SCCS/1315/10
24 March 2010

EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Melatonin

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

The SCCS adopted this opinion at its 6th plenary meeting
of 23 March 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCS

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

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ISSN 1831-4767 ISBN 978-92-79-12738-0
doi:10.2772/23346 ND-AQ-09-010-EN-N

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

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, melatonin, CAS 73-31-4, EC 200-797-7, directive 76/768/EEC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Melatonin, 23 March 2010

TABLE OF CONTENTS

1. BACKGROUND

The Commission received a request from the German authorities for a safety evaluation of the use of the substance melatonin in cosmetic products.

In the meantime also a dossier has been submitted by a company.

Melatonin is proposed to be used in hair products at a maximum concentration of 0.0033% w/w (33 µg/g).

2. TERMS OF REFERENCE

1. *Does the SCCS consider the use of melatonin in a concentration of 0.0033% w/w (33 µg/g) in hair care products safe for the consumer?*

2. *Does the SCCS foresee any other restrictions to the safe use of melatonin?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Melatonin (INCI name)

3.1.1.2. Chemical names

N-Acetyl-5-methoxytryptamine
3-(2-Acetylamino-ethyl)-5-methoxyindole
N-[2-(5-Methoxyindol-3-yl)ethyl]acetamide
N-[2-(5-Methoxy-1H-indol-3-yl)ethyl]-acetamide ()
5-Methoxy-N-acetyltryptamine

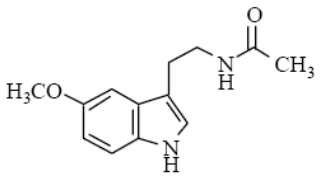
3.1.1.3. Trade names and abbreviations

Melatonin	Melovine
Circadin	NSC 113928
Melatol	NSC 56423
Melatonine	Regulin

3.1.1.4. CAS / EC number

CAS: 73-31-4
EC: 200-797-7

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{13}H_{16}N_2O_2$

3.1.2. Physical form

White-cream to yellowish crystalline powder

3.1.3. Molecular weight

Molecular weight: 232.3 g/mol

3.1.4. Purity, composition and substance codes

Substance code: 03-MELA

Batch 3/104: was used for the elucidation of structure of Melatonin using NMR,IR MS and elemental analysis. HPLC was used for testing for impurity. This batch was used for the phototoxicity study
Batch 6/104: was used for the 6-month study
Batch 6050699: was used for the teratogenicity studies

Purity
> 99.5% batch 3/104

Comments:
Batch 6/104 and Batch 6050699 have not been characterized by NMR, MS, IR or analysed by HPLC for impurities.

3.1.5. Impurities / accompanying contaminants

Substance code	each impurity max. 0.1%	
00-MELA	5-Hydroxytryptamine, serotonine	(synthesis precursor)
01-MELA	N,O-Diacetyl-5-hydroxytryptamine	(synthesis precursor)
02-MELA	N-Acetyl-5-hydroxytryptamine	(synthesis precursor and degradation product)
01-MEXA	5-Methoxytryptamine	(degradation product)

Comment
None of these substances, which according to the way of synthesis theoretically could be impurities, could be detected in batch 3/104 by HPLC with a limit of Detection of 0.01-0.02%. There was a loss of drying between 0.17 and 0.19% (solvents). Total impurities (HPLC) were < 0.5%.

In addition to batch 3/104, only batches 4/105, 5/105 and 1/106 were analysed for impurities.

3.1.6. Solubility

Ethanol	182 g/L at 20°C	
Water	2 g/L at 20°C	5 g/L at 50°C
Ethyl acetate	16 g/L at 20°C	
Ethanol/water (40/60 v/v)	64 g/L at 20°C	
Acetone	150 g/L at 20°C	

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 1.2

The test was performed according to EU method A.8. on melatonin batch 6/104.

3.1.8. Additional physical and chemical specifications

Melting point:	116 – 120 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/

pKa: /
Refractive index: /
UV_Vis spectrum (200-800 nm) /

The following methods were used for the characterization:
1. Elemental analysis
2. Infrared spectroscopy
3. ^{1}H nuclear magnetic resonance spectroscopy
4. ^{13}C nuclear magnetic resonance spectroscopy
5. Mass spectrum
6. Ultraviolet spectrophotometry

IR absorption data:
The characteristic bands of the main functions of Melatonin (03-MELA) are presented below:

Wave number (cm-1)	Assignment
3305.9	NH_2 stretching of amid
3078.0	CH stretching
2988.3	CH stretching
2928.4	CH stretching
2828.7	CH stretching
1621.7	C=O stretching (amid)
1557.5	C=O stretching (amid)
1489.0	NH_2 stretching (amid)
1309.8	C=C stretching (aromatic)

UV light absorption data:
The typical UV absorption spectrum of Melatonin (batch 3/104) shows a peak at 279 nm.

3.1.9. Homogeneity and Stability

No data

General comments

The batches of melatonin for which analytical data has been provided show a purity of >99%. In the large majority of the available studies the purity of the Melatonin batches used is not documented. However, the described procedure of synthesis starting with serotonin obviously leads to products with purities much better than 90%. The dose of Melatonin is 0.1 mg/application, and the amount of impurities simultaneously applied is by at least 1 to 2 orders of magnitude lower. Therefore, they can be assumed not to have adverse effects on health.

3.2. Function and uses

The applicant has applied for the use of Melatonin in hair care products at a concentration of 0.0033%.

Melatonin is an endogenous molecule produced in the human vertebrate pineal gland, the retina and possibly in some other organs. It displays a marked circadian rhythm, with high levels at night and low levels during the day.

Use as an anti-oxidant/cosmetic ingredient

Melatonin is a radical scavenger and functions as an antioxidant. It has been proposed that in this way melatonin may add to the protection against molecular damage by oxygen and nitrogen-based toxic reactants.

Ref.: 37

In the CosIng data base, Melatonin is indicated as an antioxidant in cosmetic products.

The applicant claims that Melatonin stimulates hair growth in hair organ culture, improves scalp in seborrheic patients and stabilizes hair loss in various forms of alopecia.

UV suppressive effect on the skin

Topically applied Melatonin (0.6 mg/cm²) has shown an UV (290-390 nm)-suppressive effect in a double-blind randomized clinical trial in 20 healthy volunteers. Melatonin suppresses erythema compared to treatment with the vehicle alone.

Ref.: 3

Use as active substance in medicines

During many years of experience with medical uses of Melatonin, various effects of the substance in humans have been studied.

Melatonin is used to treat insomnia and to minimize the effects of jet lag or of shift work. Sleep disorders, Circadian rhythm and Pituitary hormone secretion have been studied using doses between 0.1 and 5 mg/day [Chase et al. 1997 Lewy et al. 1995 Forsling et al. 1999]. An oral application of 0.1 to 0.3 mg/day at midday has been shown to lead to an increased duration of sleep during the night.

Numerous publications on Melatonin deal with behaviour, contraception and cancer. In all these examinations, Melatonin doses between 5 and 240 mg/day have been used (Lieberman et al. 1984; Silman 1993; Aldeghi et al. 1994).

3.3. Toxicological Evaluation

The purity of the test substance used in most studies is not known.

3.3.1. Acute toxicity

Single dose toxicity studies with Melatonin were performed in mice and rats following different routes of administration. The following values were obtained:

Species	Route of Administration	LD50 (mg/kg bw)	Reference
Mouse	i.p.	> 800	5
	i.p.	1168	45
	Oral	1250	
	s.c.	> 1600	
	i.v.	472	
Rat	i.p.	1131	
	Oral	> 3200	
	s.c.	> 1600	
	i.v.	356	

Essentially, similar behavioural effects were seen in both mice and rats after administration of large doses of Melatonin. At doses of 400 mg/kg bw, vasodilatation of the extremities indicated by a reddening of the ears and feet, piloerection and ptosis were common. In addition, muscle relaxation, a marked lack of motor activity and ataxia were observed. At higher doses an impairment of the righting, placing and hindlimb ipsilateral flexor reflexes, a marked reduction in body temperature and slow, labored respiration preceded death.

