

OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD
PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE
(AHTN)

adopted by the SCCNFP during the 21st plenary meeting
of 17 September 2002

1. Terms of Reference

The SCCNFP was requested to give an opinion concerning the safety for use in cosmetic products of 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and the potential need for any restrictions or conditions for its use.

Basing its assessment only on review of the cosmetic use of AHTN and considering the information provided in the 6 submissions previously made by COLIPA¹, the SCCNFP has issued on 24 October 2000 an opinion requesting :

- a new in vitro penetration study in accordance with the SCCNFP Notes of Guidance (SCCNFP/0119/99) in order to complement its information related to the percutaneous absorption of AHTN,
- a new in vitro photo-toxicity study in accordance with the validated 3T3 Neutral Red Uptake Assay.

The SCCNFP should also take into consideration the results related to the pigmentation of internal organs of rats observed in a 13-week oral toxicity study.

In submission VII, COLIPA has provided a set of additional information which is considered by the SCCNFP.

2. Toxicological Evaluation and Characterisation

2.1. Percutaneous absorption

Previous results (Submissions I to VI)

In animals

Eighteen male pigmented rats received a single dermal application of ¹⁴C-AHTN at a nominal dose rate of 0.1 mg AHTN/cm² shaved body area (4.5 mg/kg bw) in 200 µl solvent (70 % aqueous ethanol). The area was occluded with aluminium foil. At 6 h after dose administration or at sacrifice residual dose material was removed from the skin . The treated area was re-occluded with clean aluminium foil.

Pairs of animals were sacrificed at 0.5, 1, 3, 6, 12, 24, 48, 72 and 120 h. For animals sacrificed at 6 h or later urine, faeces and expired air were collected.

Results :

The total mean proportions of applied radioactivity found in tissues increased from 0.55 % at 0.5 h to 9.00 % at 6 h. A peak of 10.0 % was reached at 12 h, before the mean proportion declined to 1.72 % of the applied dose by 120 h.

An apparent level of absorption of applied radioactivity into systemic circulation of 14 % could be estimated from the mean total proportion of radioactivity in excreta and tissues.

¹ Submission n° 1 of December 1996, Submission n° 2 of April 1997, Submission n° 3 of July 1997, Submission n° 4 of July 1998, Submission n° 5 of March 1999 and Submission n° 6 of February 2000

A mean cumulative total of 1.94 % of the applied radioactivity was excreted in the urine over the 0 - 120 h experimental interval. The mean cumulative total of applied radioactivity excreted in faeces was considerably greater than in urine at 12.7 %, indicating preferential excretion in the faeces.

The highest levels of radioactivity were associated with the content of small (1.49 to 0.13 %) and large intestines (2.68 to 0.15 %). Highest tissue concentrations of radioactivity generally occurred in animals sacrificed at 6 and 12 h after dose administration. With the exception of the gastrointestinal tract and contents, the highest peak mean concentration was found in the liver with 1.35 µg/g tissue. The highest concentration observed at 120 h were in the liver with a mean of 0.544 ppm.

Ref. : 51

AHTN was applied in a fragrance vehicle of 75 % v/v ethanol: 25% v/v diethylphthalate as 0.1 and 0.5 % dose solution (15 µg/cm² and 78 µg/cm²) on fresh, full-thickness, male F344 rat skin in vitro. The skin was either occluded with a Teflon cap or left open to the atmosphere. Receptor fluid was collected every 2 h for up to 72 h. At the end of the experiment, the skin surface was washed, the skin was digested in methanolic sodium hydroxide and assayed for residual test compound.

Results :

AHTN was poorly absorbed over 24 h (0.28 ± 0.14 %, n = (8)). Occlusion of the skin surface enhanced AHTN absorption at 24 h to 3.00 ± 1.35 % (n=5). Skin residues at the end of each 24 h experiment were high (50 - 60%). AHTN was well absorbed into the skin probably due to their high lipophilicity. In contrast AHTN was poorly absorbed through unoccluded skin into the receptor fluid after 24 h. However, continued absorption from the skin into the systemic circulation would be expected to occur.

