission III:***
**Single patch test in human volunteers**

| | |
|---|---|
| Date of test: | Jan 2007 |
| Guideline: | Internal protocol |
| Species: | 21 human volunteers |
| Substance: | Climbazole |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| Patches tested: | Per volunteer 4 semi-occlusive patches with 0.3 ml of:<br>1) Climbazole 2% w/v in ethanol:water (75:25),<br>2) distilled water (negative control),<br>3) 1.5% w/v Sodium Lauryl Sulfate (SLS) in water (positive control),<br>4) ethanol:water (75:25) (vehicle control). |
| Measurements: | 30-60 min. and 24h after patch removal |
| QAU/GLP: | in compliance |

Climbazole was tested for potential irritation on human skin in a primary irritation patch test as a 2% solution. The test material, the negative control, vehicle control and positive control were applied to patch sites on the outer and upper regions of the right and/or the left arm.

Each volunteer received one application of the test and control material under semi-occlusive conditions for 24 hours. The sites were graded 30-60 minutes and 24 hours following patch removal.

Results
The results are summarized in the following table:

| Test material | | Grading time | |
|---|---|---|---|
| | | 30-60 minutes | 24 hours |
| 2% Climbazole in ethanol:water 75:25 | | 0.07 | 0.00 |
| Negative control | (distilled water) | 0.10 | 0.00 |
| Vehicle control | (ethanol: distilled water 75:25) | 0.05 | 0.00 |
| Positive control | (1.5% w/v SLS in distilled water) | 0.52 | 0.48 |

Conclusion
Under the conditions of this 24 hour single application human primary irritation patch test, the 2% aqueous and ethanolic solution of Climbazole did not differ from either negative control or the vehicle control in its relative skin mildness. All of them elicited average skin grades (average scores from 0.05 to 0.1) that are described as 'very mild' skin irritation at the readings after 30-60 minutes only. After 24 hours after patch removal, virtually no skin reaction was noted in any of these groups. The positive control, 1.5% SLS, elicitated skin grades that are described as 'mild' skin irritation.
Thus, Climbazole *per se* did not produce any skin irritation under the conditions of this test.

Ref.: 52

Comment
In the previous submission (2005), no data on the skin irritation potential of Climbazole were provided. Without any information on the compound's skin irritative properties and/or screening tests (e.g. Episkin®), it is not considered ethical to perform a safety study on human volunteers. If data from other sectors (e.g. pharmaceuticals, biocides) are available, these can also be referred to.

### 3.3.2.2. Mucous membrane irritation

***Taken from SCCP/0981/05:***

A 1972 Draize study with 0.5% Climbazole in PEG-400 was considered non-irritating to the rabbit eye. However, the test was not performed according to current standards, wherefore the results cannot be considered reliable.
In addition, some *in vivo* (Draize) and *in vitro* (HET-CAM, NR and RBC studies) were performed with Climbazole-containing shampoos. The shampoos have all been found to be irritating to the eye, but, due to the presence of SLS, these tests were not found of relevance for the evaluation of the eye irritation potential of Climbazole.

***From submission III***

**HET-CAM assay**

| | |
|---|---|
| Date of study: | Feb 2007 |
| Guideline: | INVITTOX Protocol N°47: HET-CAM Test |
| Species/strain: | Lohmann Selected Leghorn chicken |
| Replicates: | 6 eggs for the test substance |
| | 3 eggs per negative/positive control substance |
| Test substance: | Climbazole |
| Batch: | Batch N°3 |
| Purity: | 98.3% |

Dose level: 100 mg applied for 5 minutes
Negative control: 0.9% NaCl in water
Positive controls: 1% SDS, 0.1N NaOH in water
GLP/QAU: In compliance

In this Hen's Egg Chorioallantoic Membrane (HET-CAM) test, 15 fertilized eggs were incubated for 9 days, after which they were opened to expose the CAM. In 6 of the eggs, 100 mg neat Climbazole was applied for 300 seconds guaranteeing that at least 25% of each membrane was covered. Negative and positive control substances were each applied to the membranes of 3 eggs.
Endpoints investigated were haemorrhage, coagulation and blood vessel lysis. The time until occurrence of these three endpoints was recorded and the mean irritancy index was calculated.

Results
No haemorrhage, lysis or coagulation was observed for Climbazole. The severe irritancy of the positive control substances was confirmed by high scores.

Conclusion
It was concluded that, under the experimental conditions reported, Climbazole did not exhibit any ocular irritation potential *in vitro* when tested neat in the HET-CAM assay.
Ref.: 43

**CEET assay**

Date of study: Apr 2007 (date of report)
Guideline: Prinsen MK (1996 and 2006) and internal protocol
Species/strain: chicken eyes
Replicates: 3
Test substance: Climbazole
Batch: not stated
Purity: not stated
Negative control: vehicle (propylene glycol)
Positive controls: 1% SDS, 0.1N NaOH
Dose level: 0.5-1.0-2.0% Climbazole in propylene glycol
GLP/QAU: no signed documents available

In this *in vitro* Chicken Enucleated Eye Test (CEET), chicken eyes obtained from slaughterhouse animals for human consumption were exposed to a single application of 30 µl of several dilutions of Climbazole in propylene glycol for 10 seconds. The vehicle served as negative control.
Parameters investigated were corneal thickness (expressed as swelling), corneal opacity and fluorescein retention of damages epithelial cells. In addition, histopathology was performed on the cornea to assess the nature and the depth of the possible injury.