Ref.: 45

No specific studies have been performed with regard to the dermal toxicity of Melatonin. However, Sugden et al. investigated the toxicity of Melatonin applied subcutaneously to mice and rats. In both species, the LD50 (24 h) was > 1600 mg/kg body weight.

Ref.: 45

There is no information available with regard to acute toxicity of Melatonin following inhalation.

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

No *in vitro* or animal data have been submitted.

Studies in human volunteers with finished product containing 0.0033% melatonin are described below.

Cutaneous tolerability: Occlusive patch test with Melatonin-based end product

Guideline:	/
Subjects:	Healthy human volunteers, both female and male, all with a negative case-history for allergic contact dermatitis and absence of any other presently active cutaneous pathologies
Group size:	20
Observation period:	48 hours
Test substance:	Commercial Melatonin Hair Product containing 0.0033% Melatonin in an ethanol / water solution (30% ethanol), diluted 1/10
Code of test substance:	W/157/001/015
Batch:	6050699 (trade name: ASATEX®); 034107
Dose level:	0.1 mg/day
GCP:	/
Date of study:	2003

The primary skin irritation potential of Melatonin Hair Product containing 0.0033% Melatonin was investigated by application of a gauze rinsed with a 1/10 dilution of the product to the skin of the forearm or the back. The occlusive patch remained on the test area for 48 hours. Scoring of skin reactions, namely erythema, oedema, and vesiculation, was done 30 minutes, 24 hours and 48 hours after the test substance had been washed off.

Results
The mean score for each parameter (erythema, oedema, vesicles) was calculated at the observation times. The sum of the three average values corresponds to the Mean Index of Cutaneous Irritation at 30 minutes, 24 and 48 hours. The product was then classified according to the following table:

Mean Index of Cutaneous Irritation	Product Classification
0 - 0.5	Non-irritating

Mean Index of Cutaneous Irritation	Product Classification
0.5 – 2	Slightly irritation
2 – 5	Moderately irritating
5 – 8	Strongly irritating

The mean scores after 30 minutes, 24 hours and 48 hours were all in the range of 0 - 0.5 (= non-irritating).

Conclusion
Based on the results of this study, the test product is considered to be non-irritating to human skin.

Ref.: 15

Comment
The test concentration was 10% of the intended use concentration. It is not possible to draw any conclusion from this study.

Two-week use test with Melatonin-based end product (CC-02-211)

Guideline:	/
Subjects:	23 male and 25 female human volunteers (20-67 years old), suffering from hair loss
Group size:	48
Observation period:	2 weeks
Test substance:	Melatonin Hair Product containing 0.0033% Melatonin (Batch N° 6050699) in an ethanol / water solution (30% ethanol) (designation used in the study report: Hair Tonic)
Code of test substance:	W/157/001/012
Batch:	09202
Dose level:	Application once daily on the scalp. No rinsing was allowed. Exact dosage was not stated.
GCP:	/
Date of study:	November - December 2002

The volunteers were asked to apply the product, as supplied in individual doses, on their scalp, once per day before bedtime, over a period of 14 consecutive days. They were advised to distribute the test product evenly on the scalp.
At the end of the study the volunteers completed a questionnaire. They were firstly asked about any reactions they noticed after having used the test product.

Results
With regard to the possible unwanted effects, the only parameter reported to have increased during the use of the product was the stinging effect. The level of this effect reached a maximum of 3 (1 being minimal and 5 being extreme) in one volunteer only and the total number of subjects who reported stinging was 6 out of 48.

Ref.: 18

Comment
The product tested did show a stinging effect of the scalp of some subjects.

3-Month use test and compatibility study with Melatonin-based end product (MEL-COS-AS01)

Guideline:	/
Subjects:	Human volunteers with androgenetic alopecia (15 male and 15 female volunteers, 18-40 years old)

Group size:	30 at the beginning of the study, 21 at the end
Observation period:	90 days
Test substance:	Melatonin Hair Product containing 0.0033% Melatonin (Batch N° 6050699, trade name: Asatex®) in an ethanol / water solution (30% ethanol)
Code of test substance:	W/157/001/012
Batch:	09202
Dose level:	Daily topical application of 3 ml of the cosmetic product containing 0.0033% Melatonin to the scalp for 90 days with administration in the evening
GCP:	/
Date of study:	March 2002 to June 2003

The treatment scheme for the volunteers consisted in the topical application of 3 ml of the product containing 0.0033% Melatonin once a day in the evening (seven days a week) for a period of 90 days. The volunteers were assessed at the time of recruitment, after 30 days and after 90 days of treatment.
In order to evaluate the results obtained, use was made of *a)* a macrophotograph according to standard shots and *b)* a clinical evaluation of efficacy, tolerability, degree of satisfaction and cosmetic agreeability of the product.

Results
Safety
The product was generally well tolerated by the panellists during the whole period of application. The side effects recorded were itching (7/21 volunteers), temporary reddening (1/21 volunteers) and burning sensation (1/21 volunteers).

Efficiency
Melatonin for topical use proved to influence the activity of the hair follicle, inducing a statistically significant clinical response compared to baseline conditions.

Conclusion
The author concludes that the product showed a satisfactory tolerability in the test group of 21 volunteers that completed the study.

Ref.: 27

Comment
The relevance of this study is hampered by the fact that about 30% of the participants dropped out during the study.

Further studies with relevance to skin irritation are reported in section 3.3.11.

3.3.2.2. Mucous membrane irritation

No studies have been performed specifically investigating the potential of Melatonin for mucous membrane irritation.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	draft OECD 429
Species/strain:	CBA/Ca strain mice, 8-12 weeks old
Group size:	5 animals / dosage group
Observation period:	6 days
Test substance:	Melatonin (purity > 98%)
Dose levels:	0.00 - 0.25 - 2.5 - 5.0 - 10 %, w/v in olive oil:acetone (4:1)

GLP: /
Date of study: Published in 2001

After dissolving Melatonin (0.5, 2.5, 5.0 and 10.0%, w/v) in acetone:olive oil (4:1, AOO), 25 µl of the solution was applied to the dorsal surface of each ear of 5 female CBA/Ca mice/dosage group for three consecutive days. On the sixth day, [^{3}H]-methyl thymidine was administered intravenously and the uptake of [^{3}H]-methyl thymidine (dpm) by the draining lymph nodes was determined by established methods. Dinitrochlorobenzene (DNCB, 0.25%, w/v), para-aminobenzoic acid (PABA, 2.5%, w/v) and AOO were used as positive, negative and vehicle control, respectively.

Results
The mean dpm obtained with Melatonin treatment at all concentrations were not significantly different ($p>0.05$) from that of AOO. The stimulation index (SI) values of Melatonin at different concentrations were close to 1. As expected, the SI for DNCB reached a clearly positive value (approximately 12).

Conclusion
The results of this study indicate that Melatonin is not a skin sensitizer.

