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

UPDATED POSITION PAPER

CONCERNING

CONSUMER SAFETY OF ALPHA-HYDROXY ACIDS

Adopted by the SCCNFP during the 28th plenary meeting
of 25 May 2004

1. Terms of reference

The safety of α-hydroxy acids (AHA) in cosmetic products has been questioned by some Member States with regard to their dermal tolerance.
Hydroxy acids have a long history of use in dermatological preparations and recently have become important ingredients in cosmetics. Concerns on both the dermal and systemic safety of these materials has led to calls for their listing in Annex III (List of substances which cosmetic product must not contain except subject to restrictions and conditions laid down) to the Cosmetics Directive 76/768/EEC.
Despite their widespread use, there is concern on the safety of AHA. In particular, the need of new studies to be performed on:

* the investigation of the maintenance of barrier function integrity of the skin including the effects on skin absorption of other cosmetic ingredients

* the effects of AHA on skin's responses to UV exposure

* the evaluation of safety of long term use of AHA

is indicative of the lack of sufficient data to provide a full scientific assessment of the safety of AHA with restrictions on use.
Recently, new scientific data on some of the above issues have been available to better evaluate the safe use of AHA.

2. Previous position of the SCCNFP

The SCCNFP adopted a position paper (SCCNFP/0370/00) (1) on the safety of AHA based on studies on short term phototoxicity (sensitivity of human skin to UV-induced damage: sunburn cells and pyrimidine dimers production) and skin irritation. The SCCNFP considered that there was a need for more information in order to provide a full scientific assessment of the safety of AHA. However, on the precautionary principle, the SCCNFP suggested that:

- glycolic acid may be used safely at a level of up to 4 % and a pH ≥ 3.8
- lactic acid up to a maximum level of 2.5 % and a pH ≥ 5.0.

Further, it was recommended that there should be appropriate warnings to the consumer of:

- avoiding contact with the eyes
- avoiding or affording protecting from UV whilst using products containing AHA because of the suggestion of susceptibility to increased damage from UV whilst cosmetic products containing them are being used.

Moreover, the SCCNFP expressed the need for the Cosmetic Industry to provide specific information with regard to two potential effects of AHA on the skin:

- to evaluate the effect of short-term skin treatment with glycolic acid (a model AHA) on the skin penetration characteristics of a model penetrant following an appropriate study protocol.

- to know whether a NOAEL for sunburn cells (SBCs) production, as the most sensitive parameter to measure UV sensitivity, should be identified and serve as the decisive threshold concentration for consumer information (e.g. potential sun alert statement).

Recently, the Cosmetic Industry has provided a new submission of data (submission IV) on skin barrier function and UV sensitivity as well as a study on photocarcinogenicity of AHA.

3. AHA and the Skin barrier function

AHA, specially at high concentrations, are known to act as exfoliants and some concern has been expressed that the removal of dead skin surface cells may adversely affect the barrier function of the stratum corneum (SC). Impairment in skin barrier function will cause an increased transepidermal water loss (TEWL).

3.1. Effect of AHA on TEWL

Based on the log regression test by Kligman (2), a clinical evaluation of the effects of 12% ammonium lactate (pH 4.4) and 8% glycolic acid (partially neutralized at pH 4.4) was conducted in ten subjects with moderate to severe ichthyosis/xerosis of the lower legs. Product treatment consisted of a previous two-week rest period followed by three weeks of product applications (twice daily) with a one-week recovery period. Acceptable levels of TEWL were observed, indicating little to no change in the SC barrier function, as also shown histologically by the normalization of the classical SC basket weave picture (3).

In a meeting with the SCCNFP, CTFA provided data to demonstrate no compromise in the skin barrier after either short or longer term skin applications of AHA, as measured by TEWL. In all cases, it was demonstrated that AHA do not compromise the integrity of the skin barrier (4-6).

3.2. Effect of AHA on skin penetration of model compounds

Several studies were performed to determine the potential effect of a glycolic acid moisturizer (10 % glycolic acid) on percutaneous penetration of other compounds (glycerine, hydrocortisone, tritiated water, hydroquinone and musk xylene) either on humans or on hairless guinea pig skin. In all cases, no significant effects were seen (7, 8).

