EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Climbazole

COLIPA n° P64

Adopted by the SCCP during the 5th plenary meeting
of 20 September 2005

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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".

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.

Climbazole bears the symbol (+) and can therefore be used in cosmetics at higher concentrations, as long as they are not employed as preservatives. Climbazole is currently authorized as a preservative up to a maximum concentration of 0.5 % (Annex VI, Part 1, No. 32).

In its opinion of 17 February 1999 concerning the restrictions on materials listed in Annex VI of Directive 76/768/EEC on cosmetic products, the SCCNFP stated that those substances indicated by (+) in Annex VI, when incorporated into cosmetic formulations for non-preservative functions, should be subjected to the same restrictions in usage levels and warnings as when used for preservative effects.

If a preservative marked with the symbol (+) is added for non-preservative purpose to a cosmetic product in a concentration higher than that laid down in the Annex VI, data to substantiate its safety should be submitted to the SCCP.

The European Commission has received a submission from industry proposing the use of Climbazole as an anti-dandruff active ingredient in hair care formulation up to maximum concentration of 2.0% in rinse-off products and up to a maximum concentration of 0.5% in leave-on products.

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumer when Climbazole is used for non-preservative purposes as an anti-dandruff active ingredient in hair care formulation up to maximum concentration of 2.0% in rinse-off products and up to a maximum concentration of 0.5% in leave-on products.*

2. *And/or does the SCCP recommend any further restrictions or conditions for its use in cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Climbazole

3.1.1.2. Chemical names

1-(4-chlorophenoxy)-1-(imidazol-1-yl)-3,3 dimethylbutan-2-one
1-(4-chlorophenoxy)-1-imidazol-1-yl-3,3 dimethyl-2-butanone (IUPAC)
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

Ref.: 32; 69; 70

3.1.1.3. Trade names and abbreviations

Trade name: Baypival®
Crinipan AD®

(Compound tested partly under the code BAY e 6975)

COLIPA no: P 64

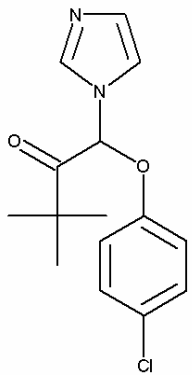
Ref.: not stated

3.1.1.4. CAS / EINECS number

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

Ref.: not stated

3.1.1.5.	Structural formula



Ref.: not stated

3.1.1.6.	Empirical formula

Formula : $C_{15}H_{17}ClN_2O_2$

Ref.: not stated

3.1.2.	Physical form

White to yellowish or brownish powder

Ref.: 32, 33, 72

3.1.3.	Molecular weight

Molecular weight: 292.76 g/mol

Ref.: not stated

3.1.4.	Purity, composition and substance codes

<u>BAY e 6975</u>

Batch no.	Pt. 2/74
n-perchloric acid method	100.2%
n-lauryl sulfate method	99.8%

Ref.: 32

<u>Crinipan AD</u>

Product Nr.	600306
HPLC method	98 - 102%

Ref.: 33

3.1.5. Impurities / accompanying contaminants

BAY e 6975

Cl^-	< 0.1%
SO_4^{2-}	< 500 ppm
Heavy metals	< 10 ppm

Ref.: 9

Crinipan AD

4-Chlorophenol:	< 150 ppm

Ref.: 33

3.1.6. Solubility

Soluble in water 49 mg/l (20°C, pH 7)

Ref.: 72

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow} : 3.6

Ref.: 32,33,72

3.1.8. Additional physical and chemical specifications

Melting point:	94 – 98 °C
Boiling point:	no information
Flash point:	not applicable
Vapour pressure:	0.001 Pa (50°)
	< 0.1 mbar (80 °)
Density:	580 kg/m^3
Explositivity/ Flammability properties:	no information
UV spectrum	λ = 276 nm: E(1/1) approx. 35.5
	λ = 283 nm: E(1/1) approx. 28

Ref.: 32, 33, 72

Climbazole is declared to be non-sensitive against long wave UV radiation and daylight (wavelengths not stated), stable against elevated temperatures up to 70°C and not hygroscopic.
The tests proving these statements lack full description; some parts of the documentation are even illegible.

Ref.: 32

3.2. Function and uses

This dossier is intended to support the safety of *Climbazole* when used as an anti-dandruff (Pityriasis capitis) active ingredient in hair cosmetic preparations up to a maximum concentration of 2% in rinse-off products and up to a maximum of 0.5% in non-rinse products for hair and scalp.

Ref.: 69, 80

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	acute oral toxicity test (1975) according to guidelines at the time (mostly in accordance with OECD 401 / EC B.1 method)
Species/strain:	Wistar rat CF1/W 86 mouse giant Chinchilla rabbit Beagle dog
Group size:	10 male rats, 10 male mice, 2 female rabbits, 2 dogs per dosage level
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	MEB 6401
Dose levels:	rats: 160, 200, 250, 320, 400, 500, 630, 800, 1000 mg/kg bw mice: 320, 400, 500, 630, 800, 1000 mg/kg bw rabbits: 125, 250, 500 mg/kg bw dogs: 50, 100, 250, 500 mg/kg bw
Observ. period:	14 days
GLP:	study performed before 1987

Climbazole (BAY e 6975) was administered by a single gavage to the rats, mice and rabbits in an aqueous suspension (tap water) with 5% Cremophor (10 ml/kg bw application volume) and to the dogs in a gelatine capsule. Some surviving animals, and, if possible, deceased animals have been necropsied. Calculation of LD_{50} was conducted with probit analysis.

Results
Symptoms noted in mice and rats consisted mainly of coordination impairment, tetanic seizures and a dose-dependent reduction of motility. In the rabbit and dog study, the limited number of animals suffered from sedation or convulsions.

The following LD_{50}-values were calculated:

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

Conclusion
The study was not conducted according to the current OECD guideline. Nevertheless, the available description allows to deduce that it has mostly been performed according to the currently deleted OECD 401 or EC B.1 acute oral toxicity test method.
Therefore, it does not seem appropriate to ask for a new acute oral toxicity study. The LD_{50}-oral-rat of 400 mg/kg bw/day is accepted.

Ref.: 1

3.3.1.2. Acute dermal toxicity

Without further details, a publication mentions a LD_{50}-dermal-rat of > 5000 mg/kg bw.

Ref.: 77

3.3.1.3. Acute inhalation toxicity

/

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

No information is available on the skin irritative properties of *Climbazole* as a compound.

The file provided by the manufacturer contains a large number of compatibility studies in human volunteers with *Climbazole*-containing end products (shampoos and lotions). The concentration of the compound in these formulations shifts between 0.0%, 0.5% and 2.0%.
All shampoos / lotions appear to be well tolerated when applied as a single patch for 48h on the human skin.
In addition, a study in which 2 different hair tonics (both containing 0.5% *Climbazole*) were applied on the back of rabbits daily for 3 weeks, revealed no adverse effects.

Ref.: 59, 60, 61, 62, 63, 64, 65, 35, 4

3.3.2.2. Mucous membrane irritation

Primary eye irritation study in rabbits (*Climbazole* in PEG-400)

Guideline:	according to the recommendations of the Department of Health, Education, and Welfare (Hazardous Substances, Test for Eye Irritants, Federal Register 37, p. 8534, 1972)
Species/strain:	rabbit, white
Group size:	8, sex not stated
Test substance:	0.5% BAY e 6975 (*Climbazole*) in polyethylene glycol 400
Batch no.:	not stated
Dose levels:	0.1 ml
Route:	ocular
Exposure:	1 application
GLP:	test performed in 1975, prior to GLP regulations

0.1 ml of an emulsion containing 0.5% *Climbazole* was applied to the conjunctival sac of one eye of each animal. The control animals received 0.1 ml polyethylene glycol 400 and were treated otherwise in the same way.

The substance remained in the eye for 5 minutes (5 animals) or 24 hours (3 animals) and was removed by 2 minutes of rinsing with water. Subsequently, the eyes were observed for 7 days. The effects on conjunctivae (reddening, swelling, ulceration) were scored after 1, 24, 48, 72 hours and after 7 days.

Results
A moderate, transient reddening (grade 1 and 2) and a slight swelling (grade 1) following 5 minute application of *Climbazole*-containing emulsion was observed in only 1 animal, that was therefore classified as positive for irritating reactions. Grade 1 reddening was sporadically observed in other animals from the 5-minute and 24-hour application duration. No irritating reactions on the conjunctivae have been observed following application of pure polyethylene glycol 400. *Climbazole* was classified by the laboratory as not irritating to the eye.