Results
The vehicle and all three concentrations of Climbazole caused comparable corneal effects, consisting of slight swelling, slight opacity and slight fluorescein retention. Microscopic evaluation revealed slight epithelial erosions. The individual results of the positive control substance were not stated.

Conclusion
All mixtures tested (as well vehicle as Climbazole solutions) showed to be Category II irritants (slightly irritating to the eyes). As the vehicle alone already caused these effects, the study authors conclude that Climbazole did not contribute to the slight eye irritative effect of the formulations tested.

Ref.: 44

**Comment with regard to the performed in vitro eye irritation tests**
At present, the HET-CAM assay has been accepted as a replacement method to identify strong eye irritants. Also, the CEET is only accepted as a screening assay. Therefore the negative results obtained do not exclude a slight irritative potential for Climbazole.
Nevertheless, from an ethical point of view, a new *in vivo* study does not appear necessary. Based upon the skin irritation results, an old Draize eye irritation test and the newly obtained *in vitro* results, 2% Climbazole is not expected to cause eye irritation in humans.

### 3.3.3. Skin sensitisation

***Taken from SCCP/0981/05:***

A Magnusson Kligman Guinea Pig Maximisation test (1983) was available, though could not be considered as valid, as the doses had been wrongly chosen. The mentioned Buehler test was not accompanied by its full test description, which made it impossible to assess its results.

***From submission III***

**Local Lymph Node Assay**

| | |
|---|---|
| Date of test: | Aug 2006 |
| Guideline: | OECD TG 429, Annex V to Dir. 67/548/EEC, Method B.42: Skin sensitisation: Local Lymph Node Assay |
| Species: | CBA/J mouse |
| Group: | 5 animals per dosage group |
| Doses tested: | 1-5-10-20% Climbazole in DMSO |
| Negative controls: | acetone:olive oil 4:1, DMSO |
| Positive control: | 35% α-hexylcinnamaldehyde in acetone:olive oil 4:1 |
| Substance: | Climbazole |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| QAU/GLP: | in compliance |

A Local Lymph Node Assay was performed to investigate the sensitisation potential of Climbazole. 4 groups of animals were each treated with one of the 4 test substance concentrations ranging from 1 to 20% Climbazole. During the induction phase, the test item was applied over the dorsal surface of each ear (25 µl per ear) for 3 consecutive days (days 1, 2, 3). On day 6, each animal was administered with radio-labelled thymidine ($^3$HTdR) by intravenous injection. After 5 hours, the animals were euthanized and the draining lymph nodes were excised and pooled to prepare single cell suspension for each animal. $^3$HTdR incorporation was measured by scintillation counting. The proliferative response of lymph node cells was expressed as the ratio of $^3$HTdR incorporation into lymph node cells of treated animals relative to that recorded in control animals.
Positive and negative controls were concurrently tested.

Results
Climbazole did not lead to any overt sign of toxicity. Very slight erythema was noted on the ears of the animal treated with 1% Climbazole on day 4 and on the ears of the animals treated with 5 to 20% on days 3 and 4.
The lymph nodes of the mice of the vehicle control and all Climbazole-treated groups were normal in size and appearance.

The simulation indexes were as follows:

| Test substance | Stimulation index (SI) |
|---|---|
| Climbazole 1% | 0.91 |
| Climbazole 5% | 0.76 |
| Climbazole 10% | 1.19 |
| Climbazole 20% | 1.08 |
| Pos. control | 7.77 |

Conclusion
It is concluded that, under the conditions investigated, Climbazole at concentrations up to 20% exhibits no potential to induce dermal sensitisation in this LLNA, as its stimulation index does not exceed 3 for any of the concentrations tested.

Ref.: 7

### 3.3.4. Dermal / percutaneous absorption

***Taken from SCCP/0981/05:***

Although an *in vitro* dermal absorption study was available, its results could not be used for further calculations as the scientific validity of the test was questionable.

***From submission III:***

***In vitro* percutaneous absorption (human skin) with Climbazole-shampoo**

| | |
|---|---|
| Date of test: | Apr-May 2006 |
| Guideline: | OECD TG 428: Skin absorption: *In vitro* method. |
| Species: | human |
| Skin samples: | 12 dermatomed (400 µm) skin samples from 4 volunteers, + 2 control samples |
| Substance: | Climbazole |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| Test formulation: | 2% radiolabelled Climbazole in 'standard shampoo formulation' (full composition stated in report) |
| Dose applied: | 10 mg/cm² |
| Exposure time: | 30 minutes (then washed off), skin remains unoccluded |
| Sampling period: | 24h (0-0.5-1-2-4-6-24h) |
| Receptor fluid: | 4% w/v polyoxyethylene-20-oleyl ether in phosphate buffered saline (PBS); pre-check of Climbazole solubility performed (up to 4%) |
| QAU/GLP: | in compliance |

Climbazole was investigated for its skin penetration *in vitro* in a 2% shampoo formulation under rinse-off conditions. Dermatomed human skin (400 µm) from 4 female donors was processed, put on static Franz diffusion cells (skin temperature = 32 ± 1°C) and treated with 10 mg/cm² radiolabelled Climbazole formulation. The amount of radioactivity and its homogeneity in the shampoo was measured by LSC and the stability in the formulation was established.

The receptor fluid (4% w/v polyoxyethylene-20-oleyl ether in PBS) ensured that the test substance freely proportioned from the skin membrane. The test material was spread over the surface using small glass rods and was not occluded for the duration of the whole period (24h). After 30 min. contact, a sample of receptor fluid was taken and the skin surface was washed. Thereafter, sampling continued at regular times. After the final receptor fluid sample had been taken, the remaining fluid in the receptor chamber was discarded and the surface of the skin was washed again. Successively, deeper layers of the SC were removed

by adhesive tapes to a maximum of 5 strips. The tape strips were digested and analysed by LSC. The remaining skin was carefully removed from the receptor chamber and analysed.