Ref. : 53, 54

Fixolide, dissolved in methylcarbinol at concentrations of 1, 3 and 10 %, was applied to an intact minipig skin area of 5 cm² at a dose of 12 µl/cm² (= 120 µg/cm², 360 µg/cm² and 1200 µg/cm²). Total penetration was determined after 16 h. Unabsorbed material from the skin surface was removed after 16 h, the amount of labelled material in the stratum corneum, in the stripped skin, and in the chamber liquid is estimated. The penetration rate per cm² skin surface is calculated.

Results :

AHTN was poorly absorbed after 16 h application through intact minipig skin into the receptor chamber liquid (0.04, 0.08 and 0.31 µg/cm²). Recovery from the stripped skin and horny layer accounted for 8.2, 5.6, 3.3 % and 5.5, 5.3, 4.4 %, respectively.

AHTN has the tendency to cumulate in the epidermis and upper layers of the dermis.

Ref. : 55

In humans

¹⁴C-AHTN was dissolved in ethanol/water (7/3) vehicle (2.4 mg/ml) and applied at the rate of 10.9 µg/cm² to a 100 cm² area of the skin on the back of each of three male subjects. The treated area was covered with gauze dressings after 30 min. After 6 hours the dressings were removed and the treated area was washed with 70 % aqueous ethanol soaked swabs. An area of 6.25 cm² was stripped with adhesive tape. The treated area was reoccluded with fresh dressings as necessary up to 120 h after which the dressings were removed and skin stripping was performed again on a different area of skin. Samples of whole-blood and excreta were taken during the 5 day study period.

Results :

The mean total recovery of radioactivity from excreta, dressings, swabs and skin strips was 82.39 % of the applied radioactivity with a range of 77.43 % - 86.72 %. A mean total absorption of 0.881 % (range 0.211 - 1.93 %) was estimated from recoveries of radioactivity in urine and faeces. Concentrations of radioactivity in plasma and whole-blood were below their limits of accurate measurement (1.04 and ca 6 ng/g resp.) at all sampling times. A mean of 4.24 % of the applied radioactivity was recovered from adhesive tape samples after 6 h. Only a small amount of radioactivity (mean 0.064 %) was detected in skin stripping at 120 h.

Ref. : 43

In vitro Additional Human Skin Penetration Study (submission VII)

An additional in vitro study of the human skin penetration rate and distribution of radio-labelled AHTN following application under non occlusive conditions has been submitted.

Radio-labelled AHTN was prepared as a 1 % (w/v) solution in ethanol (96 % v/v).

The test membranes, isolated epidermis, used for the study were proceeding from human female breast and abdominal skin, obtained from 3 donors after cosmetic surgery and stored at – 20°C. On the day of use epidermal membranes were taken up onto 25 mm diameter filter paper support and then mounted onto diffusion cells. Twelve active dosed cells were prepared plus two control cells. Membrane integrity was then assessed by measuring the penetration rate of tritiated water over a period of 1 hour.

- The applied dose was 20 µl/cm² of the AHTN 1 % solution in ethanol (corresponding to 200 µg/cm² AHTN).
- 200 µl samples were taken from each receptor chamber at 1, 2, 6, 12 and 24 hours then placed into scintillation fluid and analysed for ¹⁴C using liquid scintillation counting (LSC)
- Following the last receptor phase sample, AHTN remaining on the surface of each of the epidermal membrane was removed with a cotton bud which was then extracted in methanol for analysis. Each epidermal membrane was tape stripped 10 times (D-squame). The strips were grouped (strip 1, strips 2-3, strips 4-6 and strips 7-10) and then solubilized (Optisolve) and analysed. The remaining samples of skin were solubilized individually (Optisolve) prior to sampling and analysing.

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The diffusion cell donor samples were wiped to remove sealing grease. Wipes were extracted (tetrahydrofuran). The diffusion cell donor samples were then washed in methanol. The filter paper supports were extracted into methanol.

- Evaporation loss of AHTN in ethanol was assessed under the same experimental conditions, using polytetrafluoroethylene sheet (PTFE) dosed with 20 µl/cm² of the 1 % AHTN solution in ethanol.
- The amounts of AHTN per unit of area (µg/cm²) in the receptor phase and various compartments of the skin and diffusion cells were determined and then the percents of the applied dose of AHTN on those compartments were calculated.