Ref.: 22

3.3.4. Dermal / percutaneous absorption

***In vitro* percutaneous absorption study with Melatonin-based end product**

Guideline:	OECD 428 (1995)
Test system:	Pig skin (1000 µm), ¼ of full pig skin containing the stratum corneum, stratum germinativum and a small part of the dermis, 6 samples
Contact time:	24 hours
Test substance;	Cosmetic Melatonin Hair Product containing 0.0033% Melatonin (Batch N° 6050699) in an ethanol / water solution (30% ethanol).
Code of test substance:	W/157/001/012
Batch:	09202
Volume applied:	50 µl, 60 µl, 70 µl, 80 µl, 90 µl, 100 µl, made up to 100 µl volume with ethanol / water solution (30% ethanol) Receptor fluid: 0.14 M NaCl, 2 mM K_2HPO_4, 0.4 mM KH_2PO_4, 100 IU penicillin/ml, 100 µg streptomycin/ml, 40% ethanol
GLP:	/
Date of study:	2 – 11 September 2002

The *in vitro* cutaneous absorption of the test substance was determined using 6 full thickness porcine skin samples containing stratum corneum, stratum germinativum and part of the dermis.
On each skin sample, a total volume of 100 µl, containing different Melatonin concentrations, was applied. After 24 hours of contact, the amount of the test substance was determined in the receptor fluid, in the skin extract and in the rinsing solution.

Results
Eighty percent of Melatonin was found to remain on the skin and 20% of the Melatonin was found in the total skin extract (no separation of skin layers). In the receptor fluid, Melatonin was not detectable. The different skin layers have not been investigated separately. In total, 99.83% of the applied Melatonin was recovered.

Conclusion
The applicant stated that this study was added to complete the dossier although the results are difficult to interpret since the study design deviates from the recommendations

mentioned in the document SCCNFP/0750/03. The major differences can be identified as (1) the fact that each of the 6 skin samples used was treated with a different concentration of the test substance, (2) the lack of documentation on the choice of the receptor fluid and the solubility of the test substance in it, and (3) the fact that the skin layers have not been separated and that therefore the amount of substance present in the stratum corneum could not be detected.
In light of the availability of the results of the clinical trial [Macher 2003], this *in vitro* dermal absorption study was not repeated.

Ref.: 17

Comment
The study is considered inadequate for the MOS calculation.

14-Day pharmacokinetics and clinical and biological tolerability study with Melatonin based end product: Clinical trial in human volunteers

As described in detail under 3.3.9, a 14-day pharmacokinetics and clinical and biological tolerability study was performed with the intended finished cosmetic product containing 0.0033% Melatonin.
This study showed that daily application of the formulation under the intended conditions of use (application of 3 ml once daily before bed time) lead to an increase of Melatonin plasma levels which however was within physiological levels. Also, 6-hydroxy melatonin sulphate urine values did not exceed physiological levels.

Ref.: 29

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral toxicity

14-Day oral study in the rat

Guideline:	/
Species/strain:	Long-Evans and Fischer 344 rats
Group size:	10 males and 10 females per dosage group
Observation period:	14 days
Test substance:	Melatonin
Dosage levels:	0.0, 0.005, 0.050, 5.00, 50 and 200 mg/kg bw/day
GLP:	/
Date of study:	Published in 2003

Melatonin was administered to Long-Evans and Fischer 344 rats 5 days/week for 14 days at the following dose levels: 0, 0.005, 0.05, 5.0, 50 or 200 mg/kg bw/day. The animals were observed for 12 days before necropsy. All dosage groups and controls were exposed to one of the three different lighting and timed dosing treatments. The Core Treatment Groups (1) were exposed to full-spectrum fluorescent lighting during the 12-hour light cycle and (2) were dosed during the fourth and fifth hour of the light cycle. The Night Treatment Groups (1) were exposed to full spectrum fluorescent lighting during the 12-hour light cycle and (2) were dosed during the third and fourth hour of the 12-hour dark cycle.

The Light Treatment Groups (1) were exposed to full spectrum fluorescent lighting during their 12-hour light cycle plus either 1000 lux (Fischer 344 rats) or 1500 lux (Long-Evans rats) during the sixth and seventh hour of the light cycle seven times or approximately every other dosing day and (2) were dosed during the fourth and fifth hour of the light cycle. Body weights were recorded approximately weekly and clinical observations were recorded daily during dosing beginning with test day 2.

At termination, all Core Treatment Group rats were necropsied; organ weights were recorded only for the Core group and tissues were processed for microscopic examination only in the Core group. Specially prepared slides of the right eyes from all rats in all dosage groups and lighting treatments (Core, Light and Night Treatment Groups) were examined.

Results
No Melatonin-related clinical signs, no early deaths, no substance-related body weight changes, no organ weight changes, no gross lesions or histopathological findings were noted in this study. Administration of Melatonin did not result in dose-response related differences in retinal outer nuclear layer thickness means in any sex, strain, and lighting treatment groups up to the highest tested dose of 200 mg/kg bw/day.

Conclusion
Although not designed as a classical subacute toxicity study, and despite of the brief description of the test, a NOAEL of 200 mg/kg/day can be established for the specific effects measured in this 14-day oral study with Melatonin in the rat.

Ref.: 6

3.3.5.1. Repeated Dose (28 days) subcutaneous toxicity

28-Day subcutaneous study in the rat

Guideline:	/
Species/strain:	Sprague Dawley rat
Group size:	19 males and 19 females per dosage group
Observation period:	28 days
Test substance:	Melatonin (obtained from Regis Technologies, Morton Grove, USA)
Dosage levels:	0.0, 0.050, 0.50 and 4.8 mg/kg bw/day (males) 0.0, 0.074, 0.75 and 7.3 mg/kg bw/day (females)
GLP:	/
Date of study:	Published in 2000

In this 28-day toxicity study, Sprague-Dawley rats received subcutaneously by an osmotic pump 60 µl/day of vehicle (PEG 400) containing 0.03%, 0.3% or 3% Melatonin, continuously for 28 days. The dose of Melatonin delivered based on weekly group mean body weights (n = 10) was approximately 0.050, 0.50 and 4.8 mg/kg bw/day for the males and 0.074, 0.75 and 7.3 mg/kg bw/day for the females. An additional group (19/sex) underwent surgery, but no osmotic pumps were implanted (sham control).

Results
No deaths or changes in clinical observations occurred. No substance-related effect was noted in body weights, haematology, clinical chemistry, urinalyses or gross pathology. A dose-related trend of increasing serum Melatonin concentrations occurred in males and females. In males, there was a trend toward decreasing serum prolactin concentrations with time at all levels of Melatonin treatment. No difference in serum follicle-stimulating hormone (FSH) concentrations occurred between treated groups. Most of the samples were at the limit of detection for the serum luteinizing hormone (LH) assay (0.157 ng/ml). A dose-related increase occurred in urine 6-sulphatoxymelatonin (the primary metabolite) concentrations in Melatonin-treated male and female groups. No treatment-related organ weight or histopathology changes were noted in rats infused with 0.03% or 0.3% Melatonin. Two of 10 males administered 3.0% Melatonin had decreased testes weights and testicular degenerative changes composed of reduced or absent spermatogenesis, spermatidic giant cells and oedema.

Conclusion

Although the authors do not define a clear NOAEL value, one can propose the value of 0.50 mg/kg bw/day (corresponds to the 0.3% Melatonin solution in the male rats) as the NOAEL for Melatonin based on decreased testes weights and testicular degenerative changes.

Ref.: 35

3.3.5.3. Sub-chronic (90 days) oral

Guideline:	/
Species/strain:	Long-Evans and Fischer 344 rats
Group size:	10 males and 10 females per dosage group
Observation period:	90 days
Test substance:	Melatonin (in methylcellulose 0.5% containing ethanol 0.25%)
Dosage levels:	0.0 - 0.005 - 0.050 - 5.00 - 50 - 200 mg/kg bw/day
Administration:	gavage
GLP:	/
Date of study:	Published in 2003

In a 90-day toxicity study, Melatonin was administered by gavage to Long-Evans and Fischer 344 rats at the following dose levels: 0, 0.005, 0.05; 5.0, 50 or 200 mg/kg bw/day. There was a Special Study Group composed of Fischer 344 rats, and two Core Groups, one composed of Long-Evans rats and the other composed of Fischer 344 rats. Doses were administered daily for 90 days, excluding weekends and holidays, for a total of 17 dosing days and 68 dosing days, for the Special Study Group and Core Groups, respectively. Body weights were recorded weekly for all 3 groups and, clinical observations weekly from the 2 Core Groups only. Sperm morphology and vaginal cytology evaluations were conducted in the Core male and female rats in the 0, 5, 50 and 200 mg/kg treatment groups.