A pharmacokinetic study was performed to determine the effect of a glycolic acid treatment on the skin penetration of benzophenone-3 (9).
The objective of this study was to determine, via urinary excretion, the effect of cutaneous glycolic acid treatment on the skin penetration of benzophenone-3 following a twice daily treatment with a glycolic acid (10 %) lotion or a non-glycolic acid vehicle for 8 and 28 days. The study protocol considered:

- glycolic acid as a worst case test substance which has a comparable pK to lactic acid and a significantly lower pK compared to other hydroxy acids.
- a worst case concentration/pH (10 % glycolic acid at pH 3.5).
- a typical formulation, close to marketed AHA products for skin exfoliation.
- twice daily application.

- skin penetration measurements of benzophenone-3 at 8 days and 4 weeks.

This study was divided into two phases: The screening phase and the treatment phase.

Screening phase: Twenty-seven subjects, with a test site on the volar aspect of each forearm (100 cm² per site approximately) were included. On each test site, 2 mg/cm² of a sunscreen lotion containing benzophenone-3 was applied. The excretion of benzophenone-3 was evaluated from urine samples collected over 24 hours from each subject. LC/MS/MS methodology was used. The total amount of benzophenone-3 excreted ranged from 0.07 to 0.99 mg or approximately 0.2 to 2.5 % of the applied dose.

Treatment phase: The subjects were randomly assigned to one of two groups: an 8 day treatment group and a 28 day treatment group, each consisting of at least twelve subjects. The test substance (10 % glycolic acid lotion) and the vehicle lotion (without glycolic acid) were applied twice daily at a dose of 2 mg/ cm².
Following completion of the assigned treatment period, subjects again received an application of benzophenone-3 (2mg /cm^2) and each of the subjects collected all urine voided for a 24-hour period (range 350 – 2600 ml). The total amount of benzophenone-3 excreted in the collected urine samples ranged from 0.04 to 1.92 mg (approx 0.1- 4.8 % of the applied dose).
These experimental data are insufficient for adequate statistical evaluation.

Therefore, the study is not acceptable.

4. AHA and UV skin sensitivity

A change in UV transmission, possibly due to the exfoliant activity of AHA applied on the skin, could result in increased UV-damage to epidermal cells. This can be monitored by the formation or production of SBCs and by a lowering of the Minimal Erythema Dose (MED). SBCs are visualized in H&E sections of skin as small rounded cells with eosinophilic cytoplasm and a pyknotic nucleus and are a more sensitive indicator of UV damage than erythema. They are regarded as markers of lethal DNA damage by UV-radiation (UVR) and are produced by both UVB and UVA wavelengths (10).

4.1. AHA and SBCs production

Effects of topical treatments of AHA on the sensitivity of human skin to solar simulated radiation.
This investigation was intended to determine whether treatments for a 4-week period with various topical products change the response of human skin to solar radiation or UVR in respect to SBCs production and MED variations (11).
The following treatments were applied to designated sites over the mid-back region (5cm x 10 cm) once daily for seven days every week for four consecutive weeks:

- a vehicle lotion adjusted to pH 3.5.
- three different glycolic formulations at different glycolic acid concentrations (2.5 %, 4 % and 6 %) and adjusted at pH 3.5.

The topical dose of each product was 100 mg per test area (approx 2 mg/ cm²). The MED of the subjects was determined previously by exposing several normal skin sites (1 cm of diameter) to a

series of exposures in 25 % dose increments from the solar simulator. The same procedure for the MED determination was followed at the end of the 4 week study period.
A similar procedure was followed to obtain skin biopsies at the end of the study. Approximately fifteen minutes after the last topical application of the test products, a circular area of 1 cm in diameter was exposed once to single a dose of 1.5 MED from the solar simulator. Approximately 20 hours later, a shave biopsy (approx. 4 mm x 4 mm) was obtained from each site. The skin specimens, fixed in 10 % buffered formalin, were processed in order to visualize the number of SBCs in each biopsy. A minimum of 70 High Power Fields (HPF) were counted from each biopsy. In Table I the results obtained on SBCs per HPF and on MED are shown.

Table I: Minimal erythema dose (MED, mJ/ cm^2) and mean number of sun burn cells (SBC) per high power fields (HPF) of the skin sites treated with glycolic formulations at different glycolic acid concentrations.

		Glycolic acid formulations			Vehicle	Skin control
		2.5 %	4.0 %	6.0 %		
MED	Mean	66.4	62.6	58.5	66.8	73.1
	S.D.	13.0	11.8	12.7	15.5	17.0
SBCs	Mean	3.64	3.61	4.62	2.70	2.72
	S.D.	3.98	3.28	3.80	2.69	3.00

The study authors considered that all treatments, except for the vehicle, resulted in a statistically significant reduction in the MED compared with the control untreated site. With a glycolic formulation, in the concentration range 2.5-4.0 %, an increase in SBCs production was observed. Results on the effect of glycolic formulations at glycolic acid concentrations lower than 2.5% are not available. No NOEL has been found with regard to an effect on SBC formation.