Results
The quantities that penetrated during the 30 minute exposure to Climbazole-containing shampoo and within the 48 hours after application are shown in the following table. Both the amounts absorbed and penetrated are taken as systemically available.

| ANALYSED SAMPLE | Climbazole measured [µg/cm²] | [% of dose] |
|---|---|---|
| Donor chamber | 0.173 ± 0.187 | 0.088 ± 0.094 |
| Skin wash (0.5h) | 192 ± 8.84 | 96.9 ± 4.476 |
| Skin wash (24h) | 1.32 ± 1.66 | 0.669 ± 0.841 |
| Stratum corneum | 0.149 ± 0.103 | 0.075 ± 0.052 |
| Remaining epidermis | 0.216 ± 0.151 | 0.109 ± 0.077 |
| Receptor fluid | 0.081 ± 0.076 | 0.041 ± 0.038 |
| **Bioavailable** | **0.297 ± 0.209** | **0.150 ± 0.106** |

Conclusion
In this *in vitro* dermal penetration study in human skin, the amount of Climbazole systemically available from a standard shampoo formulation containing 2% of the preservative was found to be 0.297 µg/cm² (0.15%).

Ref.: 42

### *In vitro* percutaneous absorption (pig skin) with Climbazole in leave-on formulations

Date of test: Feb-March 2008
Guideline: OECD TG 428: Skin absorption: *In vitro* method.
Species: pig
Skin samples: 12 dermatomed (400 µm) skin samples from 12 pigs per test formulation.
Substance: Climbazole
Batch: Batch N°425
Purity: 98.8%
Test formulations: Hair Serum (aqueous hair lotion) and Skin Serum (complex w/o emulsion), both containing 0.5% Climbazole
Dose applied: 10 mg/cm²
Exposure time: 24h
Sampling period: 24h (0-0.5-4-8-12-16-20-24h)
Receptor fluid: phosphate buffered saline (PBS)
QAU/GLP: in compliance

Climbazole was investigated for its skin penetration *in vitro* at 0.5% in the formulations Hair Serum and Skin Serum under 24h leave-on conditions.
Dermatomed skin (400 µm) from 24 pigs was processed, put on static Franz diffusion cells (skin temperature = 32 ± 1°C) and treated with 10 mg/cm² Climbazole formulation. Before that, the integrity of the skin was ascertained by conductivity measurements (acceptable range < 900 µS/cm). Positive and negative controls with Benzoic acid and 2-Ethylhexyl *trans*-4-methoxycinnamate are described to be used to check the performance of the skin penetration system every 3 - 4 months. The sufficient solubility of Climbazole in the receptor solution (PBS) and in the extraction solution is stated to be analytically demonstrated and guaranteed to be ≤ 4 µg/ml.

The test material was applied on the skin surface and left in contact for 24h without occlusion. After 30 min. contact, a first sample of receptor fluid was taken, where after sampling continued at regular times. After the final receptor fluid sample had been taken, the remaining fluid in the receptor chamber was discarded and the surface of the skin was washed. Successively, deeper layers of the SC were removed by adhesive tapes to a maximum of 5 strips. The tape strips were digested and analysed by HPLC. The remaining skin was carefully removed from the receptor chamber and analysed.

Results
The quantities that penetrated during the 24h exposure to Hair Serum and Skin Serum are shown in the following table. Both the amounts in the receptor fluid and the amounts in remaining epidermis + dermis are taken as systemically available.

*Hair serum (0.5% Climbazole)*

| ANALYSED SAMPLE | Climbazole measured (n=9)* [µg/cm²] | [% of dose] |
|---|---|---|
| Receptor fluid | 0.746 ± 0.471 | 1.51 ± 0.85 |
| Stratum corneum | 0.060 ± 0.001 | 0.125 ± 0.018 |
| Remaining epidermis + dermis | 0.356 ± 0.194 | 0.723 ± 0.366 |
| Washing solution | 45.4 ± 7.56 | 92.9 ± 9.83 |
| **Bioavailable** | **1.10 ± 0.619** | **2.23 ± 1.12** |

* For 3 skin samples, the mass balance was not within the (100 ± 15)% range. Therefore, the results of those samples were not used in further calculations.

*Skin serum (0.5% Climbazole)*

| ANALYSED SAMPLE | Climbazole measured (n=9)* [µg/cm²] | [% of dose] |
|---|---|---|
| Receptor fluid | 0.628 ± 0.264 | 1.75 ± 0.718 |
| Stratum corneum | 0.102 ± 0.120 | 0.284 ± 0.329 |
| Remaining epidermis + dermis | 0.617 ± 0.490 | 1.71 ± 1.33 |
| Washing solution | 34.2 ± 3.37 | 95.2 ± 6.03 |
| **Bioavailable** | **1.25 ± 0.469** | **3.46 ± 1.25** |

* For 3 skin samples, the mass balance was not within the (100 ± 15)% range. Therefore, the results of those samples were not used in further calculations.

Conclusion
In this *in vitro* dermal penetration study in pig skin, the mean amounts of Climbazole systemically available from a hair serum and skin serum leave-on formulation, both containing 0.5% of the preservative, were found to be 1.10 µg/cm² (2.23%) and 1.25 µg/cm² (3.46%), respectively.