Conclusion
The scoring system is not stated, which makes the results difficult to judge. In addition, the eyes have been rinsed after 5 minutes. Normally, rinsing is only allowed after 24 hours or in case the substance appears to cause severe irritation.
Therefore, the results of this study cannot be used for the evaluation of the eye irritative potential of *Climbazole*.

Ref. : 8.

Other *in vivo* studies

The documentation provided by the manufacturer contains descriptions of 4 additional Draize eye irritation tests, all performed with 0.3 to 0.5% *Climbazole* containing shampoos, based on a sodium lauryl sulfate (SLS) solution. All shampoos have been found to be irritating.
Due to the presence of the SLS, this result was to be expected and does not provide any information on *Climbazole* as a compound.

Therefore these studies are not taken into account for the evaluation of the eye irritative potential of *Climbazole*.

Ref. : 5, 6, 7.

***In vitro* studies**

The documentation provided by the manufacturer contains descriptions of a large number of *in vitro* studies, consisting of 8 HET-CAM studies, 8 NR assays and 8 RBC studies with *Climbazole*-containing shampoos. The concentration of the compound in these formulations shifts between 0.0%, 0.5% and 2.0%. All shampoos reveal to be irritating through these tests.

Due to the presence of SLS in the shampoos, this result was to be expected and does not provide any information on *Climbazole* as a compound.

Therefore these studies are not taken into account for the evaluation of the eye irritative potential of *Climbazole*.

Ref. : 10, 11, 12, 13, 41, 39, 40, 42, 45, 48, 46, 50, 44, 49, 47, 43, 53, 58, 55, 57, 51, 56, 54, 52.

Conclusion
No information is available on the eye irritative properties of *Climbazole* as a compound.

3.3.3. Skin sensitisation

Magnusson Kligman Guinea Pig Maximisation test

Guideline:	Magnusson Kligman Maximisation test, published in EU legislation (1979).	
Species/strain:	Bor:DHPW albino guinea pig	
Group size:	20 females in substance group; 10 females per control group	
Test substance:	BAY e 6975 (*Climbazole*, purity 100%)	
Batch no.:	570327	
Concentrations:	intradermal induction:	10% test substance in propylene glycol
	dermal induction:	10% test substance in propylene glycol, occluded
	challenge 1:	1 + 10% test substance in propylene glycol, occluded
	challenge 2:	0.1 + 1% test substance in propylene glycol, occluded
GLP:	no information (study performed in 1983)	

For the intradermal induction a 10% *Climbazole* solution in propylene glycol was applied either pure (2 injections of 0.1 ml each) or in a 1:1 emulsion with Freund´s Complete Adjuvant (FCA) (2 injections of 0.1 ml each). 2 further injections with FCA/water (1:1) were applied. Application sites have been either the shaved neck or back. 1 week later the epidermal induction was conducted for 48 h under occlusion with a 10% *Climbazole* solution. On day 21 and 35 the animals were challenged by epidermal application of *Climbazole* under occlusive dressing (24 hours), either with 1 + 10% test substance (challenge 1) or 0.1 + 1% test substance (challenge 2), each on opposite flanks. Control animals received either FCA (10 animals) or propylene glycol (10 animals) for intradermal induction, and propylene glycol for dermal induction and challenge. Cutaneous reactions were evaluated at 24, 48, and 72 h after removal of the dressing.
A range finding study with 8 animals was conducted to determine primary irritating effects of *Climbazole*.

Results
1 animal of the substance group (challenge 1) died due to a circulatory shock and 1 control animal was sacrificed in moribund status. Reddening at the challenge application site was observed within all dose groups:

<u>Challenge 1:</u>	
10% challenge:	9 of 19 animals at least 2 scoring time points; 4 of 10 control animals
1% challenge:	3 of 19 animals; no control animal
<u>Challenge 2:</u>	
1% challenge:	2 of 19 animals at at least 2 scoring time points; no control animal
0.1% challenge:	1 of 19 animals at at least 2 scoring time points; no control animal

Some animals with reddening showed scurf. The intensity of skin reactions did not differ markedly between treated and control animals. The highest grade of skin reaction (sum 2.5; moderate to intense) was observed at the 2 animals treated with 1% of challenge 2 group.

The observed skin reactions in the 10% challenge group were judged as results of a primary skin irritation, because intensity and incidence (about 40 – 50%) was comparable between treatment and control animals. For the other challenge groups, a lower incidence was determined, which could have been expected due to the irritating effects observed in the range finding study: 1 of 4 animals treated with 0.3% *Climbazole* solution with propylene glycol showed positive skin reactions.

Conclusion
The laboratory concludes that *Climbazole* showed no skin sensitizing properties in a maximisation test with guinea-pigs.

Evaluation of validity
The study was not conducted in compliance with the OECD guideline 406. In the preliminary test, the experimenters appeared to have difficulties to determine the highest dose to cause minimal to moderate irritation (induction dose in the final study) and the highest non-irritating dose (challenge dose in the final study). Moreover, in the final study, the challenge concentration caused irritation in animals of nearly all dosage groups, which makes it nearly impossible to distinguish between irritative and sensitising effects.
Therefore, the results of this study cannot be considered as valid.

Ref.: 14

Buehler test

The test substance is declared to having been found negative in the Buehler test, but the full reference is not available.

Ref.: 15 (not included in data package)

3.3.4. Dermal / percutaneous absorption

Dermal / percutaneous absorption *in vitro*

Guideline:	*in vitro* percutaneous absorption study, 1984 internal protocol
Tissue:	human abdominal skin
Method:	diffusion chamber
Test substance:	Ceox® = shampoo containing 1% *Climbazole* (purity not stated)
Batch no:	not stated
Concentrations:	Ceox® dissolved 1:2 or 1:10 with water
Amount applied:	0.5 ml/4.91 cm^2
No. of chambers:	6
GLP:	no information (performed in 1984)

For *in vitro* determination of *Climbazole* penetration through skin the abdominal human skin of dead bodies (different donors) was used. Skin parts with macroscopic lesions were rejected. A shampoo (Ceox®) containing 1% *Climbazole* was used as test item dissolved either 1:2 or 1:10 with water. A test volume of 0.5 ml was used for all skin penetration tests.

The test item was uniformly placed on the upper site of each skin part (4.91 cm^2). Below the skin parts, a subcutaneous medium (consisting of bovine serum albumin, phosphate buffer and antibiotics) that has a direct, air free contact with the skin, collects the penetrated amount of *Climbazole*. The tests were conducted under non-occlusive and occlusive conditions. Measurement of *Climbazole* concentration in the subcutaneous medium (13.5 ml) after 24 hours was conducted by thin-layer densitometric assay (detection limit: 1 ng/ml).

Results
The amount of *Climbazole* penetrated into the subcutaneous medium was measured as follows (mean, S.D.):

study conditions:	concentration of *Climbazole* in subcutaneous medium	penetration rate (24 h)
1:2 dilution (2.5 mg), non-occlusive	111 ± 44 ng/ml	304 ± 122 ng/cm^2
1:2 dilution (2.5 mg), occlusive	121 ± 112 ng/ml	554 ± 503 ng/cm^2
1:10 dilution (0.5 mg), non-occlusive	13 ± 15 ng/ml	61 ± 62 ng/cm^2
1:10 dilution (0.5 mg), occlusive	150 ± 105 ng/ml	681 ± 461 ng/cm^2

Conclusion
The study was not conducted according to the current dermal absorption guidelines. No measurements have been performed in the skin. The diluted formulation was applied at a dosage level of 0.5ml/4.91cm², corresponding to ± 102 mg/cm². In a finite dose experiment 2-5 mg/cm² is recommended. Therefore, the calculated percentages of 0.04% to 0.09% are not acceptable. There was no dose-dependency in the penetration rates of the tests under occlusion. Many other shortcomings can be noted.

Therefore, the results of this study cannot be taken into consideration for the calculation of the MoS of *Climbazole*.