On study day 23, the Special Study rats were bled for Melatonin, thyroid hormone, and clinical chemistry analyses. During week 12, the Core rats were bled for thyroid hormone analyses, and at termination for Melatonin, haematology and clinical chemistry analyses. At termination, all Core rats were necropsied, organ weights were recorded, and tissues were processed for microscopic examination. Slides of the left eyes from all Core rats of all dose groups were examined for retinal morphometric assessment.

Results

Dark-coloured faeces were observed in the two highest dosage groups (50 and 200 mg/kg bw/day). No treatment-related individual organ weight changes were observed during the study. However, mean weight gains over the entire study in all the female Long-Evans Melatonin treated groups were 7 to 10% less than their control. Also in the Fischer rats, a reduction in body weight gain was observed, though only in dosages starting from 5 mg/kg bw/day. As far as clinical biochemistry is concerned, increases in T_3 and T_4 were observed at dosages starting from 0.05 mg/kg/day, but these measurements have been declared as not clinically significant, since no concurrent effects on thyroid histopathology were observed. Cystic uterine endometrial hyperplasia was observed in a number of treated Long-Evans female rats, but also in their respective control group. Finally, one treatment-related finding in a 50 mg/kg bw/day treated Long-Evans female was a dilated uterus at necropsy.

Conclusion

In light of the poor description of the test and given the fact that the raw data cannot be consulted, it is very difficult to establish a NOAEL value based on this study. An additional issue is that there is a large gap between the dosages of 0.05 and 5 mg/kg bw/day.
Without full description of the test and without the raw data, the NOAEL value can be temporarily set on 5 mg/kg bw/day (based upon the significant treatment-related adverse effects, being the coloured faces and the dilated uterus).

Ref.: 14

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

General comment on repeated dose toxicity
The repeated dose toxicity studies suffer from inadequate design (Batelle, 2003) or poor description and documentation (Gerken et al., 2003). So there is only one study (Prevo et al., 2000) from which a NOAEL can be derived. The NOAEL of 0.5 mg/kg bw/day is based on decreased testes weights and testicular degenerative changes.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial reverse mutation test

In order to determine the mutagenic potential of Melatonin and its major metabolite 6-hydroxymelatonin, a Bacterial reverse mutation test tests were performed using three strains of *Salmonella typhimurium* (TA 97, TA 98 and TA 100) without and with metabolic activation (Arochlor 1254 induced rat liver) in two independent experiments.
Concentrations ranging from 5 to 5,000 µg of Melatonin/plate were employed. No mutagenicity was observed up to the top concentration of 5,000 µg Melatonin/plate in any of the bacterial strains used, either in the presence or absence of metabolic activation. Similarly, 6-hydroxymelatonin exhibited no mutagenicity at the concentrations used, a maximum of 100 µg/plate without and with metabolic activation.

Comment
These tests show some shortcomings, such as the fact that the purity of the chemical was not given and that only a part of the classical strain battery was tested.

Ref.: 34

The so-called "WP2 Mutoxitest" is an assay under development that could be an analogue of the Ames test. It involves the tester strain IC 203, deficient in OxyR, together with its *OxyR*$^+$ parent WP2 *uvrA*/pKM101 (denoted IC188) and it was used to detect the mutagenic activity of 80 chemical compounds, including melatonin. Melatonin was tested at the concentration of 5,000 µg/plate with negative results.

Ref.: 31

Comment
In light of the fact that this test is not validated yet and that Melatonin was only tested without metabolic activation, the results can only be considered as indicative.

The effect of Melatonin on N-nitroso-N-methylurea (NMU) induced mutagenicity was investigated in A bacterial reverse mutation test with preincubation. Two strains were used: Salmonella TA 100 which detects base-pair substitution at the G-C site, and TA 102 detecting damage at the A-T site substitutions at the point mutation level. The experiment was performed without metabolic activation only. Melatonin was tested at the concentration of 0.5 up to 2 µmol/plate and gave negative results. Although the raw data are not given, the authors describe that Melatonin did not show mutagenic activity by itself and had no protective effect against NMU induced lesions.

Ref.: 33

By analogy with the experiments performed by Musatov et al. [1999], the effect of Melatonin on 7,12-dimethylbenz[a]anthracene (DMBA) inducedmutagenicity was investigated in a bacterial reverse mutation test with preincubation. Two strains were used: Salmonella TA 100 which detects base-pair substitution at the G-C site, and TA 102 detecting damage at the A-T site substitutions at the point mutation level. The experiment was performed with and without metabolic activation.
Melatonin was tested at the concentration of 0.25 up to 2 µmol/plate and inhibited DMBA mutagenicity in a dose-dependent manner. Although the raw data are not given, the authors describe that Melatonin did not show mutagenic activity by itself and that it had a protective effect against the DMBA-induced lesions.

Ref.: 2

In vitro comet assay

To investigate the effect of Melatonin on the mutagenicity activity of N-nitroso-N-methylurea (NMU) a Single Cell Gel Electrophoresis assay (SCGE assay or COMET assay) was performed on Chinese hamster ovary cells (CHOK1 cells). Melatonin itself revealed no clastogenic activity. However a slight, but statistically significant ($P<0.001$), dose-related anticlastogenic effect of melatonin (10^{-10}-10^{-7} M) was observed. This indicates that melatonin may have a slight but significant and dose related inhibitory effect towards NMU mutagenicity in CHOK1 cells.

Ref.: 33

To study if melatonin have an inhibitory effect on 7,12-dimethylbenz*[a]*anthracene induced mutagenesis in vitro, the COMET assay was performed on Chinese hamster ovary (CHOK1) cells.
While Melatonin revealed no clastogenic activity in the assay by itself, it induced a slight but significant and dose-related inhibitory effect towards DMAB in CHOK1 cells.

Ref.: 2

***In vitro* Chromosome Aberration Test**

The protective role of Melatonin on radiation-induced DNA damage in human lymphocytes was investigated by studying the chromosomal rearrangement on metaphases stained with the fluorescence plus Giemsa technique. Cells treated with Melatonin before irradiation showed less chromosomal rearrangements than cells irradiated without any Melatonin treatment. Although raw data are not given, the authors state that Melatonin by itself was not found to be clastogenic.

The experiment performed did not allow detecting whether the mechanism of action of Melatonin involved scavenging free radicals or activating repair enzymes, neither did it provide information on the primary DNA damage.

Ref.: 48

Comment
The chromosomal aberration assay performed indicates the protective effect of melatonin on gamma radiation-induced damage in human lymphocytes. Unfortunately, it was not designed to test Melatonin itself for a clastogenic potential, due to the non-conformity with the guideline for the chromosomal aberration test (no mitotic index, dose selection, harvest time, etc.).

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo mammalian micronucleus test

In an *in vivo* micronucleus test, the ability of Melatonin to influence lipopolysaccharide (LPS)-induced genotoxicity was tested using micronuclei as an index in both bone marrow and peripheral blood cells of rats. Melatonin (5 mg/kg bw) was injected prior to a single dose of 10 mg/kg bw LPS and thereafter at 6-h intervals up to 72 h. The number of micronucleated polychromatic erythrocytes increased significantly after LPS administration both in cells from peripheral blood and bone marrow. Melatonin administration to LPS-treated rats highly significantly reduced micronuclei formation in both peripheral blood and bone marrow cells beginning at 24 h after LPS administration and continuing to the end of the study (72 h). In blood, the increase in micronuclei formation was time-dependent in LPS-treated rats with peak values being reached at 36 – 48 h. According to the authors, the ability of Melatonin to reduce LPS-related genotoxicity is likely related to its antioxidant activity.