Results : Following 24 hour exposure the distribution of AHTN in all compartment (mean ± SE) is summarized in the following tables :

AHTN dosed cells (µg/cm²)

Nber of cells = 12	1 surface wipe	2 strip 1	3 strip 2-3	4 strip 4-6	5 strip 7-10	6 remaining skin	7 receptor phase	8 donor chamber wash	9 filter paper	10 total recovered
Mean	117	3.03	2.30	1.43	0.868	7.00	0.760	52.4	0.389	186
SD	8	1.02	0.54	0.44	0.259	2.24	0.405	8.0	0.205	6
SE	2	0.31	0.16	0.13	0.078	0.68	0.122	2.4	0.062	2

AHTN dosed cells (% applied dose)

Mean	58.5	1.51	1.14	0.711	0.432	3.49	0.379	26.9	0.194	92.5
SD	4.1	0.50	0.26	0.215	0.126	1.90	0.200	3.9	0.102	2.4
SE	1.2	0.15	0.08	0.065	0.038	0.33	0.060	1.2	0.031	0.7

According to the results :

- the exact amount of AHTN applied to the skin membranes was 200.0 ± 1.1 µg/cm²,
- the total recovery of AHTN is 92.5 ± 0.7 % of the applied dose (column 10). It results from the study performed with the PTFE sheets that a small quantity of AHTN evaporates In 24 hours, allowing to consider that the overall recovery of AHTN moves to a value closer to 96 % ,
- the majority of the applied doses found in the surface wipe (column 1) and donor wipe chamber wash (column 8), corresponding to 84.1 ± 1.0 % of the applied dose,
- a significant amount of AHTN is found in all stripped layers of the stratum corneum (column 2, 3, 4, 5) corresponding to 3.8 ± 0.3 % of the applied dose. (It can be observed that the distribution within the layers strongly decreases with skin depth),
- human skin absorption of AHTN corresponding to the amounts found in receptor phase (column 7), filter paper (column 9) and remaining stratum corneum and epidermis (column 6) is 4.06 ± 0.38 %.

Using this figure, the percentage of absorption to be retained for the calculation of the safety margin should be 4.1 %.

Discussion

This additional assay does not meet the requirements for practical and technical reasons and cannot be considered for the assessment of the safety margin :

- it does not conform to the SCCNFP Notes of Guidance,
- the use of pure ethanol as vehicle strongly affects the tissue and therefore the potential skin penetration of the substance.

Basing on all the information it seems therefore more convenient to consider the previous *in vivo* human study (ref. 43) which methodology remains more pertinent in spite of using a low concentration of AHTN.

According to this study it can be retained that the mean total absorption after 120 hours was 0.881 % AR (range 0.211 – 1.930 % AR). Therefore 2 % can be assumed as conservative absorption for the new calculation of the safety margin.

2.2. Phototoxicity

A new Neutral Red Uptake toxicity assay had been submitted.

Test method	:	Neutral Red Uptake Phototoxicity Assay (OECD 432 (draft); EC/ B41; 19 th May 2000)
GLP	:	U.S. FDA 21 CFR 58; OECD
Test article	:	No. 99AC87; Sample C; white solid; 04/21/99; (data on purity of sample not provided) ($\geq 98\%$)
Cell line	:	Balb/c 3T3 mouse fibroblast (ATCC)
Positive control	:	Chlorpromazine
Principle of the test method	:	Absorption of UV light by the chemical can result in a phototoxic response (cell damage). Comparison of cytotoxicity of the chemical when tested in the presence and in the absence of UV exposure.
UV radiation	:	Dermalight SOL 3 solar simulator, equipped with UVA H1 filter (320-440 nm). Delivery of 1.7 ± 0.1 mW/cm ² of UVA energy resulting in a dose of 1 J/cm ² per 10 min
Replicate	:	4-6 replicate wells; duplicate plates
Doses	:	6-8 decreasing concentrations
Results (PIF)	:	Test Article; 0.949; 1.233; (NEGATIVE)* Positive control: 17.48; 13.62 (POSITIVE)
Laboratory	:	Institute for <i>In Vitro</i> Science, Inc, USA
Date	:	20 th Sept. 2001

* A chemical is considered phototoxic is the PIF > 5.0 (Photo Irritancy Factor)

Conclusion : AHTN is not phototoxic when tested with the Neutral Red Uptake Phototoxicity Assay on Balb/c 3T3 mouse fibroblast cells treated *in vitro*

2.3. Characterisation of the pigmentation in internal organs

Abnormal green to dark brown coloured livers and mesenteric lymph nodes were observed in 11/12 males and 4/12 females in the high dose group (50 mg/kg bw/day) of the 13-week oral toxicity study in the rat reported in submission 1 :

* It has been suggested that this pigmentation in both organs may be related to the signs of haemotoxicity observed with the high dose of AHTN (anaemia).