Ref.: 117

## 3.3.5. Repeated dose toxicity

***Taken from SCCP/0981/05:***

The original submission contained subacute oral studies with rats and dogs, a subacute dermal toxicity study with the rabbit and subchronic oral studies with rats and dogs. The main problem was that the dossier suffered from the age of the studies. Nearly all of them were performed between 1975 and 1983, before GLP-regulations were in place. The descriptions were brief and the raw data incomplete. For ethical reasons and after a thorough re-examination of the available information, the SCCP proposed to accept the use of a cautious NOEL-value of 5 mg/kg bw/day, deduced from the 90d oral study with the rat.

## 3.3.6. Mutagenicity / Genotoxicity

***Taken from SCCP/0981/05:***

The SCCP described the presented tests as old and not performed according to recent guidelines. Although they all showed negative results, the SCCP recommended performing the currently defined basic set of mutagenicity tests as carcinogenicity data were not available either. The applicant followed this advice and introduced the following mutagenicity/genotoxicity data package.

### 3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

**Ames test**

| | |
|---|---|
| Date of study: | May-Jun 2006 |
| Guideline: | OECD TG 471 |
| Species/strain: | *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537<br>*Escherichia coli* WP2 *uvr*A (pKM101) |
| Replicates: | 2 for the preliminary and main study<br>3 for the confirmatory study and retest |
| Test substance: | Climbazole |
| Vehicle used: | Dimethylsulfoxide (DMSO),<br>except for positive control sodium azide (diluted in water) |
| Batch: | Batch n°3 of 600136 |
| Purity: | 98.3% |
| Concentrations: | Preliminary study: 25-50-100-250-500-1000-2000-5000 µg /plate<br>Initial study: 1.5-5.0-15-50-150-500-1500-5000 µg/plate<br>Main study: 50-100-250-1000-2000-5000µg/plate for *Escherichia*<br>5.0-10-25-50-100-250-500 µg/plate for *Salmonella*<br>Retest main study: 1.5-5.0-15-50-150-500-1500-5000 µg/plate |
| GLP/QAU: | In compliance |

The strains were exposed to the test material dissolved in DMSO on plated agar in the presence and absence of rat liver metabolic activating system (S9 mix prepared from livers of male Sprague-Dawley rats that had received a single intraperitoneal injection of Aroclor 1254 five days before). The concentrations tested ranged from 1.5 to 5000 µg/plate. DMSO alone served as negative control. As a positive standard requiring metabolic activation 2-aminoanthracene was used. 2-Nitrofluorene (for TA98), sodium azide (for TA100 and TA1535), 9-aminoacridine (for TA1537) and methyl methanesulfonate (for WP2 *uvr*A) were used as positive controls without metabolic activation.

Results
In the preliminary mutagenicity assay, the dose levels tested were 25, 50, 100, 250, 500, 1000, 2000 and 5000 µg per plate. Precipitate was observed at 2000 or at 5000 µg per plate. Toxicity was observed at 250, 500, 1000 or 2000 µg per plate.

In the main mutagenicity assay, an increase in the number of revertants was not observed. The dose levels tested were 50, 100, 250, 500, 1000, 2000 and 5000 µg per plate with tester strain WP2 uvrA (pKM101) and 5.0, 10, 25, 50, 100, 250 and 500 µg per plate with all *Salmonella* tester strains. Precipitate was observed at 500, 2000 or at 5000 µg per plate with some test conditions. Toxicity was observed at 500, 2000 or at 5000 µg per plate with tester strain TA98 in the absence of S9 activation and tester strain WP2 uvrA (pKM101) in the presence and absence of S9 activation. No toxicity was observed with the remaining test conditions; therefore, those test conditions were retested.
In this main study retest, toxicity was only observed at 1500 µg per plate. No mutagenic effects were noted.

Conclusion
It was concluded that, under the conditions of this study, Climbazole was found negative in the Ames test with *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A (pKM101) in the presence and absence of Aroclor-induced rat liver S9.

Ref.: 46

### *In vitro* mammalian cell gene mutation test

| | |
|---|---|
| Date of study: | Jun-Jul 2006 |
| Guideline: | OECD TG 476 |
| Species/strain: | L5178YTK$^{+/-}$ mouse lymphoma cell line |
| Replicates: | 2 |
| Test substance: | Climbazole |
| Vehicle used: | Dimethylsulfoxide (DMSO) |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| Concentrations: | Preliminary toxicity study 1 (4h exposure): |
| | ± S9 mix: 0.5-1.5-5-15-50-150-500-1500-3000 µg/ml |
| | Preliminary toxicity study 2 (24h exposure) |
| | ± S9 mix: 0.5-1.5-5-15-50-150-500-1500-3000 µg/ml |
| | Cloning study (4h exposure) |
| | +S9 mix: 10-25-30-50-60 µg/ml |
| | -S9 mix: 10-25-30-50-60-80 µg/ml |
| | Repeat cloning study (4h exposure) |
| | +S9 mix: 40-60-65-70-72.5-75 µg/ml |
| | Repeat cloning study (24h exposure) |
| | -S9 mix: 5-10-15-17.5-20 µg/ml |
| GLP/QAU: | In compliance |

Climbazole was investigated for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells with and without metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Test concentrations and exposure times were based on the level of toxicity. Methyl methanesulfonate and 7,12-Dimethyl-benz(a)anthracene served as positive controls for the cultures without and with metabolic S9 activation, respectively.

Results
In the preliminary toxicity assay visible precipitate was observed in concentrations ≥ 150 µg/ml in treatment medium at the beginning of treatment and ≥ 500 µg/ml at the end of treatment.
In the experiments with 4 hour exposure, concentration-related increases in mutant frequencies over those of the solvent controls, were not observed in the non-activated or S9-activated systems. However, in the initial experiment the reduction in total growth was 36 and 35% without and with S9 mix respectively and thus the appropriate level of toxicity (10-20% survival in the highest concentration tested) was not reached.