Ref.: 42

In a further *in vivo* micronucleus test in mice, the protection afforded by Melatonin against paraquat-induced genotoxicity in both bone marrow and peripheral blood cells was tested using micronuclei as an index of induced chromosomal damage. Melatonin (2 mg/kg bw) or an equal volume of saline were injected intraperitoneally (i.p.) into mice 30 min prior to the i.p. administration of paraquat (2 injections of 15 mg/kg bw; the paraquat injections were given with a 24-h interval) and thereafter at 6-h intervals to the end of the study (72 h). Using fluorescence microscopy, the number of micronuclei (MN) in polychromatic erythrocytes (PCE) per 2,000 PCEs (1,000 PCEs/slide) per mouse was counted both in blood and bone marrow, and the ratio of PCEs to normochromatic erythrocytes (NCE) (PCE/NCE) was calculated.

Paraquat treatment increased the number of MN-PCE at 24, 48 and 72 h, both in peripheral blood and bone marrow cells, while no differences were observed in the PCE/NCE ratio. Melatonin inhibited the paraquat-induced increase in MN-PCE by more than 50% at 48 and 72 h. The proposed mechanism of action of Melatonin is its free radical scavenging ability.

Ref.: 32

General comment on mutagenicity
In vitro and in vivo mutagenicity tests showed that melatonin itself exerts neither mutagenic nor clastogenic effects.

Melatonin was found to be negative in the bacterial reverse mutation test, in the WP2 Mutoxitest, in the COMET assay using CHOK 1 cells and in the micronucleus test.

In vitro tests revealed an anticlastogenic potency of Melatonin. In the Comet assay, Melatonin was found to inhibit clastogenic effects when applied together with mutagenic substances. It also reduces the frequencies of chromosomal aberrations and micronuclei.

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

To address possible toxic effects of Melatonin, the applicant provided an expert statement based on an extensive discussion of the available literature.

It can be taken from publicly available data that high doses of Melatonin (1 - 4 mg/kg body weight) and the resultant elevated plasma levels can affect male and female reproductive function. If melatonin levels in the serum reach a supraphysiological range, a possible effect on the reproductive system (e.g. menstrual cycle, fertility, course of pregnancy,

puerperium, subsequent pregnancy and outcome of pregnancy in women or spermatogenesis and fertilisation capacity of sperm in men) needs to be investigated. In the context of the proposed cosmetic use of Melatonin in a concentration of 0.0033% in hair products, the question is whether low doses like these could also give rise to health concerns. This depends on the amount of Melatonin applied and the resulting plasma levels (Rabe 2003). It can be supposed that an interaction with reproductive function is not to be expected when the Melatonin plasma levels remain within the physiological range after an application of the product in question. As described in chapter 3.3.9., plasma levels after the topical application of 0.1 mg Melatonin stay indeed within the physiological range; therefore toxic effects on reproduction are not to be expected.

3.3.8.2. Teratogenicity

Guideline:	/
Species/strain:	Crl:CD®(SD)BR VAF/Plus® rats
Group size:	25 females/group
Observation period:	25 days
Test substance:	Melatonin in aqueous methylcellulose
Test substance purity:	≥ 99.9%
Batch:	tca 6040568
Dosage levels:	0 - 50 - 100 - 200 mg/kg bw/day
GLP:	yes
Date of study:	22/04/1997 - 03/07/1997

A developmental toxicity study was performed in CD® Sprague-Dawley rats. The rats (25/group) were dosed by gavage with 50, 100 or 200 mg Melatonin/kg bw/day or with vehicle (0.5% aqueous methylcellulose; administration volume: 5 ml/kg bw/day) on gestation day 6 through gestation day 19. Maternal food/water consumption, body weight and clinical signs were monitored at regular intervals throughout gestation. At termination (gestation day 20), maternal liver and gravid uterine weights, number of ovarian corpora lutea, conceptus survival, foetal sex and foetal body weight were evaluated. Foetal morphological examination included external structures as well as visceral and skeletal structures.

Results
In a dose-range finding study in rats (0, 1, 10, 100, 150 or 200 mg Melatonin/kg bw on gestation days 6 through 19), prenatal growth, viability, and external morphological development were not affected. Mild maternal toxicity (aversion to treatment, reduced maternal weight gain) was noted at ≥ 100 mg/kg bw/day.
In the definitive study, no maternal deaths occurred, and clinical signs associated with Melatonin exposure were minimal. Aversion to treatment was noted at ≥ 50 mg/kg bw/day, and mild sedation, reduced maternal food intake, and reduced body weight gain were found during initial treatment with 200 mg/kg bw/day. Melatonin had no effect on prenatal survival, foetal body weight, or incidence of foetal malformations/variations.

Conclusion
The NOAEL for maternal toxicity was 100 mg/kg bw/day. The NOAEL for developmental toxicity was 200 mg/kg bw/day (the highest dose tested).

Ref.: 19; 20

3.3.9. Toxicokinetics

Melatonin administered intravenously

Intravenous administration of Melatonin to the rat has shown to result in a rapid distribution into plasma and all tissues of the animal, including cerebrospinal fluid and brain [Kopin et

al. 1961, Vitte et al. 1988]. When the compound was injected at 5 mg/kg, the apparent elimination half-life appeared to be 19.8 min [Yeleswaram et al. 1997].

When Melatonin was administered intravenously to the dog and the monkey at 3 mg/kg, the apparent elimination half-lifes were measured to be 18.6 and 33.9 min respectively [Yeleswaram et al. 1997].

When 23 µg/person of Melatonin was administered by intravenous injection to humans, an apparent half-life of 36 to 42 minutes in the systemic circulation was determined [Fourtillan et al. 2000].

The toxicokinetics of Melatonin in man after intravenous administration are generally characterized by a very short distribution phase in the order of minutes, followed by a steady state concentration in the examined tissues (serum, blood, plasma, brain, etc.) in the order of a couple of hours, and a rapid elimination (half-life of about 40 minutes) after metabolisation in the liver [Vitte et al. 1988, Mallo et al. 1990, Le Bars et al. 1991, DeMuro et al. 2000, Fourtillan et al. 2000].

As displayed in Figure 1, the major metabolic pathway of Melatonin in man has been identified as being the hydroxylation of position 6, followed by conjugation, primarily with sulfate (70%) and, to a smaller extent, with glucuronic acid (6%) [Kopin et al. 1961, Ma et al. 2005]. Melatonin 6-hydroxylation and O-demethylation have been identified as being mainly CYP1A2 mediated [Facciola et al. 2001, Ma et al. 2005].