* It has been established that in spite of this pigmentation, the high dose used in this study induced no histopathological changes in the organs concerned. The discoloration was reversible when treatment was interrupted, suggesting that the pigmentation may also have resulted from a metabolite of AHTN.

* There is no scientific reason to reconsider the previous SCCNFP opinion concerning the NOAEL dose which was adopted for the calculation of the safety margin (5 mg/kg bw).

2.4. New Calculation of the Safety Margin

In a previous submission COLIPA provided a table corresponding to the estimated total consumer exposure to AHTN through fragranced cosmetic products, considering that the range of cosmetic products selected covers all those that are likely to be used in any one weekly period.

Calculation of Exposure to AHTN in Cosmetic Products, the retention factors of bath products, shower gel, toilet soap and hair spray are corrected according to the “Notes of guidance for testing of cosmetic products for their safety evaluation – Annex 5” :

Type of cosmetic product	Application quantity in grams per	Application frequency per day	Retention factor (%)	Fragrance compound in product	AHTN in fragrance compo-und	AHTN in product	Exposure to AHTN (mg/day)	Exposure to AHTN for 60 kg person
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	application			(%)	(%)	(%)		(µg/kg/day)
Body lotion ⁽¹⁾	8	0.71	100	0.4	12	0.048	2.726	45.4
Face cream ⁽²⁾	0.8	2	100	0.3	12	0.036	0.576	9.6
Eau de toilette ⁽³⁾	0.75	1	100	8.0	12	0.960	7.200	120.0
Fragrance cream ⁽¹⁾	5	0.29	100	4.0	12	0.480	6.960	116.0
Anti-perspirant /deodorant	0.5	1	100	1.0	12	0.120	0.600	10.0
Shampoo	8	1	1	0.5	12	0.060	0.048	0.8
Bath products ⁽⁴⁾	17	0.29	1	2.0	12	0.240	0.12	2.0
Shower gel ⁽⁴⁾	5	1.07	10	1.2	12	0.1440	0.770	13.0
Toilet soap	0.8	6	10	1.5	12	0.1800	0.860	14.0
Hair spray	5	2	10	0.5	12	0.060	0.600	10.0
						Total	20.460	340.8

- (1) Assumes use of conventional body lotion 5 times per week and a fragranced cream twice per week.
- (2) Including make up and foundation.
- (3) Including perfume and after shave, but these three products are not used concurrently. The quantity used is inversely proportional to the fragrance concentration so these values include all hydroalcoholic products.
- (4) Assumes use of bath products twice per week and an average use of shower gel 1.5 times per day, 5 times per week.

Based on such exposure the safety margin can be calculated :

Maximum daily exposure to AHTN I (mg)	=	20.460 mg
Maximum absorption through the skin A (%)	=	2 %
Maximum dermal absorption per day I x A	=	0.409 mg
Typical body weight of human	=	60 kg
Systemic exposure dose (SED) I x A / 60 kg	=	6.82 µg/kg
No observed adverse effect level (mg/kg) NOAEL	=	5 mg/kg

Margin of Safety	NOAEL x 1000 / SED =	733
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2.5. Opinion

On review of the information currently available, the SCCNFP is of the opinion that AHTN can be safely used as a fragrance ingredient in cosmetic products, up to a maximum concentration of 12 % in the fragrance compound.

The above has been formulated only as a review of the cosmetic use of AHTN. For a full safety assessment of AHTN, it is necessary to consider other sources of consumer exposure from non-food products e.g. laundry products.

2.6. References

92. Harbell et all. Neutral Red Uptake Phototoxicity Assay in Balb/c 3T3 Mouse fibroblasts. Institute for in Vitro Science. September 20, 2001.
93. M. Green et all. In vitro human skin penetration of radiolabelled fragrance material AHTN. An-eX Analytical Services. September 10, 2001.
94. Api et all. Evaluation of oral Subchronic toxicity of AHTN in the Rat. Toxicology Letters 2001 (submitted for publication).