In the main experiment with 24-hour exposure (-without S9 mix), a biological relevant and dose dependent increase in the mutant frequency was observed. In this experiment the appropriate level of toxicity (10-20% survival in the highest concentration tested) was reached.

Conclusion
It was concluded that, under the conditions of the study, Climbazole induced gene mutations at the *tk* locus of mouse lymphoma cells after 24 h exposure in the absence of

metabolic activation and thus Climbazole is mutagenic in this *in vitro* gene mutation assay in mammalian cells.

Ref.: 48

### *In vitro* micronucleus test

| | |
|---|---|
| Date of study: | May-Sep 2006 |
| Guideline: | OECD Draft TG 487: *In vitro* mammalian cell micronucleus test |
| Species/strain: | human peripheral blood lymphocytes (pooled blood from two donors) |
| Replicates: | 3 independent experiments |
| Test substance: | Climbazole |
| Vehicle used: | Dimethylsulfoxide (DMSO) |
| Batch: | Batch N°3 of 600136 |
| Purity: | 98.3% |
| Concentrations: | *Experiment I*:<br>- 20, 40 and 60 µg/ml without metabolic activation for 20 h, 24h after PHA stimulation, 28h recovery;<br>- 160, 180 and 200 µg/ml with metabolic activation for 3 h, 24h after mitogen stimulation, 45h recovery.<br>*Experiment II*:<br>- 40, 60 and 80 µg/ml without metabolic activation for 20 h, 48h after PHA stimulation, 28h recovery;<br>- 100, 175 and 200 µg/ml with metabolic activation for 3 h, 48h after mitogen stimulation, 45h recovery.<br>*Experiment III*:<br>- 30, 60 and 85 µg/ml without metabolic activation for 20 h, 24h after PHA stimulation, 28h recovery;<br>- 30, 50 and 85 µg/ml without metabolic activation for 20 h, 48h after PHA stimulation, 28h recovery;<br>- 170, 190, 200 and 210 µg/ml with metabolic activation for 3 h, 48h after mitogen stimulation, 45h recovery. |
| GLP/QAU: | In compliance |

Climbazole was investigated for the induction of micronuclei in cultured human lymphocytes 24 h (experiments I and III) and 48 h (experiments II and III) after mitogen stimulation with phytohaemagglutinin (PHA). The experiments were performed with and without liver S9 fraction from Aroclor 1254-induced rats as an exogenous metabolic activation system. Concentrations were selected on the basis of preliminary tests. Toxicity was determined by measuring reduction in replication index (RI). 4-Nitroquinoline 1-oxide (NQO) and Vinblastine (VIN) were employed as clastogenic and aneugenic positive control chemicals, respectively, in the absence of liver S9. Cyclophosphamide (CPA) was employed as a clastogenic positive control chemical in the presence of liver S9.

Results
Treatment of cells with Climbazole in the presence of metabolic activation (S9) did not result in frequencies of micronucleated binucleate (MNBN) cells that were significantly ($p \leq 0.05$) different from those observed in the concurrent controls for the majority of concentrations analysed. The one exception to this was a small but statistically significant increase at the highest concentration analysed (200.0 µg/ml, inducing 63% cytotoxicity) following treatment in Experiment II.

However, this increase was small (mean MNBN cell frequency = 1.1% compared to 0.55% in the concurrent control), with numbers of MNBN cells exceeding historical vehicle control (normal) data present in just one of the two replicate cultures analysed. The MNBN cell frequency of all other Climbazole treated cultures fell within the observed historical control ranges. In order to clarify the small increase observed in Experiment II, a third 3+45 hour

+S9 treatment was performed in Experiment III (following 48 hour PHA stimulation). Concentrations selected for analysis encompassed a similar range as analysed in

Experiments I and II, up to the maximum practicable concentration that could be analysed. The analysed concentrations of 170.0, 190.0, 200.0 and 210.0 µg/ml induced 12%, 29%, 41% and 47% reduction in RI respectively. Frequencies of MNBN cells showed to be not significantly ($p \leq 0.05$) different from those observed in concurrent vehicle controls for all concentrations analysed. The MNBN cell frequency of all Climbazole treated cultures fell within historical vehicle control (normal) values.
Treatment of cells with Climbazole in the absence of S9 did not result in frequencies of MNBN cells, which were significantly different from, those observed in concurrent vehicle control cultures for all concentrations analysed. The MNBN cell frequency of the majority of Climbazole treated cultures analysed fell within historical vehicle control (normal) ranges. The single exception to this was observed in a single treated culture at the highest concentration analysed in Experiment II (80.00 µg/ml) where the MNBN cell frequency marginally exceeded the 95% reference range, but was still within the observed historical range. Given that the increase was small, restricted to a single culture and that there was no statistical elevation as compared to the concurrent control, this observation was not considered of biological importance.
Nevertheless, in an attempt to ensure that concentrations were analysed up to those inducing as close to 60% cytotoxicity as possible, further 20+28 hour –S9 treatments (following 24 and 48 hour stimulation with PHA) were also performed in the third experiment (Experiment III). Maximum concentrations analysed in Experiment III were 85.00 µg/ml that induced either 59% cytotoxicity (24 hour PHA) or 63% cytotoxicity (48 hour PHA).
Frequencies of MNBN cells were not significantly ($p \leq 0.05$) different from those observed in concurrent vehicle controls for the majority of concentrations analysed. The single exception to this was observed at the highest concentration analysed (85.00 µg/ml) following 24 hour PHA treatment. However, as both replicate cultures at this concentration (and all other Climbazole treatments) exhibited MNBN frequencies that fell within historical vehicle control (normal) ranges, this increase was not considered of biological importance.