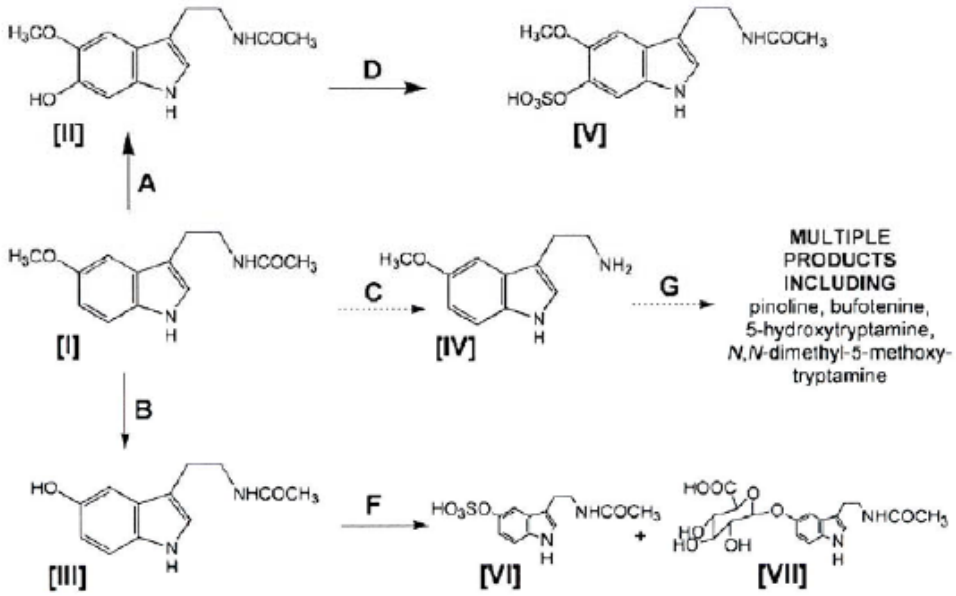


Fig. 1: The common metabolic pathways of melatonin [I] by 6-hydroxylation (A) to 6-hydroxymelatonin [II], which is sulfated (D) to 6-sulfatoxymelatonin [V], and by O-demethylation (B) to N-acetyl-5-hydroxytryptamine [III], which is further conjugated (F) to its sulfate [VI] and its glucuronide [VII]. A minor pathway is deacetylation (C) to 5-methoxytryptamine [IV], which can be further metabolized (G) to a range of minor metabolites, including pinoline, bufotenine, N,N-dimethyltryptamine, and 5-hydroxytryptamine, which itself can be reconverted to melatonin [I] in the serotoninmelatonin cycle [Ma et al. 2005]

Melatonin administered orally

When melatonin is administered orally to rats, dogs and monkeys at 10 mg/kg, the absolute bioavailability appeared to be moderate in rats and high in dogs and monkeys [Yeleswaram et al. 1997].

Oral administration to human volunteers of up to 3 times 80 mg of Melatonin / person in one hour showed that the test compound is rapidly absorbed and distributed throughout the human body, the half-life for the first part of the biphasic distribution phase being in the order of minutes. Highest Melatonin values were measured in serum, 60 to 150 minutes after administration. Elimination of the molecule appeared to be slower than in rodents, since the Melatonin concentration plateau lasted for several hours. The half-life of the first part of the biphasic elimination phase was found to be 20 to 50 minutes [Waldhauser et al. 1984].

When a lower dose of 3 mg of Melatonin / person was administered orally, an increase in serum Melatonin within 20 minutes after oral administration was reported, followed by a rapid decrease at 240 minutes [Shirakawa et al. 1998].

Fourtillan et al. [2000] reported a terminal half-life of 36 to 45 minutes when Melatonin was measured in plasma after oral administration of a dose of 250 µg / person to human volunteers.

Vakkuri et al. [1985] reported that maximum blood and saliva concentrations of Melatonin were reached 60 minutes after oral uptake of 100 mg of Melatonin. The half-life of the molecule in blood was determined as 41 minutes [Vakkuri et al. 1985].

When 2 or 4 mg Melatonin / person were administered to human volunteers, only 15% of the ingested dose actually reached the systemic circulation. The majority of the administered Melatonin disappears through first-pass metabolisation [DeMuro et al. 2000] and is excreted in urine as sulphatoxy-melatonin, the major conjugation product of 6-hydroxymelatonin. Unchanged melatonin renal clearance was lower than 1% [Fourtillan et al. 2000].

Melatonin topically applied

When 20 or 100 mg Melatonin were dissolved in a 70% ethanol solution and applied on the scalp of human volunteers, elevated serum Melatonin levels were measured after 1 hour and lasted for up to 8 hours, the maximum observation period in the study. These findings indicate that topically applied Melatonin is easily taken up through the skin in the blood system and that the stratum corneum appears to act as a continuously releasing depot for the substance [Bangha et al. 1997b].

14-Day pharmacokinetics and clinical and biological tolerability study with Melatonin-based end product: A randomised, double-blind, placebo-controlled study using a cross-over design (MEL-COS-1)

Guideline:	/
Species/strain:	Healthy female volunteers
Group size:	8 (4 fertile and 4 menopausal women)
Observation period:	7-8 weeks
Test substance:	Melatonin Hair Product containing 0.0033% Melatonin (Batch N° 6050699) in an ethanol / water solution (30% ethanol)

	(designation used in the study report: Melatonin-containing cosmetic hair solution)
Code of test substance:	W/157/001/012
Batch:	09202
Dose level:	Daily topical application of the content of one unit-dose plastic ampoule (3 ml) to the scalp for 14 days prior to bedtime
GCP:	in compliance
Date of study:	January - April 2003.

The 8 female volunteers were asked to apply the test substance (product containing 0.0033% Melatonin) / placebo, as supplied in individual 3 ml doses, on their scalp once per day before bedtime over a period of 14 consecutive days. This period was followed by a 14-day non-treatment "wash-out" period and finally by a second identical 14-day treatment period, with the only difference that the volunteers who had received the test substance during the first treatment period received placebo during the second treatment period and vice versa.
Blood samples for Melatonin assay were withdrawn from day 14 to day 15 during bedtime every 30 minutes for 8 hours from pre-application to T+8 h post application and then at T+10h, T+12h, T+16h and T+20h post application.
Urine was collected from day 13 to day 14 over 24 hours in 3 periods of 8 hours for the 6-hydroxy melatonin sulphate assay.
With regard to possible neurocognitive effects, Leeds psychomotor tests (Critical Flicker Fusion Test and Multi Choice Reaction Time) were performed on day 1 (baseline) and on day 14 at T+11 and T+18 h post application.
Physical examinations of the volunteers were carried out on days 13 and 15 and vital signs were checked on day 13, 14 and 15.
Clinical laboratory tests were performed on day 13 and included haematology, plasma biochemistry and urine analysis, drugs of abuse, alcohol screen and pregnancy test for nonmenopausal women. Plasma levels of estradiol, FSH and LH were analyzed on day 15.
Adverse event reporting occurred continuously throughout the study.

Results
The mean concentration-time profiles of Melatonin appeared not to be different between periods after 14 days of topical administration of the cosmetic product containing 0.0033% Melatonin or placebo.

On day 14, plasma concentration of Melatonin reached C_{max} earlier (4.7 h compared to 6 h) and was slightly higher after application of the cosmetic product containing 0.0033% Melatonin than after placebo application (83.4 pg/ml compared to 71.2 pg/ml).
The four randomized fertile women showed higher plasma concentrations measured over 20 h than the four randomized menopausal women, both after application of the cosmetic product containing 0.0033% Melatonin or after placebo application.

The area under the plasma curve (AUC_t) was similar, after application of the cosmetic product containing 0.0033% Melatonin or placebo respectively (492 and 427 pg x h/ml).

The cumulative amount of the 6-hydroxy melatonin sulphate excreted in the urine up to 24 h after application of the product containing 0.0033% Melatonin was comparable to the amount excreted after placebo application, with higher inter-subject variability in both periods.
With regard to neurocognition (Leeds psychomotor tests), no significant effect of the product containing 0.0033% Melatonin was observed.
Blood and urine biochemistry and haematology tests revealed only few values out of their normal ranges. These scarce abnormalities were without clinical relevance.
The product containing 0.0033% Melatonin was not associated with any significant changes in vital signs (blood pressure and heart rate), or ECG parameters.
The product containing 0.0033% Melatonin was well tolerated, and reported adverse events in the volunteers were similar whether they were receiving the melatonin containing product

or placebo. A causal relationship between the application of the investigational products and the adverse events was therefore considered unlikely. There was no cutaneous reaction at the site of application.