Ref.: 24

Dermal / percutaneous absorption *in vivo*

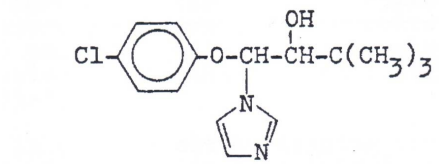
Human volunteer studies

The dossier contains 4 human volunteer studies, summarized below:

	Study n° 1	*Study n° 2*	*Study n° 3*	*Study n° 4*
Date of test	1980	1979	1979	1979
Test substance	0.5% *Climbazole* in isopropanol/water	2.0% *Climbazole* shampoo	2.0% *Climbazole* shampoo	1.0% *Climbazole* hair lotion
N° of volunteers	6 male volunteers	6 male volunteers	6 female volunteers	6 male volunteers

	Study n° 1	*Study n° 2*	*Study n° 3*	*Study n° 4*
Application	10 ml solution on scalp	6 g shampoo on scalp, twice	40 times 6 g shampoo rubbed on back and palm of the hands, followed by rinsing	20 ml solution on scalp
Sampling	1, 2, 4, 6, 8 & 24h after application	1, 2, 4, 6, 8 & 24h after application	2, 4, 6, 8 & 24h after application	2, 4, 6, 8 & 24h after application
Measurement	*Climbazole* and BAY g 5919* in blood plasma	*Climbazole* and BAY g 5919* in blood plasma and urine	*Climbazole* and BAY g 5919* in blood plasma and urine	*Climbazole* and BAY g 5919* in blood plasma and urine
Results	*Climbazole* : up to 3 ng/ml, BAY e 5919 : up to 6 ng/ml. Complete lack of time-dependency.	Plasma : *Climbazole* : up to 4 ng/ml, BAY e 5919 : up to 5 ng/ml. Complete lack of time-dependency. Urine : No individual data given. Declared not to be detected in any sample	Plasma : *Climbazole* and BAY e 5919 : up to 20 ng/ml. Concentrations appear to peak after 6h. Urine : *Climbazole* and BAY e 5919 : up to 5 ng/ml. No time-dependency.	Plasma : *Climbazole* : up to 20 ng/ml, BAY e 5919 : up to 34 ng/ml. Lack of time-dependency. Urine : *Climbazole* up to 3 ng/ml; BAY e 5919 not detected. No time-dependency.

* BAY g 5919 = major metabolite of *Climbazole* :



BAY g 5919

None of the above studies were performed according to an existing guideline. Their descriptions are very brief and no control groups have been included, which makes the results difficult to assess. Nevertheless, it cannot be denied that *Climbazole* and its major metabolite are measured in blood plasma and urine after application of a shampoo under in-use (mimicking) conditions. This is a clear indication that percutaneous absorption does take place. One of the studies mentions an absorption value for *Climbazole* of 2.3%.

Ref.: 20, 21, 23, 68

Rabbit study

The dossier contains a study (1976) in which 2 *Climbazole*-containing formulations (0.5% isopropanol/water/salicylate hair lotion and 0.5% in PEG-400) were applied twice per day at a dose level of 1 ml on the intact or abraded skin of 6 rabbits by rubbing in.

No *Climbazole* could be detected in plasma with the PEG-400 based solution. Application of the hair lotion, on the other hand, generated plasma levels of 13 ng/ml for *Climbazole* and 10 ng/ml for its metabolite.

The study was not performed according to an existing guideline, which makes its results difficult to interpret.

Ref.: 19

3.3.5. Repeated dose toxicity

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

28-day oral toxicity study with the rat

Guideline:	28-day oral toxicity test (1974) according to guidelines at the time
Species/strain:	Wistar rat
Group size:	10 males
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	1/73
Dose levels:	0, 50, 100 mg/kg bw/day
Route:	oral; gavage
Exposure:	4 weeks, 7 days/week
GLP:	no information (test performed in 1974, prior to GLP regulations)

Climbazole (BAY e 6975) was applied in 0.5% aqueous tylose suspension in a constant volume of 10 ml/kg. Control animals received the tylose suspension. The animals have been observed permanently for signs of toxicity. Body weight, food consumption and water consumption were recorded weekly. Blood glucose was determined in 5 animals of each dose group after 3, 10, and 23 treatment days. *Climbazole* concentration in blood was determined after the 2nd and 23rd application, as well as 24 hours after last application. Clinical investigations (haematology, clinical chemistry, enzymes, and urinalysis) were conducted at the end of treatment period. After 4 weeks, animals were sacrificed, and investigated by macroscopy. Comprehensive histopathology was conducted for liver, adrenal glands, thyroid, and testes of 5 animals each of every dose group. Statistical calculations have been conducted with the Wilcoxon rank sum test.

Results

No animal died within 4 weeks of study duration, and no signs of toxicity have been observed. Body weight gain of the high dose group animals were significantly reduced after 1st and 4th week and a significantly increased blood glucose level was measured after 23rd application in this group. Activity of aminopyrin-N-demethylase and triglycerides levels were significantly increased in both dose groups, however no dose-dependency was observed. Glutamate-pyruvate-transaminase activity was significantly increased only at 50 mg/kg bw/day.

At necropsy 5 animals of each treatment group revealed an altered liver tissue (pale; lobuli-like pattern), and histopathology showed fatty degeneration. Absolute and relative liver weight was significantly enhanced at both dose groups. At 100 mg/kg bw/day also the absolute and relative weight of thyroid glands were significantly elevated. *Climbazole* plasma levels 2 hours after application were between 2.6 and 3.75 µg/ml, not depending on application dose and administration days. No *Climbazole* was measured 24 hours after administration.

Conclusion
The study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. However, due to the availability of detailed information on the study design and findings (including some individual animal data), limited validity in terms of scientific criteria can be assigned. The study is acceptable as a dose-finding (pilot) experiment. Its results indicate that the NOAEL for *Climbazole* following repeated (sub-chronic) daily per oral dosing in the rat is lower than 50 mg/kg bw/day. The observed adverse effects were blood biochemical changes and morphological organ damage in the liver.

Ref.: 30

42-day oral toxicity study with the dog

Guideline:	42-day oral toxicity test (1974) according to guidelines at the time
Species/strain:	Beagle dog
Group size:	2 males + 2 females
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	483751
Dose levels:	0, 100 mg/kg bw/day week 1, 2; 0, 50 mg/kg bw/day week 3 - 6
Route:	oral; capsules
Exposure:	6 weeks, 7 days/week
GLP:	no information (test performed in 1974, prior to GLP regulations)

The animals received *Climbazole* (BAY e 6975) for 2 x 50 mg/kg bw/day for the first 2 weeks, and 1 x 50 mg/kg bw/day for the following weeks in gelatine capsules. Control animals received the empty gelatine capsule. The animals have been observed permanently for signs of toxicity. Body weight was recorded weekly. Clinical investigations (haematology, clinical chemistry including enzymes, and urinalysis) were conducted before treatment and after 2, 4, and 6 weeks. *Climbazole* concentration in serum was determined at days 1, 8, 15, 29, and 43, before treatment as well as 1, 2, 4, and 24 hours after application. After 6 weeks, animals were sacrificed, and investigated by macroscopy and comprehensive histopathology.

Results
No animal died within 6 weeks of study duration. Signs of toxicity have been observed at the first 2 weeks (sedation, anorexia), but stopped with reduced dose. Elevated GTP (glutamate pyruvate transaminase) and a slight anaemia were judged as treatment-related. No other parameters of the clinical investigation were affected, and no substance-related macroscopic and microscopic alterations have been observed.

Conclusion
The study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. However, due to the availability of detailed information on the study design and findings (including some individual animal data), limited validity in terms of scientific criteria can be assigned. The study results indicate that the NOAEL for *Climbazole* following repeated (sub-chronic) daily per oral dosing is probably lower than 50 mg/kg bw/day. The observed adverse effects were some clinical findings and blood biochemical/haematological changes.

There was no indication of morphological organ damage, though it must be stated that the group size of this experiment was too small to draw any final conclusion.

Ref.: 74

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

90-day oral toxicity study with the rat

Guideline:	90-day oral toxicity test (1978) according to guidelines at the time
Species/strain:	Wistar rat
Group size:	15 males + 15 females
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	323551
Dose levels:	0, 5, 15, 45 mg/kg bw/day
Route:	oral; gavage
Exposure:	13 weeks, 7 days/week
GLP:	no information (test performed in 1978, prior to GLP regulations)

Climbazole (BAY e 6975) was applied in 0.5% aqueous tylose suspension in a constant volume of 10 ml/kg. Control animals received the tylose suspension. The animals have been sorted by weight (light, medium, heavy) and randomly assigned to the dose groups afterwards. The animals have been observed daily for clinical signs of toxicity. Body weight, food consumption and water consumption were recorded in weekly intervals. Clinical laboratory investigations (haematology, blood biochemistry, enzymes, and urinalysis) of 5 animals of each sex and dose group have been conducted after 6 weeks and 13 weeks. After 13 weeks, survivors were sacrificed and submitted to necropsy (including macroscopic organ examination). Comprehensive histopathology was conducted in 10 animals each of the control and high dose groups. Individual animal data were condensed to give group mean values.