Conclusion
It is concluded that Climbazole did not induce micronuclei in cultured human peripheral blood lymphocytes following treatment in the absence and presence of a rat liver metabolic activation system (S9) in three independently performed experiments.

Ref.: 47

| 3.3.6.2 Mutagenicity/Genotoxicity *in vivo* |
|---|

***In vivo* micronucleus test**

| | |
|---|---|
| Date of study: | Jan-Feb 2007 |
| Guideline: | OECD TG 474, Annex V to Dir. 67/548/EEC, Method B.12: Mutagenicity: *In vivo* mammalian erythrocyte micronucleus test |
| Species: | ICR mice |
| N° of animals: | 3 male and 3 female mice per dose in dose-range finding studies<br>5 male mice per dose group and sacrifice time in main study |
| Sacrifice times: | 3 days post-dose in dose-range finding studies<br>24h post-dose for all micronucleus test doses<br>48h post-dose for vehicle and high dose of micronucleus test |
| Test substance: | Climbazole |
| Vehicle used: | Polyethylene glycol 200 (PEG 200) |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| Doses tested: | Dose-range finding study 1:<br>50-100-300-400-500-1000 mg/kg bw |

| | |
|---|---|
| | Dose-range finding study 2:<br>100-150-200 mg/kg bw<br>Micronucleus test (main study):<br>37.5-75-150 mg/kg bw |
| GLP/QAU: | In compliance |

Climbazole was investigated for the induction of micronuclei in the bone marrow polychromatic erythrocytes (PCEs) of mice. Negative (vehicle) and positive (Cyclophosphamide monohydrate) controls were in accordance with the OECD guideline. The test article was formulated in PEG 200 and administered once. Its final dose selection was based on two dose-range-finding assays.
The proportion of PCEs among all erythrocytes was used as a measure of bone marrow toxicity.

Results
Based on mortality and severity of the clinical signs observed at doses between 150 and 1000 mg/kg bw in the dose-range finding assays, doses of 37.5, 75 and 150 mg/kg bw were selected and tested in the micronucleus test. Since no differences in the toxicity between male and female mice were observed in the dose-range finding assays, the micronucleus test was conducted using male mice only. In the main study, one male died at 150 mg/kg bw 24 hours post-dose. Hyperactivity was observed at 37.5, 75 and 150 mg/kg bw and piloerection was seen at 75 and 150 mg/kg bw. Reductions in mean (group) body weights, up to 10%, were observed in some of the test article-treated groups.
Bone marrow analysis revealed no appreciable reductions in the ratio of PCEs to total erythrocytes in the test article-treated groups relative to the respective vehicle control groups. However, the clinical signs described above indicated sufficient systemic exposure. Neither was there any significant increase in the incidence of micronucleated PCEs in any of the test article-treated groups relative to the respective vehicle control groups. The positive control induced a significant increase in the incidence of micronucleated PCEs.

Conclusion
It is concluded that a single oral administration of Climbazole at doses up to and including the maximum tolerated dose of 150 mg/kg bw did not induce a significant increase in the incidence of micronucleated PCEs in bone marrow of male ICR mice. Therefore, Climbazole was shown to reveal no genotoxic potential *in vivo* in the mouse micronucleus assay, when tested up to the maximum tolerated dose level.

Ref.: 49

### *In vivo* Unscheduled DNA Synthesis

| | |
|---|---|
| Date of study: | Jan-Feb 2007 |
| Guideline: | OECD TG 486, Annex V to Dir. 67/548/EEC, Method B.39: Mutagenicity: Unscheduled DNA Synthesis test with mammalian liver cells *in vivo* |
| Species: | Sprague Dawley rat |
| N° of animals: | 3 male rats per dose group and sacrifice time |
| Sacrifice times: | Dose-range finding study: 48h following dosing<br>Main study: 2h to 4h and 12h to 16h following dosing |
| Test substance: | Climbazole |
| Vehicle used: | Polyethylene glycol 200 (PEG 200) |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| Doses tested: | Dose-range finding study: 0-100-200-400-600-800 mg/kg bw<br>Main study: 100-200 mg/kg bw |
| GLP/QAU: | In compliance |

Climbazole was investigated for the *in vivo* induction of DNA damage, as measured by unscheduled DNA synthesis, in hepatocytes of rats. Negative (vehicle) and positive (dimethylnitrosamine) controls were included according to the guidelines.

Results
Based on mortality and clinical signs observed in the dose-Range Finding Study, 200 mg/kg bw was determined to be the maximum tolerated dose and doses of 100 and 200 mg/kg bw were selected for the UDS test. There were no significant differences in toxicity between males and females so the UDS test was conducted in males.
In the main study, there were no clinical signs of toxicity observed following dosing, or at the 2 to 4 hour or 12 to 16 hour harvest times. No significant increases in the group mean net nuclear grain (NG) count for animals treated with Climbazole were observed at any harvest time. The positive control group mean NG counts were significantly increased relative to the vehicle control.

Conclusion
The study authors conclude that, under the described test conditions, Climbazole did not induce a significant increase in the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the negative control) in hepatocytes isolated from treated male rats. Therefore they conclude that Climbazole was found negative in the *in vivo* UDS test in rats, when tested up to the maximum tolerated dose level.