Conclusion
Overall, Melatonin plasma profiles following application of either the product containing 0.0033% Melatonin or placebo solution were in agreement with the concentration-time profiles reported in the literature concerning the circadian rhythm of endogenous Melatonin. The study authors concluded that the treatment resulted in Melatonin plasma levels and 6-hydroxy melatonin sulphate urine levels not exceeding physiological levels.

There were a few complaints (headaches, diarrhoea) which occurred during both periods, and there were no changes in laboratory parameters, ECG and vital signs associated with the application of the cosmetic product containing 0.0033% Melatonin. Finally, no effects on reaction times and cortical arousal were evidenced at day 14 with the cosmetic product containing 0.0033% Melatonin confirming that repeated application of the product does not induce central effects.

Ref.: 29

Comment
In most subjects the plasma levels after application of Melatonin were higher than after placebo, with increases of less than 20 ng/l. Some of the subjects showed no increase of plasma levels after application of Melatonin. A shortcoming of the study is that no blood samples were taken before day 0.

Physiological Melatonin levels are on average in the order of 10 (day) to 100 ng/L (night). There are high variations in interindividual physiological serum melatonin levels. Influencing factors are, besides others, age, gender, exposure to light. Intraindividual serum levels are well reproducible. Thus, the observed increases in Melatonin plasma levels due to the application of 0.1 mg melatonin on the scalp were within the range of physiological levels.

This study shows that low amounts of Melatonin applied to the scalp are in part absorbed through the skin and lead to measurable, albeit minor increases in Melatonin plasma levels.

The results of Bangha et al. (1997b) are in line with the study of Macher 2003. When applying 20 mg Melatonin on the scalp, plasma levels between 760 and 3440 ng/l were observed during the next 8h. For an application of 0.1 mg instead of 20 mg Melatonin, plasma levels of 3.8 to 17.2 ng/l can be calculated.

After an oral application of 0.1 mg melatonin, plasma levels of 97.2 to 249.2 ng/l can be calculated from the results of Fourtillanet al. (2000) who applied 0.250 mg Melatonin orally. This indicates that dermal absorption is about 10 fold lower than oral absorption.

3.3.10. Photo-induced toxicity

3.3.10.1. Photoirritation and photosensitisation

Guideline:	Method B.41 of Annex V to Dir. 67/548/EEC
Species/strain:	BALB/3T3 (ATCC, CCL-163, clone A31) mouse fibroblast cell line
Group size:	5 animals / dosage group
Observation period:	6 days
Test substance:	Melatonin
Batch:	3/104
Purity:	100.0%
Dose levels:	0.032 - 0.10 - 0.32 - 1.0 - 3.2 - 10 - 32 - 100 µg Melatonin/ml, dissolved in Earle's balanced salt solution (EBSS)
Negative control:	EBSS

Positive control:	Chlorpromazine (0.032 - 0.10 - 0.32 - 1.0 - 3.2 - 10 - 32 - 100 µg /ml)
GLP:	Yes
Date of study:	23 - 30 November 2004

In a preliminary experiment, the molar absorption coefficient of the 100 µg Melatonin/ml stock solution was determined to be > 10 l/mol/cm, indicating photoreactive potential.
In the main study, Melatonin was dissolved and diluted in EBSS. The cells in two parallel plates were treated for 1 hour with the following concentrations of the test item: 0.032 - 0.10 - 0.32 - 1.0 - 3.2 - 10 - 32 - 100 µg/ml.

One of the parallel samples was treated for 50 minutes in absence, the other in presence of a non-toxic dose of UVA light. One day after treatment cytotoxicity was analyzed as a measure of reduction of neutral red uptake and compared to the untreated negative controls.

Results
The cells treated with the test item did not show significant cytotoxic effects either in absence (-UVA) or in presence (+UVA) of UVA light. Relative cell viability in the -UVA experiment was 92%, in the +UVA experiment 79% compared to the untreated negative controls. Thus, no EC50 values could be determined and only a formal photo-irritation factor (PIF) = 1 was calculated.

The controls confirmed the validity of the study. The negative controls of the +UVA experiment showed a viability of 83% of the untreated negative controls. The mean OD550 of the untreated negative controls was ≥ 0.2. The ECB50B values of the positive controls of the -UVA (13.58 µg/ml) and the +UVA experiment (0.38 µg/ml) were within the validity ranges, the PIF was 35 (> 6).

Conclusion
The test item showed no phototoxic potential under the conditions of this phototoxicity study.

Ref.: 7

3.3.10.2. Photomutagenicity / photoclastogenicity

Vijayalaxmi et al. [1999] examined the potential radioprotective properties of pharmacological doses of Melatonin in whole-body irradiated mice. CD2-F1 male mice were treated with Melatonin, and the whole body irradiated with an acute dose (150 cGy) of ^{137}Cs gamma rays. Peripheral blood and bone marrow cells were examined for genetic damage, which was determined by comparing the incidence of micronuclei (MN) in both Melatonin pre-treated and non-treated irradiated animals (and control mice).
Whole-body irradiation resulted in a dramatic increase in the incidence of MN (ca 10-fold) in both tissues. Melatonin treatment significantly and dependent on dose attenuated this increase by less than 20%

The authors explain these findings with an antioxidative effect of Melatonin which protects "the genetic material of haematopoietic cells" from the damaging effects of acute whole-body irradiation

Ref.: 49

Laser irradiation-induced phototoxicity has been intensively applied in clinical photodynamic therapy for the treatment of a variety of tumors. It has been shown that, similar to Vitamin E, the antioxidant Melatonin (100 µM) was able to largely attenuate laser irradiation-induced mitochondrial reactive oxygen species formation and to prevent apoptosis when applied 30 min before irradiation.

Ref.: 21

Comment
In therapeutic doses Melatonin seems to have a protective potential against radiation caused cell damage, possibly due to its antioxidant effect.

3.3.11. Human data

Topical tolerability and compatibility

Human studies assessing skin compatibility are described in sections 3.3.2.1. and 3.3.9. of this opinion.

In addition, the following efficacy/compatibility studies, all involving daily topical application of 3 ml hair product containing 0.0033% Melatonin, have been performed:

Study duration	Endpoints	N° of volunteers	
6 months	- efficacy: hair count and hair density - skin compatibility	35 males	Lorenzi, 2005
3 months	- efficacy: appearance and texture of thinning and fine hair - skin compatibility	30 males 13 females	39
3 months	- efficacy: hair loss and stimulation of hair growth - skin compatibility	901 males 990 females	8

In the above mentioned studies around 1900 test persons were examined either by physicians and/or by using questionnaires which were filled in by the patients or their hairdressers. Photographs also have been used in these examinations. The authors of these studies stated that the application of 0.0033% melatonin causes a decrease in seborrhoea, of seborrhoic dermatitis and an increase in hair growth and "cosmetic satisfaction".
Adverse effects like redness, itching, stinging, or burning were reported in these studies with a low frequency. These studies have been mainly performed to prove the efficacy of Melatonin-containing end products. Little detail on safety aspects is given in these studies, so they can only provide supportive evidence for the safe use of the compound at the intended use concentration.

Clinical trial assessing the toxicology of chronic Melatonin treatment

Guideline:	/
Species/strain:	Healthy male human volunteers (22-55 years old), with a normal sleeping pattern and a normal clinical and laboratory exams.
Group size:	30 for the treatment group, 10 for the placebo group
Observation period:	35 days
Test substance:	Melatonin 10 mg capsules, donated by Ind. Chím. Farmacêutica Schering-Plough
Dose level:	10 mg/person/day, administered for 28 days
GCP:	/
Date of study:	Published in 2000

40 male volunteers were randomized into 2 groups: group 1 (30 volunteers) received Melatonin capsules containing 10 mg as a daily dose and the 10 volunteers in group 2

received placebo over the same period of 28 days, in a double-blind study design. The capsules were applied 1 hour before sleep time.