Results

No animals of the dose groups showed any substance-related clinical symptoms. All test animals survived until termination of the study. A significant reduction of weight gain was observed in males of the high dose group as of the 10^{th} week. Some parameters of clinical laboratory investigations (reduced erythrocyte number in males of the 45 mg/kg bw/day group; reduced activity of alkaline phosphatase in females of all dose groups; reduced creatinine in both sexes) were altered, but were within the physiological range. No other altered parameter has been observed, or has been related to *Climbazole*. A dose-dependent increase in absolute liver weight was observed in the females, that was significant in the 15 mg/kg bw/day, and the 45 mg/kg bw/day dose groups. Histopathology revealed no indications of organ damage.

The increased liver weight was seen as related to increased N-demethylase activity and increased cytochrome P450 content. These findings were thus interpreted as signs of adaptive reactions such as increased metabolic activity rather than as indications of damage to the liver parenchyma.

Conclusion

The study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. However, due to the availability of detailed information on the study design and findings (including some individual animal data), satisfactory validity in terms of scientific criteria can be assigned to this experiment.

Test substance-related effects were observed following daily p.o. dosing of rats over 13 weeks at the intermediate and high dose levels (15 and 45 mg/kg bw/day). The liver was identified as target organ for the effects of *Climbazole*. Because the effects on the liver at 15 mg/kg bw/day (increased liver weight without histopathological evidence of toxicity, P450 induction) were not judged to be clinical relevant, this dose is regarded as a NOAEL by the experimenters.

Conclusion
Viewing the absence of the full raw data package, it appears to be safer to use the NOEL value of 5 mg/kg bw/day for the calculation of the MoS of *Climbazole*.

Ref.: 2

90-day oral toxicity study with the dog

Guideline:	90-day oral toxicity test (1980) according to guidelines at the time
Species/strain:	Beagle dog
Group size:	3 males + 3 females
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	483751
Dose levels:	0, 5, 10, 20 mg/kg bw/day
Route:	oral; capsules
Exposure:	13 weeks, 7 days/week
GLP:	no information (test performed in 1980, prior to GLP regulations)

Climbazole (BAY e 6975) was administered perorally to three groups of male and female dogs in gelatine capsules daily 4 to 6 hours before feeding. Control animals received gelatine capsules without the test substance according to the same treatment scheme. The animals have been observed daily for clinical signs of toxicity. Body weight was recorded in weekly intervals. Haematological investigations (hematocrit, haemoglobin, erythrocyte, leucocyte thrombocyte, and reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, hemogram, erythrocyte sedimentation rate), clinical-chemical investigations (blood biochemistry, serum enzymes, and urinalysis), neurological investigations (papillary reflex, patellar reflex, flexor reflex, startle reflex), and measurement of body temperature of all animals have been conducted after 2, 5, and 12 weeks. Ophthalmoscopic investigations have been conducted after 5, and 12 weeks. Electrocardiograms of all animals were recorded one hour before and after the 20th, 43rd, and 92nd application. After 13 weeks, all surviving animals were sacrificed and submitted to necropsy (including macroscopic examination of organs and tissues).
Comprehensive histopathology was conducted on a number of organs and tissues (urinary bladder, gallbladder, aorta, muscles, adrenal gland, thyroid gland, pituitary gland, esophagus, stomach, intestines, ovaries, uterus, testis, epididymis, prostate, brain, bone marrow, bones, salivary glands, pancreas, lymph nodes, nervus ischiducus, nervus opticus, eyes, liver, lung, spleen, kidney, heart, thymus) in all animals of the control and the high dose group.

Results
None of the animals in the dose groups showed any substance-related clinical symptoms. All animals survived until termination of the study. Body weight development was comparable between the control and dose groups.

Ophthalmoscopy, determination of body temperature, neurological investigations, electrocardiograms, haematological and blood biochemical parameters, urinalysis, as well as gross pathological (including organ weights) and histopathological investigations revealed no *Climbazole*-related alterations. Activity of N-demethylase (dose-dependent) and cytochrome P450 content (high dose group) were elevated at the end of the study.

Conclusion

The study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. However, due to the availability of detailed information on the study design and findings (including some individual animal data), satisfactory validity in terms of scientific criteria can be assigned to this experiment.

Based on the reported study results, the laboratory proposes to use the highest tested dose of 20 mg/kg bw/day as the NOAEL in this sub-chronic peroral toxicity study in dogs. The only test substance-related effects observed exclusively in the high dose group were not considered as adverse effects but rather as signs of adaptive changes indicating increased metabolic activity.

Viewing the absence of the full raw data package, it appears to be safer to take into account the NOEL value of 10 mg/kg bw/day.

Ref.: 3

3.3.5.3. Chronic (> 12 months) toxicity

No chronic toxicity studies have been conducted with *Climbazole*.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Ames test 1 (parent compound)

Guideline:	Ames test (1977) according to guidelines at the time
Species/strain:	*Salmonella typhimurium*, TA100, TA1537, TA98
Replicates:	2
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	323568
Concentrations:	0, 3.15, 10, 31.5, 100, 315, 1000, 2000 µg/plate with metabolic activation 0, 31.5, 100, 315, 1000, 2000 µg/plate without metabolic activation
GLP:	no information (test performed in 1977, prior to GLP regulations)

A standard plate-incorporation assay was performed with *Climbazole* (BAY e 6975), dissolved in DMSO. S9 mix from Aroclor 1254 induced male Sprague-Dawley rats was used for metabolic activation. The experiments were performed according to the Ames test, with minor modifications. The *Climbazole* solution, 500 µl S9 mix or 500 µl 150 mM KCl, 100 µl of bacterial culture, and 2000 µl top agar were mixed in a test tube and poured on a minimal agar petri dish. After incubation for 2 – 3 days in the dark the his^+ revertants were counted.

In assays without metabolic activation, the positive controls for all strains were N-methyl-N´-nitro-N-nitrosoguanidine, and benzo(a)pyrene 4,5-oxide. In assays with rat-liver S9, the positive controls for all strains were 3-methylcholanthrene, benzo(a)pyrene, and 2-aminoanthracene.

Results
Climbazole revealed no mutagenic activity in the plate-incorporation test with TA100, TA1537, TA98, neither in presence nor in absence of a metabolic activating system. A slight toxic effect was observed at the highest *Climbazole* concentration in presence of the S9 mix only. Many distinct small his^- colonies were obvious instead of a his^- lawn, and the number of his^+ revertants of TA1537 and TA100 were lower than in the control group.
In the test series without metabolic activation at a concentration of > 315 µg/plate the test substance precipitated on the plates, what might have reduced the availability for liver enzymes and bacteria. Both, negative and positive control groups gave the expected results.

Conclusion
The study was not conducted in compliance with the recent OECD guideline 471 and many study details were not in accordance (e.g. 3 instead of 5 strains were used; only 2 replicates have been tested, ...). However, the test conditions applied are sufficient to demonstrate that the test substance does not induce gene mutations in various strains of Salmonella typhimurium.

Ref.: 26

Ames test 2 (BAY g 5919)

Guideline:	Ames test (1983) according to guidelines at the time	
Species/strain:	*Salmonella typhimurium*, TA 1535, TA100, TA1537, TA98	
Replicates:	4	
Test substance:	BAY g 5919 (*Climbazole* metabolite, purity not stated)	
Batch no.:	08111982	
Concentrations:	main tests:	0, 20, 100, 500, 2500, 12500 µg/plate with and without metabolic activation
	additional tests:	0, 125, 250, 500, 100, 2000 µg/plate with and without metabolic activation
GLP:	no information (test performed in 1983, prior to GLP regulations)	

A standard plate-incorporation assay (Ames test) was performed with BAY g 5919, a metabolite of *Climbazole* (BAY e 6975), dissolved in DMSO. S9 mix from Aroclor 1254 induced male Sprague-Dawley rats was used for metabolic activation.
The positive control for all strains was 2-aminoanthracene. As a second positive control cyclophosphamide (endoxan) was used for TA1535 and TA100 and trypaflavin was used for TA1537 and TA98.
Due to the toxic effect observed at higher concentrations, additional tests were conducted with supplementary concentrations.

Results
The *Climbazole* metabolite BAY g 5919 revealed no mutagenic activity in the plate-incorporation test with TA 1535, TA100, TA1537, TA98, neither in presence nor in absence of a metabolic activating system.