Ref.: 50

### 3.3.7. Carcinogenicity

No data submitted

### 3.3.8. Reproductive toxicity

***Taken from SCCP/0981/05:***

A 1-generation reproduction study was presented, though its relevance was questioned.
With regard to the teratogenic effects of the substance, the SCCP opinion mentions a study of 2001 providing a useful NOAEL (embryotoxicity) of 30 mg/kg bw/day and a NOAEL (maternal toxicity) of 15 mg/kg bw/day.

### 3.3.9. Toxicokinetics

***Taken from SCCP/0981/05:***

*In vivo* metabolic disposition studies in humans, dogs, rats and mice were presented in the previous SCCP submission. They showed that Climbazole appeared to be rapidly metabolised into its major metabolite through first pass metabolism. Excretion mainly occurred through the bile. Again, the presented data package contained old tests, which reduced their scientific validity. The SCCP was of the opinion that, since the mother compound was metabolised, the metabolite BAY g 6975 should have also been considered in the safety assessment.

***From submission III:***

**Oral bioavailability of $^{14}$C Climbazole in mice**

| | |
|---|---|
| Date of test: | Mar - Apr 2007 |
| Guideline: | OECD TG 417: Toxicokinetics was 'considered'. |
| Species: | CD-1 mouse (male) |
| N° of animals: | 3 animals per terminal blood sample + 3 spare animals |

| | |
|---|---|
| Substance: | Climbazole, radiolabelled |
| Batch: | Batch N°3 |
| Purity: | 98.3% |
| Dose level: | 150 mg/kg bw, single oral dose (10 ml/kg bw of Climbazole in PEG 200) |
| Blood sampling: | 0.25-0.5-1-2-4-8-24h post-dose |
| QAU/GLP: | in compliance |

The bioavailability of Climbazole in the plasma following a single oral administration of $^{14}C$ Climbazole to male CD-1 mice was investigated. 21 males received a single administration of 150 mg/kg bw by oral gavage.
During dosing and the times of sample collection, the animals were observed for clinically relevant abnormalities. Terminal blood samples were collected from the anesthetized animals (3 animals / time point) at 0.25, 0.5, 1, 2, 4, 8 and 24 h after administration via intracardiac puncture. The blood was centrifugated to obtain plasma, which was subsequently analysed for total radioactivity by means of liquid scintillation counting (LSC).

Results
$^{14}C$ Climbazole was detected in the plasma as early as 15 minutes after administration. The highest mean concentration of radioactivity in the plasma was observed 8 hours after administration. Thereafter the concentration declined. 24 hours after oral gavage, small amounts could be detected.

Conclusion
According to the study authors, the results clearly show that Climbazole is rapidly absorbed and bioavailable in the plasma of male mice, when administered as a single oral dose of 150 mg/kg bw.

Ref.: 51

### 3.3.10. Photo-induced toxicity

No data submitted

### 3.3.11. Human data

The newly provided single human patch test has been addressed in Section 3.3.2.1.

### 3.3.12. Special investigations

No data submitted

### 3.3.13. Safety evaluation (including calculation of the MoS)

**Calculation of the Margin of Safety**

**Calculation for the use of Climbazole at 2% as an anti-dandruff agent in shampoo**

| | | | |
|---|---|---|---|
| **Dermal absorption through human skin** | **A (µg/cm$^2$)** | **=** | **0.297 µg/cm²** |
| **Skin Area surface (hand + ½ head)** | **SAS (cm$^2$)** | **=** | **1440 cm$^2$** |
| **Typical body weight of human** | | **=** | **60 kg** |
| **Systemic exposure dose (SED)** | **Ax0.001xSAS/60** | **=** | **0.007 mg/kg bw** |
| **No observed effect level (90 day, oral, rat)** | **NOEL** | **=** | **5 mg/kg bw/day** |
| **Margin of Safety** | **NOEL / SED** | **=** | **701** |

**Calculation for the use of Climbazole at 0.5% in aqueous hair lotions**

| | | | |
|---|---|---|---|
| **Dermal absorption through human skin** | **A (µg/cm²)** | **=** | **1.10 µg/cm²** |
| **Skin Area surface (hand + ½ head)** | **SAS (cm²)** | **=** | **1440 cm²** |
| **Typical body weight of human** | | **=** | **60 kg** |
| **Systemic exposure dose (SED)** | **Ax0.001xSAS/60** | **=** | **0.026 mg/kg bw** |
| **No observed effect level (90 day, oral, rat)** | **NOEL** | **=** | **5 mg/kg bw/day** |
| **Margin of Safety** | **NOEL / SED** | **=** | **189** |

**Calculation for the use of Climbazole at 0.5% in a cosmetic face cream**

| | | | |
|---|---|---|---|
| **Dermal absorption through human skin** | **A (µg/cm²)** | **=** | **1.25 µg/cm²** |
| **Skin Area surface (1/2 head female)** | **SAS (cm²)** | **=** | **565 cm²** |
| **Typical body weight of human** | | **=** | **60 kg** |
| **Systemic exposure dose (SED)** | **Ax0.001xSAS/60** | **=** | **0.012 mg/kg bw** |
| **No observed effect level (90 day, oral, rat)** | **NOEL** | **=** | **5 mg/kg bw/day** |
| **Margin of Safety** | **NOEL / SED** | **=** | **425** |

**Calculation for the use of Climbazole at 0.5% in leave-on body lotion**

| | | | |
|---|---|---|---|
| **Dermal absorption through human skin** | **A (µg/cm²)** | **=** | **1.25 µg/cm²** |
| **Skin Area surface (whole body)** | **SAS (cm²)** | **=** | **18,000 cm²** |
| **Typical body weight of human** | | **=** | **60 kg** |
| **Systemic exposure dose (SED)** | **Ax0.001xSAS/60** | **=** | **0.38 mg/kg bw** |
| **No observed effect level (90 day, oral, rat)** | **NOEL** | **=** | **5 mg/kg bw/day** |
| **Margin of Safety** | **NOEL / SED** | **=** | **13** |

### 3.3.14. Discussion

*Physico-chemical specifications*
The physico-chemical section has been updated and completed and can now be accepted.