The study was carried out according to the following time table:

Start of the study:	Explaining and checking the terms of consent and inclusion criteria
Day 7:	Evaluation of polysomnography, laboratory examinations, Epworth Somnolence Scale, and Melatonin serum concentration
Day 14:	Evaluation of sleep diary, Melatonin serum concentration and possible side effects
Day 21:	Evaluation of polysomnography, Melatonin serum concentration, Epworth Somnolence Scale and possible side effects
Day 28:	Evaluation of laboratory examinations, possible side effects and Melatonin serum concentration
Day 35:	Evaluation of polysomnography, Epworth Somnolence Scale and possible side effects

Laboratory examinations included complete blood count, urine analysis, sodium, potassium and calcium levels, total protein levels, albumin, blood glucose, triglycerides, total cholesterol, highdensity lipoprotein, and very low-density lipoprotein, urea, creatinine, uric acid, glutamic-oxalacetic transaminase, glutamic-pyruvate transaminase, bilirubin, alkaline phosphatase, γ-glutamic transaminase, T_3, T_4, TSH, LH/FSH and cortisol.

Results
Several volunteers reported somnolence and headache, but these complaints occurred as frequently in the treated group as in the placebo group. No other differences between the placebo and melatonin groups were observed.

Conclusion
The authors concluded, that according to the parameters analysed, there is no toxicological effect that might compromise the use of melatonin at a dose of 10 mg for a period of 28 days in this study.

Ref.: 41

3.3.12. Special investigations

No documents submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Melatonin

Applied dose of 0.1 mg was considered as 100% absorbed			
Dermal absorption per treatment		=	**0.1 mg**
Typical body weight of human		=	**60 kg**
Systemic exposure dose	**SED**	=	**1.7 µg/kg bw**
No observed adverse effect level (28-day, rat, subcutaneous)	**NOAEL**	=	**0.5 mg/kg bw**
Margin of Safety	**NOAEL / SED**	=	**294**

3.3.14. Discussion

Physico-chemical properties
The batches of melatonin for which analytical data has been provided show a purity of >99 %. In the large majority of the available studies, the purity of the Melatonin batches used is not documented. However, the described procedure of synthesis starting with serotonin obviously leads to products with purities much better than 90%. The dose of Melatonin is 0.1 mg/application, and the amount of impurities simultaneously applied is by at least 1 to 2 orders of magnitude lower. Therefore, they can be assumed not to have adverse effects on health.

Skin/eye irritation and sensitisation
No classical rabbit Draize test with Melatonin is available. Several human studies were performed on the intended finished product (0.0033% Melatonin in an ethanol/water solution) and do not indicate a significant irritative potential of the substance under the intended conditions of use.
No studies have been performed to investigate the potential of melatonin for mucous membrane irritation. The skin sensitisation has been investigated in a LLNA. The results of this LLNA indicate that Melatonin is not to be considered as a skin sensitizer.

Percutaneous absorption and Toxicokinetics
An *in vitro* percutaneous absorption is available which, due to many shortcomings, was not considered adequate for risk assessment. A human clinical study is available, in which daily application under the intended conditions of use (topical application for 14 days of 3 ml of a 0.0033% Melatonin solution in ethanol/water) was used. Compared to the placebo group, a tendency to higher Melatonin plasma levels and 6-hydroxy Melatonin sulphate urine values was observed in the treated group. Most test persons showed somewhat elevated plasma concentrations when dosed, compared to application of placebo. The concentration of Melatonin in the dose group also reached C_{max} earlier than the placebo group. These results indicate that Melatonin, even when applied in very low amounts, is absorbed through human skin, which is in line with a study where much higher doses (20 mg) have been applied on the scalp.
However, it needs to be considered that physiological Melatonin levels change during the day, and are on average in the order of 10 (daytime) to 100 ng/L (night). There is high variability in inter-individual physiological serum melatonin levels. Influencing factors are e.g. age, gender or exposition to light. Intra-individual serum levels, however, are well reproducible. The observed increases in Melatonin plasma levels due to the application of 0.1 mg melatonin on the scalp remained within the range of physiological levels.

General toxicity
With an acute oral LD_{50}-value of > 3200 mg/kg bw, Melatonin is considered as acutely non-harmful.
The 28 days repeated dose toxicity study of Prevo et al. (2000) was chosen as the critical one, as this study showed adverse effects at the lowest doses and other studies had significant shortcomings. Subcutaneous administration of Melatonin lead to decreased testis weights and testicular degenerative changes at doses higher than the NOAEL of 0.5 mg/kg bw/day in rats.
In a 14 days study (oral administration) a NOAEL of 200 mg/kg bw/day was observed. An oral 90-day study in the rat resulted in a NOAEL value of 5 mg/kg bw/day. The NOAEL value of 0.5 mg/kg bw/d (from the 28 day study of Prevo et al. 2000) was used in the calculation of the MoS.

Since no reliable data is available for quantification of dermal absorption, SCCS assumed a worst case scenario, i.e. that the total applied dose of 0.1 mg is absorbed through the skin. This equals a dose of 1.67 µg/kg bw/d (60 kg bw). Comparing this dose with the lowest NOAEL of 0.5 mg/kg bw/d observed in rodents (see below), would then result in a MOS of 294.
The NOAEL of 0.5 mg/kg bw/d can be used for the interpretation of the increase of Melatonin plasma levels caused by topical application of 0.1 mg Melatonin. Serum levels of Melatonin in the dose group at the NOAEL were 1 to 2 orders of magnitude higher than the physiological levels of Melatonin in humans. In contrast, after topical application of 0.1 mg Melatonin the plasma levels remained well within physiological levels. Even if average day time plasma levels of the population are exceeded by up to 20 ng/l, no adverse neurophysiological effects e.g. such influencing the fitness to drive, are expected. An oral application of 0.1 to 0.3 mg Melatonin at midday has been shown to only increase the duration of sleep at night (Dollins et al 1994).

No chronic toxicity or carcinogenicity animal studies are available.
With regard to risk from teratogenicity and reproduction toxicity, the applicant provided an expert statement based on an extensive discussion of the available literature. In conclusion, at the proposed maximum amount of 0.1 mg Melatonin to be applied daily, and considering the fact that resulting plasma concentrations are within physiological levels, effects on reproductive function are highly unlikely to occur. A separate teratogenicity study reveals a NOAEL of 100 mg/kg bw/day for maternal toxicity and a NOAEL of ≥ 200 mg/kg bw/day for developmental toxicity.

Mutagenicity/ Genotoxicity
Melatonin was found to be negative in the bacterial reverse mutation test, in the WP2 Mutoxitest, in the COMET assay and in the micronucleus test. Its anticlastogenic activity was shown in experimental studies where Melatonin reduced the frequencies of chromosomal aberrations and micronuclei induced by genotoxic agents.
Although the documentation provided consists of public domain scientific publications that clearly suffer from the absence of raw data and GLP certificates, they follow a common pattern since all of them indicate the absence of mutagenic and clastogenic properties of Melatonin, together with the protective role of the substance against DNA damages induced by physical or chemical mutagens.

The presented publications do not cover the classical mutagenic / genotoxic testing battery as requested by the SCCS. However, in light of the fact that Melatonin is an endogenously produced molecule, that a clinical study under in-use conditions of finished product showed only marginal increases of the physiological melatonin levels, and that the available literature data on its mutagenicity/genotoxicity do not point to any mutagenic or genotoxic effect, the SCCS does not consider further mutagenicity testing necessary.

Phototoxicity
According to a phototoxicity study Melatonin is not to be considered as a phototoxic substance.

4. CONCLUSION

The SCCS is of the opinion that the use of melatonin in hair products in a concentration of 0.0033% w/w (33 µg/g) does not pose a risk for the health of the consumer.

5. MINORITY OPINION

Not applicable

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