No toxic effects were observed up to 250 µg/plate. Above this, toxicity was observed in presence and in absence of the S9 mix. From 2000 µg/plate onwards, the test substance precipitated therefore an evaluation of these results were not possible.
Positive control groups gave the expected results in the experiments with metabolic activation, but failed to produce increased mutant rates without activation in some experiments.

Conclusion
The Ames test was not entirely conducted in compliance with the recent OECD guideline 471. However, the test conditions used are sufficient to exclude a relevant mutagenic potential of the test substance in Salmonella typhimurium. BAY g 5919 was found negative under the conditions applied here.

Ref.: 27

Ames test 3 (literature data)

Results of an Ames test with Salmonella typhimurium strains TA100, TA1535, TA98, TA1537, TA1538, and E. coli strains WP2 UVRA were reported in the database CCRIS Chemical Carcinogenesis Research Information System). *Climbazole* was tested up to 5000 µg/plate in presence and absence of PCB (polychlorinated biphenyls) induced rat liver S9 mix. All tests revealed a negative result.

Ref.: 73 (not included in data package)

***In vitro* mammalian chromosome aberration test**

Guideline:	mammalian chromosome aberration test (1983) according to guidelines at the time
Species/strain:	human lymphocyte culture
Replicates:	1 - 2
Test substance:	*Climbazole*, purity 99.3%
Batch no.:	570327
Concentrations:	0, 25, 50, 100 µg/ml with and without metabolic activation
GLP:	no information (test performed in 1983, prior to GLP regulations)

The effect of *Climbazole*, dissolved in DMSO, on mitotic activity and chromosome damage was investigated in human lymphocytes from 2 male and 2 female healthy donors. The test item was added to 48-h old cultures. Negative controls received DMSO alone. The positive control was mitomycin C in absence of S9 mix, and endoxan in presence of S9 mix. Cultures with S9 mix were washed 2.5 h after substance administration. 21 hours after administration colchicine was added in a final concentration of 0.4 µg/ml. 3 hours later the cells were fixed (2 – 3 slides per culture). For determination of the mitotic index 1000 nuclei were counted for each culture. For determination of chromosomal aberrations, 100 metaphases of each sex were examined for gaps, breaks, fragments, deletions, exchanges, multiple aberrations, and disaggregation of chromosomes. Statistical calculations were conducted with the Wilcoxon rank sum test.

Results
A significant, dose-dependent reduction of the mitotic index was observed in absence of a metabolic activation system, but not in presence of S9 mix, possibly due to the washing after 2.5 hours. At the highest concentration the mitotic index was about 13% of the index in the negative control. The reduction of mitotic rate indicates that the tested concentrations were in a toxic range. Metaphases with aberrations (without gaps) were slightly but statistically significantly increased at the highest concentration without metabolic activation. Metaphases with aberrations (with gaps at 25 and 50 µg/ml, but not at 100 µg/l, without gaps at 50 µg/ml only) were also slightly, but statistically significantly increased with metabolic activation. As these increases were not concentration-related, within the biological range of variability, and within the range observed in historical controls they were not judged as related to the test compound and of biological significance. The controls revealed the expected results.

Conclusion
The study was not conducted according to the recent OECD guideline 473. In deviation of the guideline, e.g. the negative result was not confirmed by repeating the experiment and toxic ranges were not reached by the applied test concentrations with S9. The substance was found to be negative in the *in vitro* mammalian chromosome aberration test under the conditions applied here.

Ref.: 34

3.3.6.2. Mutagenicity / Genotoxicity *in vivo*

Mouse bone marrow micronucleus test

Guideline:	micronucleus test (1978) according to guidelines at the time
Species/strain:	mouse, NMRI
Group size:	5 males + 5 females
Test substance:	BAY e 6975 (*Climbazole,* purity not stated)
Batch no.:	323568
Dose level:	2 x 150 mg/kg bw, 2 x 300 mg/kg bw
Route:	oral
Sacrifice times:	6h after 2nd administration
GLP:	no information (test performed in 1978, prior to GLP regulations)

Climbazole (BAY e 6975), dissolved in 0.5% aqueous cremophor emulsion, was administered in two treatments with an interval of 24 hours. Methylmethansulfonate (MMS) (2 x 20 mg/kg bw) was used as positive control, and 0.5% aqueous cremophor emulsion as negative control. The administration volume was 10 ml/kg in all test groups. 6 hours after 2nd administration, the animals were sacrificed and femoral marrow was prepared for examination. 1000 polychromatic erythrocytes per animal were examined for cells with micronuclei. The incidence of micronuclei as well as the ratio of polychromatic to normochromatic erythrocytes was determined. The results were statistically evaluated by the distribution-free Wilcoxon rank test ($p < 0.05$ significance level).

Results
The highest dose (2 x 300 mg/kg bw) caused acute lethal effects in 2 male animals. Further, ruffled coat, marked somnolence, and weight loss were observed, as well as immobility and shortness of breath in one survivor. One of the deceased animals was replaced. 1 animal of the 150 mg/kg bw dosage group was replaced due to a bone marrow depression that was presumably unrelated to *Climbazole*.
No statistical significant increase in polychromatic erythrocytes with micronuclei was seen. Comparison of polychromatic to normochromatic erythrocytes revealed, that *Climbazole* did not induce inhibition of erythropoiesis. MMS gave positive results.

Conclusion
The study design deviates from the recent OECD guideline 474: e.g. animals were sacrificed 6h after the final treatment and only 1000 polychromatic erythrocytes instead of 2000 were evaluated. The test substance was found to be negative in the mouse bone marrow micronucleus test under the conditions applied here.

Ref.: 29

3.3.7. Carcinogenicity

No carcinogenicity studies with *Climbazole* are available.

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

Guideline:	1-generation reproduction toxicity (1979) according to guidelines at the time
Species/strain:	Charles River CD®rat
Group size:	phase I and II: 10 males and 20 females
Test substance:	*Climbazole*, purity not stated
Batch no.:	not stated
Dose levels:	0, 7.2, 36, 100 mg/kg bw/day
Route:	oral; gavage
Exposure:	phase I: males 10 weeks before mating, females 14 days before mating, both males and females throughout mating, gestation and lactation phase II: males 86 days before mating, throughout mating, gestation and lactation; females were not treated
GLP:	no information (test performed in 1979, prior to GLP regulations)

Phase I: The males were treated with *Climbazole* (indicated as compound No. 34054) for 10 weeks and the females for 14 days prior to mating. Both males and females received the test material throughout the mating, gestation and lactation periods or until sacrifice. *Climbazole* was administered orally as a single daily dose in a 3.0% aqueous carboxymethyl cellulose solution with an application volume of 10 ml/kg/day.

The control animals received an adequate volume of the carboxymethyl cellulose solution, and were treated in the same way otherwise. Mating was continued until evidence of copulation was observed or until the 15-day mating period ended. If mating was not successful after 10 days the unmated females were housed for five additional days with a different male within the same

treatment group. The females were vaginally smeared daily until sperm or a copulatory plug was observed. On gestation day 13, approximately one-half of the bred females from each group were sacrificed and their uterine contents examined. The remaining females in each group were allowed to deliver. The pups were counted, sexed, weighed and observed at designated intervals during lactation. At weaning the pups were examined for external abnormalities, sacrificed and discarded.
The dams were examined for external abnormalities, sacrificed and gross examination of the viscera was conducted.

Phase II: This additional phase of the study was implemented to evaluate the compound effects on the male rats, as signs of toxicity were observed in phase I 100 mg/kg bw/day females. Male rats continuing on study from the phase I segment remained on their respective test material regimen. The test material was administered to the males throughout this second phase in an identical regimen as in the phase I segment, starting 86 days before mating. Female rats were not treated. In contrast to phase I females were housed with the same male until evidence of mating or until the end of the 15-day mating period. Vaginal smears for estrous were not taken.
After the second phase male rats were examined for external abnormalities, sacrificed and discarded.