Because the standard deviations (SD) of these data are so large, nothing can be concluded.

4.2. Combination of AHA and sunscreens to prevent UV sensitivity.

Clinical studies with moisturizer products containing AHA and a sunscreen to evaluate the skin's sensitivity to ultraviolet light.

In two clinical studies, examining the effects of several commercially available moisturizers containing a sunscreen, exposures to 1 MED did not significantly increase SBCs in the presence of AHA. The quantitative composition of the formulations were not available.

In an initial study, 4 subjects were treated daily with 2 formulations containing AHA with sunscreens and exposed after the last application to 1 MED. Twenty hours after the exposure, shave biopsies were taken to assess presence of SBCs. Both products had a sun protection factor (SPF) of 2.9 and an AHA content in the range of 4-8% (glycolic acid). No evidence of SBCs formation versus untreated controls was observed (Table II) (13).

In a second study, subjects were treated daily with a known amount of moisturizer for 22 days, then they were exposed to 1 MED after the last moisturizer application. SBCs were measured from shave biopsies taken 20 hours after the exposure. All moisturizer products that had sunscreen protection in the range 2.9-3.5 and AHA in the range of 2-8% (lactic acid and /or

glycolic acid) showed no evidence of SBCs formation compared with untreated controls (Table III) (12).

Table II: sun burn cells (SBC) formation in human skin treated with two lotion products after an UV exposure of 1 minimal erythema dose (MED)

Lotion product (AHA content)	Panelists	Study duration	SBCs	UV Exposure (daily)	SPF
Untreated control	4	4 days	1.06	1 MED	
Test product A 4% Glycolic acid 1.5% EHMC *	4	4 days	0.024	1 MED	2.9
Test product B 8% Glycolic acid 1.5% EHMC *	4	4 days	0.044	1 MED	2.9

* EHMC : ethylhexyl methoxycinnamate

Table III: sun burn cells (SBC) formation in human skin treated with several lotion products after an UV exposure of 1 minimal erythema dose (MED)

Lotion product (AHA content)	Panellist	Study duration	SBCs	UV Exposure (daily)	SPF
Untreated control(mean)	15	22 days	0.277	1 MED	
Test product B 8% Glycolic acid 1.5% EHMC *	5	22 days	0.088	1 MED	2.9
Test product C 1% Lactic acid 1% Glycolic acid 2.0% EHMC *	5	22 days	0.044	1 MED	3.1
Test product D 1% Lactic acid 1% Glycolic acid 2.5% Benzophenone-4	5	22 days	0.040	1 MED	2.4
Test product E 6% Lactic acid 1.9% EHMC *	5	22 days	0.028	1 MED	3.3
Test product F 6% Lactic acid 1.9% EHMC *	5	22 days	0.036	1 MED	3.5

* EHMC : ethylhexyl methoxycinnamate

In both studies, complete composition of the formulations used was not provided and the generic information given in the corresponding reports are weak (12, 13).

Moreover, it was noted that these two studies, submitted for evaluation by the SCCNFP, were undertaken between 1996-1997, some years before the Committee requested new data on AHA and UV sensitivity (SCCNFP/0370/00).

Effects of sunscreen administration on SBCs production in hairless mice topically treated with glycolic acid or salicylic acid formulations for four weeks.

A study was designed to evaluate the potential for toxic or possible interactive effects associated with repeated daily administration of glycolic acid (10 %, pH 3.5), salicylic acid (4 %, pH 4.0) and sunscreen (SPF 4, 8, or 15) formulations with simulated sunlight five days a week for a period of four weeks (14).
Female hairless mice were assigned to nine dosage groups (12 mice per dosage group). Mice were administered glycolic acid or salicylic acid alone or with sunscreen formulations, or left untreated.
All test formulations were applied to the back and sides of the mice (approximately 25 cm^2). The first test formulation applied was the appropriate hydroxy acid formulation at a volume of 150 µL per mouse. The second test article administration was the sunscreen formulation at a volume of 50 µL per mouse, approximately 30 min after the first application and to the same body area. UVR exposure for all groups began approximately 30 min after the completion of the second application.
Twenty-four hours following irradiation, the mice were sacrificed. Skin samples (approximately 3cm x 3cm) were collected for histopathological evaluation and morphometric analysis of SBCs (Table IV).