*Irritation / sensitisation*
The previous results of an old Draize eye test and a combination of two newly carried out *in vitro* screening tests for eye irritation (HET-CAM and CEET) suggest that Climbazole is not an eye irritant.
Its potential to cause skin irritation is assessed through a human single patch test and shows only mild to no skin irritation.
Considering the results of the above studies and considering the dilution of the compound in its intended use, there appears to be no reason to suspect any irritation problems with the use of Climbazole in cosmetic products.
As far as its sensitising potential is concerned, a well-performed LLNA shows Climbazole to be non-sensitising in the performed mouse assay.

*Dermal absorption*
A well-performed dermal absorption study for Climbazole contained at 2% in a shampoo formulation is available and reveals dermal absorption values of 0.297 ± 0.209 µg/cm² or 0.150 ± 0.106%.
As far as application under leave-on conditions and a Climbazole concentration of 0.5% is concerned, a recent *in vitro* dermal absorption study reveals absorption levels of 1.10 µg/cm² (2.23%) and 1.25 µg/cm² (3.46%) for an aqueous hair lotion and a water-in-oil skin preparation, respectively.

*General toxicity*
As already stated in SCCP/0981/05, several subacute and subchronic studies with rodents and non-rodents were available, though were performed before the introduction of GLP. Based upon the available test descriptions and raw data, the SCCP deduced a conservative NOEL value of 5 mg/kg bw/day from the presented 90-day study with the rat.

*Mutagenicity / genotoxicity*
The recent submission contains a full mutagenicity/genotoxicity dossier as requested by the SCCP. Climbazole showed to be negative in the Ames test, in the *in vitro* micronucleus test and in the *in vitro* mammalian cell gene mutation test (with the exception of the prolonged exposure scheme, where some mutagenic potential was apparent).
The *in vivo* micronucleus test and the *in vivo* UDS assay with Climbazole showed the substance to be negative, meaning that no mutagenic/genotoxic effects are to be expected.

*Reproduction toxicity*
As mentioned earlier (SCCP/0981/05), Climbazole was tested in a 1-generation reproductive toxicity test which was considered questionable as far as its overall scientific validity is concerned, and in well-performed teratogenicity study, leading to a NOAEL (embryotoxicity) of 30 mg/kg bw/day and a NOAEL (maternal toxicity) of 15 mg/kg bw/day.

*Toxicokinetics*
A newly performed oral bioavailability assay of $^{14}C$ Climbazole in mice confirms the results that were described earlier, namely that Climbazole is rapidly absorbed and excreted and that its maximum concentration in plasma is reached after approximately 8 hours.

*Calculation of the Margin of Safety*
Taking into account the mean values measured in the scientifically acceptable *in vitro* dermal absorption study for the use of Climbazole in a shampoo (rinse-off), together with the conservative NOEL value of 5 mg/kg bw/day, the MoS calculated for the use of Climbazole as anti-dandruff agent at 2% in cosmetic shampoos, is above 100.
For the leave-on applications, the calculations show that the use of Climbazole at 0.5% in an aqueous hair lotion and in a face cream, can be considered safe, as the MoS's are 189 and 425, respectively.
The use of Climbazole at 0.5% for whole body applications, however, generates a MoS of 13. To generate an acceptable MoS (≥ 100), the treated surface area for leave-on products containing 0.5% Climbazole should not exceed 2400 cm².

*Remark*
For Climazole, no specific information regarding the potential development of (cross-) resistance has been provided.
Also no specific data on possible biopersistance has been made available.

## 4. CONCLUSION

**Question 1:** *Does the SCCP consider with the scientific data provided that Climbazole is safe for the consumers, when used as a preservative in cosmetic products up to a maximum concentration of 0.5%?*

The SCCP is of the opinion that the use of Climbazole as a preservative at a maximum concentration of 0.5% in all cosmetic products cannot be considered safe. However, when used as a preservative in hair cosmetics and face cosmetics at 0.5%, climbazole does not pose a risk to the health of the consumer.

**Question 2:** *Does the SCCP consider with the scientific data provided that Climbazole is safe for the consumers, when used for non-preservative purposes as an anti-dandruff active ingredient in hair care formulations up to a maximum concentration of 2.0% in rinse-off products?*

The SCCP is of the opinion that the use of Climbazole in rinse-off hair cosmetics up to a maximum concentration of 2.0% does not pose a risk to the health of the consumer.

**Question 3:** *Does the SCCP consider with the new scientific data provided that Climbazole is safe for the consumers, when used for non-preservative purposes as an anti-aging ingredient in leave-on products up to a maximum concentration of 0.5%, even though this application might already be covered by Question 1?*

The SCCP is of the opinion that the non-preservative use of Climbazole in hair cosmetics and face cosmetics at 0.5% does not pose a risk to the health of the consumer. The use of Climbazole at 0.5% in leave-on products other than those mentioned above, however, is not considered safe.

The inhalation exposure to Climbazole from spray products was not assessed in this opinion.

## 5. MINORITY OPINION

Not applicable

## 6. REFERENCES

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