Results
Phase I:
No toxic effects were observed in males of the 7.2 and 36 mg/kg bw/day dosage group and in females of the 7.2 mg/kg bw/day group. Increased activity, hair loss and salivation were seen with the 100 mg/kg bw/day males and the 36 and 100 mg/kg bw/day females. Red ocular and nasal discharge, abdominal urine stains and self-mutilation of the extremities and abdomen were also observed in the 100 mg/kg bw/day females. These observations decreased as treatment progressed, but the increase in the activity level of the 100 mg/kg bw/day males was evident until sacrifice, with exception of study week 8. One female died during gestation in the 36 and 100 mg/kg bw/day groups. Mean body weights of the males at the highest dose group were slightly lower than the control males. No changes of body weight were observed in the other groups. Females of the 100 mg/kg bw/day treatment group showed increased length of gestation, reduced fertility, elongated stages of diestrus. Findings in the females of the other dose groups were comparable to controls. The following effects were observed in phase I litters: decreased number of pups born alive/total pups born in the 100 mg/kg bw/day treatment group; decreased number of live pups per litter at birth in the 36 and 100 mg/kg bw/day dosage group, statistic significant only in the highest dose group. General behaviour, appearance and mean pup body weights were not affected in all treatment groups. In the 100 mg/kg bw/day dose group at the 13 day uterine examination the mean number of viable and total implantations and the ratio of implantation sites to corpora lutea were decreased and resorptions were increased when compared to the control group. These effects were not observed in the two other dose groups.

Phase II:
General behaviour and appearance, survival, body weight gains, gestation length or male and female fertility in the phase II segment were comparable to the phase I control group.

There were no differences in the general behaviour, appearance, survival or body weight gains of the phase II litters that were attributed to treatment with *Climbazole*. Uterine examination did not reveal any biologically meaningful difference between treated and untreated groups.

Conclusion
This study on fertility and general reproductive performance was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. It strongly resembles the study design of a classical 1-generation reproduction toxicity test (OECD 415).
This study revealed slight behavioural changes in male rats at the 100 mg/kg bw/day treatment level, but did not inhibit their reproductive performance as supported by the findings in Phase II of the study.
The females in the 100 mg/kg bw/day dosage group exhibited severe toxicity during the initial stages of treatment which caused disruption of the estrous cycles and an increased number of stillbirths. At the 36 mg/kg bw/day treatment level, the female rats exhibited slight-to-moderate toxicity during the initial stages of treatment with no observable effects on estrous or stillbirths. Embryotoxicity observed at 36 and 100 mg/kg bw/day consisted in decreased numbers of life pups at birth and was judged to be a secondary effect to maternal toxicity.

Viewing the lack of full raw data, the following NOAEL values are proposed:

NOAEL (maternal toxicity) = 7.2 mg/kg bw/day,
NOAEL (embryotoxicity) = 36 mg/kg bw/day.

Ref.: 87

3.3.8.2. Teratogenicity

Teratogenicity study in the rabbit

Guideline:	OECD guideline no. 414 (adopted 22nd January 2001)
Date of test :	May-July 2001
Species/strain:	Chinchilla rabbit
Group size:	24 mated females
Test substance:	HR 00/600306 (*Climbazole*, purity min. 98%)
Batch no.:	20072001
Dose levels:	0, 15, 30, 60 mg/kg bw/day
Route:	oral; gavage
Exposure:	days 6 through 27 of pregnancy
GLP:	in compliance

Climbazole (indicated as HR 00/600306) was tested in a 4% carboxymethyl cellulose (CMC) solution with bi-distilled water. Each group of 24 mated Chinchilla rabbits was administered 4 ml/kg bw orally once daily from day 6 through day 27 post coitum. The dosage was adjusted daily to the actual body weight in order to obtain the requested 15, 30 or 60 mg of *Climbazole*/kg bw/day. The control animals received the CMC solution alone.
Females treated with the test item and vehicle controls were sacrificed on day 28 post coitum and the foetuses were removed by Caesarean section.

Examination of dams and foetuses was performed in accordance with international recommendations and included body weights and sex ratios, external and fresh visceral examination, examination of fixed foetal heads (including brains) and fixed foetal thoracic organs, and skeletal and cartilage examinations.

Results
2 animals of the 60 mg/kg bw/day dosage group died or were sacrificed in extremis, and 1 animal died at the 30 mg/kg bw/day dose group. One female of the control group and the 15 and 30 mg/kg bw/day treatment group was sacrificed after abortion (post-coital days 26, 28, 26, listed according to increasing dosage). In absence of a dose-relationship these abortions were considered to be incidental. At the 30 mg/kg bw/day and 60 mg/kg bw/day dosage group some animals showed local alopecia, possibly substance related (dose-related incidence). At the highest dose group one case of self-mutilation was observed. A dose-related reduction of food consumption was noticed at the 2 highest dose groups from day 6 to 18 of gestation, but for the following days of gestation up to caesarean section food consumption was similar to control group. A corresponding course of reduced body weight gain was observed. The pathological examination revealed a total post-implantation loss in 7 animals of the highest dose group, compared to 1 animal of the control group and 30 mg/kg bw/day group, and no animal of the 15 mg/kg bw/day group. By calculating for all females surviving until scheduled necropsy, the number of foetuses was significantly decreased in the highest dose-group, and was also, but not statistically significant, decreased in the 30 mg/kg bw/day dose group. A slight increase in post-implantation loss was observed in the two highest dose groups by calculating for only dams with live foetuses (statistically not significant). At macroscopic examinations no substance-related alterations have been observed in dams.
A higher proportion of female foetuses was seen in the 30 mg/kg bw/day and 60 mg/kg bw/day dose group that was significant in the highest dose group. This difference in sex ratio was not considered to be test item-related. Examination of viscera, skeleton, and cartilage revealed no *Climbazole*-related foetal abnormalities.

Conclusion
No teratogenic effects were observed in this study with rabbits. Maternal toxicity (alopecia, reduced weight gain) was seen at 30 and 60 mg/kg bw/day. Embryotoxic effects (increased post-implantational losses) seen in the highest dose group are considered to be secondary to the effects on dams.
The proposed NOAEL (embryotoxicity) is 30 mg/kg bw/day. Based on maternal toxicity at the two higher doses, 15 mg/kg bw/day is considered a NOAEL for maternal toxicity.

Ref.: 17

Teratogenicity study in the rat (1)

Guideline:	teratogenicity study (1981) according to guidelines at the time
Species/strain:	BAY: FB_{30} rat
Group size:	25 inseminated females
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	570327
Dose levels:	0, 10, 30, 100 mg/kg bw/day
Route:	oral; gavage
Exposure:	days 6 to 15 of pregnancy, inclusive
GLP:	no information (test performed in 1981, prior to GLP regulations)

Climbazole was administered daily from day 6 to day 15 of gestation (10 exposures) in a 0.5% aqueous tylose solution with an application volume of 10 ml/kg. The control animals received an adequate volume of the tylose solution, and were treated in the same way otherwise. On day 20 of pregnancy, females were narcotised and foetuses were delivered by Caesarean section.

All foetuses were weighted, and sexing was conducted. The investigation for external malformations was conducted with all animals. A part of the foetuses was further investigated for visceral malformations, the other part was investigated for skeletal deformation.

Results
During exposure no behavioural alterations or signs of toxicity have been observed up to 30 mg/kg bw/day. At the highest dosage group, 21 of 25 females gave an unhealthy impression and showed self-mutilation. Lacerations were observed on extremities or on stomach, but no animal died. During treatment days, but not during whole pregnancy, a significant reduced weight gain was noticed. At 10 mg/kg bw/day, but not at any other dose group, the mean placenta weight was increased. A significant increase in the number of resorptions was observed at the highest dose group. In 8 females, all of the embryos have been resorbed during pregnancy. The percentage of male foetuses was lower than that of female foetuses in the 100 mg/kg bw/day dosage group. No difference in the malformation incidence between control group and treatment group was observed.
The effect on the foetuses (increased number of resorptions) was seen as result of maternal toxicity, and therefore *Climbazole* was judged as not primary embryotoxic and teratogenic up to 100 mg/kg bw/day. At 30 and 100 mg/kg bw/day, the test substance showed maternal toxic effects.

Evaluation of validity
The study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. However, due to the availability of detailed information on the study design and findings (including individual animal data), satisfactory validity in terms of scientific criteria can be assigned to this experiment. In particular, the selected dose levels, the dose gradation, time and frequency of dosing, group size and the frequency and type of examinations meet scientific requirements. In view of the detailed information given in this study and the overall consistent pattern of effects which is obvious by comparing results of this study with those from a number of additional reproductive and developmental toxicity studies, the safety data obtained in this particular study appear to be reliable.

Conclusion
The study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. This makes the results difficult to interpret, especially in the case of such a specialised area as teratogenicity.
The laboratory concludes that no signs of teratogenic effects were seen in this teratogenicity study with rats with applied doses of 10 to 100 mg/kg during gestation days 6 through 15. A NOAEL of 10 mg/kg has been identified for maternal toxicity. Embryotoxic effects were observed at 100 mg/kg and were judged to be secondary effects to the maternal toxicity.

Ref.: 16

Teratogenicity study in the rat (2)

Guideline:	teratogenicity study (1979) according to guidelines at the time
Species/strain:	Charles River CD® rat
Group size:	25 inseminated females
Test substance:	Compound No. 34054 (*Climbazole,* purity not stated)

Batch no.:	not stated
Dose levels:	0, 7.2, 36, 100 mg/kg bw/day
Route:	oral; gavage
Exposure:	days 6 to 15 of pregnancy, inclusive
GLP:	no information (test performed in 1979, prior to GLP regulations)

Climbazole (indicated as compound No. 34054) was administered daily from day 6 to day 15 of gestation (10 exposures) in a 3.0% aqueous carboxymethyl cellulose solution with an application volume of 10 ml/kg/day. The control animals received an adequate volume of the carboxymethyl cellulose solution, and were treated in the same way otherwise. On day 20 of gestation, all females were sacrificed and foetuses were delivered by Caesarean section. All foetuses were weighed, and sexing was conducted. The abdominal and thoracic cavities of the females were examined. The resorptions, total implantations and corpora lutea were recorded. All foetuses were subjected to gross examinations to determine external abnormalities. One-third of the foetuses was further investigated for visceral malformations, the remaining two thirds of the foetuses were investigated for skeletal anomalies and variations. Mean number of corpora lutea, total implantations, viable foetuses and foetal body weights were compared.

Results

There were no signs of maternal or foetal toxicity at the lowest dose group of 7.2 mg/kg bw/day. Hair loss and minimal self-mutilation (scabbing) of the extremities and abdominal area were seen at 36 mg/kg bw/day. Most rats of the 100 mg/kg bw/day dosage group had varying degrees and combinations of red ocular and nasal discharge, anogenital staining, red vaginal discharge, hair loss of the forelimbs and abdominal area and self-mutilation (scabbing of open wounds) of the extremities and abdominal area. These observations decreased as the treatment period progressed. Increased activity was observed in many of the rats in the 100 mg/kg/day dosage group during the first few days of treatment, but was not evident after the fourth day of treatment. Reduced body weight gains at 36 mg/kg/day and body weight losses at 100 mg/kg bw/day were observed in the first three days of treatment. But these reductions were offset by increased body weight gains for the remainder of the gestation period. Four rats in the 100 mg/kg bw/day dosage group died by gestation day 10.

There were no biologically meaningful differences in the mean number of implantations, corpora lutea, live foetuses, mean foetal body weights or male to female sex ratios between any of the treated groups and the control groups. The number of post implantation losses was significantly reduced in the 36 mg/kg/day dosage group, but not in other dose groups.

The mean number of corpora lutea and implantations in all of the treatment groups were increased in comparison to the control group. Since ovulation and implantation in this study occurred prior to treatment these effects were not considered treatment related. The number of non-pregnant females was increased at 100 mg/kg bw/day. As implantation is considered to be a pre-treatment event this occurrence was not considered to be compound-related. An increased incidence of numbers of foetuses and litters with 27 presacral vertebrae, 14th rudimentary ribs, and 14th full ribs was noted at 36 and 100 mg/kg bw/day.

Numbers of foetuses with malformations were 1, 2, 2, and 3 at 0, 7.2, 36, and 100 mg/kg bw/day, respectively. These malformations were of dissimilar type. Therefore, the study authors concluded that they were not indicative of a teratogenic response. At 36 and 100 mg/kg/d, the test substance showed maternal toxic effects.

Conclusion
This prenatal developmental toxicity study was neither conducted in full compliance with the respective current OECD Guideline, nor has formal adherence to GLP principles been documented and reported. The lack of the full raw data package makes the results difficult to interpret.
The authors conclude that skeletal variations have been observed in pups at maternal doses of 36 and 100 mg/kg bw/day. Maternal toxicity was obvious at 36 and 100 mg/kg bw/day, 4 dams died at the highest dose. The lowest dose (7.2mg/kg) was found to be a NOAEL. There were no clear-cut indications of specific teratogenic effects at any dose level.

Ref.: 85

Additional studies

The dossier contains a teratogenicity study from 1977, in which only one dosage level (100 mg/kg bw/day) was tested. Viewing the restricted aim (dose-range finding study) and the age of the study, it is not discussed in detail.

Ref.: 31

Finally, a so-called "peri-/postnatal toxicity study" is provided, in which 20 inseminated female rats were administered Compound N°34054 (*Climbazole*, purity not stated) orally as a single daily dose beginning on day 15 post coitum and continuing throughout gestation and lactation until sacrifice or death. The administered dosages were 0, 7.2, 36 and 100 mg/kg bw/day. Since this procedure is not common in the present evaluation of reproduction toxicity / teratogenicity for cosmetic ingredients and viewing the age of the study, it is not described in detail.

Ref.: 86

3.3.9. Toxicokinetics

***In vivo* metabolic disposition in humans**

Guideline:	/ (1979, according to an internal protocol)
Species/strain:	human
Group size:	1, sex not stated
Test substance:	*Climbazole*, purity not stated
Batch no.:	323568
Dose levels:	135 mg (2 mg/kg bw)
Route:	oral; in drinking water
GLP:	no information

135 mg of *Climbazole* were suspended in water and taken on an empty stomach (except a cup of black coffee without sugar 15 min before administration). Blood sampling was conducted 1, 2, 4, 6, 8, and 24 hours after ingestion.

Urine was sampled for the following durations: 0 to 2 hours, 2 to 4 hours, 4 to 8, and 8 to 24 hours. *Climbazole* and its metabolite BAY g 5919 were determined in plasma and urine by thin-layer densitometric assay (detection limit in plasma: 10 ng/ml for both substances; detection limit in urine 1 ng/ml for both substances).

Results
Unchanged *Climbazole* was found in plasma only 1 and 2 hours after administration (29 ng/ml, and 10 ng/ml). The metabolite BAY g 5919 reached a peak plasma level of 1500 ng/ml (C_{max}) 1 hour after administration (t_{max}). A half-life of 2 hours for BAY g 5919 removal from plasma was observed. The AUC (area under the plasma level curve) was 4.99 µg/ml.h. Only 0.05% of the administered *Climbazole* was excreted with urine up to 24 hours, mainly as BAY g 5919 (63.39 µg; 0.047%).
These results point to a fast absorption of *Climbazole* as well as a fast metabolism to BAY g 5919 within 1 hour. The authors assume that *Climbazole and* BAY g 5919 are mainly excreted via the bile.
No negative effects were reported after ingestion of *Climbazole.*

Conclusion
The study, which is considered to be unethical in this context, was not conducted according to a guideline. The validity of this study is limited due to the investigation of only 1 test person.
Ref.: 25

***In vivo* metabolic disposition in dogs after subchronic administration**

Guideline:	1980 internal protocol
Species/strain:	Beagle dog
Group size:	3 males + 3 females
Test substance:	BAY e 6975 (*Climbazole*, purity not stated)
Batch no.:	483751
Dose levels:	0, 5, 10, 20 mg/kg bw/day
Route:	oral; capsules
Exposure:	13 weeks, 7 days/week
GLP:	no information

In a 13-week sub-chronic study, the toxicity of *Climbazole* was tested in beagle dogs (see Section 4.5.2.2). Within this study, the concentrations of *Climbazole* and its metabolite BAY g 5919 were measured in blood plasma. Blood sampling was conducted on days 1, 47, and 92, every 4 hours and 24 hours after application. By mistake, blood sampling was also conducted 24 hours after the 46th application. These samples were also examined. Plasma concentration of *Climbazole* and its metabolite BAY g 5919 were measured by a thin-layer densitometric assay. The lower limit of the determination was fixed at 10 ng/ml.

Results
4 hours after application blood plasma content of *Climbazole* and BAY g 5919 correlated with increasing administered dose. No accumulative effect was observed.

The 4-hour concentrations range combined for all 3 days were as follows:

5 mg/kg group:	17 – 36 ng/ml
10 mg/kg group:	47 – 97 ng/ml
20 mg/kg group:	167 – 357 ng/ml

The 4-hour plasma levels of BAY g 5919 were on average 2 to 5 times higher than those of *Climbazole*.
24 hours after application the concentrations of *Climbazole* and BAY g 5919 were at or below 10 ng/ml in nearly all plasma samples. Concentrations above the detection limit were measured in the 10 mg/kg dosage group only on day 47 in 2 samples of male dogs (*Climbazole:* 24 and 26 ng/ml; BAY g 5919: 27 and 65 ng/ml).
Large individual differences in plasma concentrations were observed.
The authors assume a first pass effect of 95% of the administered dose, and a plasma level maximum 2 hours after administration with twice as high concentration values.

Conclusion
The study was not conducted according to a guideline. Combined with the brief test description, the results are difficult to assess.

Ref.: 22

***In vivo* metabolic disposition in rats**

Guideline:	1977 internal protocol
Species/strain:	Wistar rat
Group size:	2 males
Test substance:	*Climbazole*, purity not stated
Batch no.:	323568
Dose levels:	50 mg/kg bw
Route:	oral; gavage
GLP:	no information

The absorption of *Climbazole* (BAY e 6975) and the formation of its metabolite BAY g 5919 were investigated in male rats following single administration (*Climbazole* suspended in 0.2% agar water; 2 ml application volume). Blood sampling was conducted between 0.5 hours and 16 hours after administration (time points: 0.5, 1, 2, 3, 4, 6, 8, and 16 h). Plasma concentration of *Climbazole* and its metabolite BAY g 5919 were measured by a specific thin-layer densitometric assay. This assay measures no other metabolite of *Climbazole* than BAY g 5919, thus only the latter was quantified.

Results
Peak levels of *Climbazole* were gained already at the first time point (0.5 h) with plasma concentrations of 4.2 µg/ml, and 4.5 µg/ml, respectively, for 2 rats. The half-life of disappearance from plasma was estimated as about 3 to 4 hours.
At the 16 h-time point, the concentrations were in the range of the detection threshold (0.05 µg/ml).

The metabolite BAY g 5919 was detected already after 0.5 h (1.16 µg/ml, mean). Peak concentrations of BAY g 5919 in plasma were reached 6 hours after administration (3.9 µg/ml, 6.2 µg/ml). These results suggest a rapid absorption of *Climbazole* after oral administration and an elimination of the parent compound mainly by biotransformation. Only a part of the administered *Climbazole* was found in the plasma, either as parent substance or as metabolite.

Conclusion
The study was not conducted according to a guideline. Data are given to assess the velocity of absorption of *Climbazole* and formation of metabolites. The small number of animals and dose levels reduce the validity.

Ref.: 18

***In vivo* metabolic disposition in mice**

Guideline:	1976 internal protocol
Species/strain:	CF_1/W 68 mouse
Group size:	8 and 10, sex not stated
Test substance:	BAY e 6975 (*Climbazole,* purity not stated)
Batch no.:	not stated
Dose levels:	100 mg/kg bw
Route:	oral; gavage
GLP:	no information

The absorption of *Climbazole* (BAY e 6975), suspended in dimethylformamide and agar water, and the formation of a metabolite was investigated in mice following single administration (0.5 ml application volume). Two different study parts were conducted with blood concentration measurement in 8 animals (part 1), and plasma concentration measurement in 10 animals (part 2), respectively. Blood sampling was conducted between 15 minutes and 8 hours after administration (time points: 15 min, 30 min, 1, 2, 4, 6, and 8 h (part 1); 30 min, 1, 3, and 6 h (part 2)) and blood from all animals per study part was pooled. Blood and plasma concentration of *Climbazole* and its main metabolite were measured by a specific thin-layer densitometric assay. It was stated, that the concentrations of the metabolite were minimum concentrations and likely higher than indicated.

Results
In the first study part, peak levels of *Climbazole* in blood were gained already at the first time point (15 min) with concentrations of 9.9 µg/ml. At the 8 h-time point, the concentrations were reduced to 2.0 µg/ml. The half-life of disappearance from blood was estimated as about 3 hours. The metabolite was detected already 15 min after administration (1.6 µg/ml) and peak concentrations were reached 6 hours later (5.5 µg/ml). The second study part revealed higher plasma concentrations and a similar course of blood concentrations with time. A peak concentration of 12.0 µg *Climbazole* /ml 30 minutes after administration and of 10.1 µg metabolite/ml at the 6 hour time point were measured. The authors assume that biotransformation of *Climbazole* is mainly responsible for the *Climbazole* plasma clearance. The generally higher plasma concentrations of *Climbazole* compared with blood concentrations indicate that only small amounts of parenteral substance were taken up by erythrocytes.

Conclusion
The study was not conducted according to a guideline. Data are given to assess the velocity of absorption of *Climbazole* and formation of its metabolite. The age of the test and its brief description reduce the validity.

Ref. : 67

Additional information

Two published studies reported also a high potency of *Climbazole* to induce, but also to inhibit different rat hepatic microsomal cytochrome P450 enzymes and other drug-metabolizing enzymes (e.g. glutathione-S-transferase) after i.p. administration of 0.1 – 0.8 mmol/ kg (approx. 30 – 235 mg/kg)

Ref.: 78, 79

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

/

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

/

3.3.11. Human data

/

3.3.12. Special investigations

/

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

NOT APPLICABLE

3.3.14. Discussion

This dossier contains a large number of shortcomings, of which the most important ones are summarized below:

1. The summary is dated 24 June 2004 and should therefore have been written under the new standard format of the opinions of the SCC(NF)P. This is not the case.
2. The references are numbered at random, which makes the evaluation of the data package difficult.
3. Some full texts of specific references are "available on demand". However, these should have been included in the data package provided to the SCCP.
4. With regard to the identification of the compound:
   - the copies of the most relevant analytical reports are illegible and the report is outdated (reference 9, 1975). Full identification should have been performed on a much more recent batch of the product

- a material safety data sheet has been used to provide the physico-chemical properties of the compound. This is not an appropriate source of reference for a SCCP-dossier.

5. With regard to the acute toxicity data package:
   Although the performed test is 30 years old, there is no need to sacrifice any additional animals for its repetition. The LD_{50}-oral-rat of 400 mg/kg/day can be accepted.
6. With regard to the presented data on skin and eye irritation :
   No relevant information on *Climbazole* as a compound is provided. The majority of the tests described are *in vivo* and *in vitro* studies with *Climbazole*-containing end products. The observed irritative effects are completely or partly caused by other constituents in the formulations, but no information on *Climbazole* itself can be retrieved from the tests performed.
7. With regard to the sensitising properties of Climbazole:
   - the presented Magnusson Kligman Guinea Pig Maximisation test (1983) cannot be considered as valid, since the doses have been wrongly chosen.
   - the mentioned Buehler test was not accompanied by its full description, which makes it impossible to assess its results.
8. With regard to the percutaneous absorption
   No valid percutaneous/dermal absorption study is provided. The presented *in vivo* studies in which *Climbazole*-containing formulations were applied to human scalp or hands, showed that low concentrations of the compound and its major metabolite were measured in blood plasma and urine. Therefore, the absorption value for the calculation of the MoS for *Climbazole* will be considered 100% until sound dermal absorption data are provided.
9. With regard to long term toxicity studies :
   The dossier clearly suffers from the age of the studies. Nearly all of them have been performed between 1975 and 1983, before GLP-regulations were in place. The descriptions are brief and the raw data are incomplete.
   Nevertheless, based on the available information, a cautious NOEL-value of 5 mg/kg/day can be deduced from the 90d oral study with the rat.
   Chronic and carcinogenicity studies are not available.
10. With regard to the teratogenic effects of the substance, a relatively recent study (2001) provides a NOAEL (embryotoxicity) of 30 mg/kg bw/day and a NOAEL (maternal toxicity) of 15 mg/kg bw/day.
11. With regard to the mutagenicity/genotoxicity data
    The presented tests are old and have not been performed according to recent guidelines. They all show negative results. Due to the lack of carcinogenicity data, however, it is recommended to perform the currently defined basic set of mutagenicity tests.
12. With regard to toxicokinetics:
    Climbazole appears to be rapidly metabolised into its major metabolite BAY g 6975 through first pass metabolism. Excretion mainly occurs through the bile. Again, the presented data package contains old tests, which reduces their scientific validity.
    Since the mother compound is heavily metabolised, the metabolite BAY g 6975 should have also been considered in the safety assessment.

The Margin of Safety will be calculated once all missing data have been provided.

4. CONCLUSION

The dossier is an example of an inadequate and poor submission provided by industry.
Due to the many shortcomings mentioned under section 3.3.14, it is impossible for the experts of the SCCP to assess whether *Climbazole* is safe for use in rinse-off (2.0%) and leave-on (0.5%) anti-dandruff cosmetic products.

The SCCP notes that *Climbazole* is taken up in Annex VI and used as a preservative. In view of the poor quality of the toxicological data presented in the current dossier, the SCCP recommends a re-evaluation of the safety of this compound for preservative uses.

5. MINORITY OPINION

Not applicable

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