Table IV: Sunburn cell histomorphometry

Group	Descriptor[a]	SBCs/Mouse ± S.D.
1	No formulation administration	3.1 ± 2.0
2	10 % Glycolic acid	2.2 ± 1.6
3	10 % Glycolic acid + SPF 4 Sunscreen	0.7 ± 0.6
4	10 % Glycolic acid + SPF 8 Sunscreen	0.7 ± 0.8
5	10 % Glycolic acid + SPF 15 Sunscreen	0.9 ± 1.0
6	4 % Salicylic acid	1.4 ± 1.7
7	4 % Salicylic acid + SPF 4 Sunscreen	0.8 ± 0.6
8	4 % Salicylic acid + SPF 8 Sunscreen	1.2 ± 0.9
9	4 % Salicylic acid + SPF 15 Sunscreen	0.6 ± 0.7

[a]All groups received 240 RBU (Robertson-Berger Units) daily of UV.

Because the standard deviations (SD) of these data are so large, nothing can be concluded.

Influence of immobilization and free-ranging activity on the effect of the sunscreen application on hairless mice treated with glycolic acid or salicylic acid formulations for four weeks.

The purpose of this study was to determine the effect of a single application of a SPF 4, 8 or 15 sunscreen formulation on cutaneous histopathology and the production of SBCs in the skin of female hairless mice that were topically administered either a glycolic acid (10 %, pH 3.5) or a salicylic acid (4 %, pH 4.0) containing formulation for four weeks. Restrained or free-moving

irradiation conditions were investigated (15). The basic experimental protocol was similar to the above study.

Because of the high variability of the data, no conclusion can be drawn.

12-month study to determine the influence of topically administered glycolic acid in combination with sunscreen (SPF 15) on photocarcinogenesis in hairless mice.

The purpose of this study was to determine the potential of the topical administration of glycolic acid (4 % or 10 %, pH 3.5) plus the topical administration of sunscreen (SPF 15) to influence the development or growth of skin tumours in hairless mice when exposed to solar simulated UVR (16).
One hundred forty-four Crl:SKH1-*hr*BR mice per sex were randomly assigned to six dosage groups and glycolic acid (pH 3.5) was administered at 4% and 10% to mice. Approximately three hours after glycolic acid formulation administration, the test article, sunscreen (SPF 15), was administered. 30 minutes after sunscreen formulation administration, UVR exposure of all groups began (120 RBU/day or 240 RBU/day). Formulation administration and UVR exposure were conducted five days per week, for 40 weeks. Mice were maintained for an additional 12 weeks for a total of 52 weeks.

During study weeks 1 through 52, individual skin tumour data were collected once each week for each mouse. Tumour prevalence and time for tumour formation were estimated.
In comparison to the controls (mice receiving neither glycolic acid or sunscreen treatment) there was evidence of tumour prevention at both concentrations of glycolic acid and sunscreen (table V).

Table V: Tumour Potency Ratios (≥ 1 mm tumour size)

Dosage Group	**1**	**2**	**3**	**4**	**5**	**6**
Formulation [a]	none	4% GA+SS	10% GA+SS	4% GA+SS	10% GA+SS	none
UVR Exposure (RBU/week)	600	600	600	1200	1200	1200
Sexes Combined	1	0.64[b]	0.64[b]	0.64[b]	0.64[b]	2
Males	1	0.68	0.68	0.68	0.68	2
Females	1	0.61	0.61	0.61	0.61	2

a. GA 4% + SS : 4% Glycolic acid plus sunscreen
GA 10% + SS : 10% Glycolic acid plus sunscreen
b. Unbiased median latent period was not achieved for groups 2-5 (52 weeks) by the end of the study. The tumour potency ratio (TPR) for these groups were estimated using the unbiased median latent period of 53 weeks to indicate this fact.

From the results obtained, it may be suggested that topical administration of glycolic acid together with sunscreen formulations did not enhance photocarcinogenesis. However, no control using the sunscreen formulation alone was included in the experiment.

5. Conclusion

Potential impairment of the skin barrier function after topical application of AHA

Whilst the SCCNFP agreed previously that available data showed no increase in TEWL or dermal penetration of reference compounds after long-term use of AHA (up to 10 % at pH 3.5), concerns were raised over effects potentially occurring after short-term uses prior to adaptive changes of the skin. Considering skin renewal rate, a maximum effect (if any) on skin barrier function should be visible between 8-14 days.
In Submission IV presented by the Cosmetic Industry, several studies are included evaluating the skin barrier function by TEWL as well as the influence of AHA in the potential increase of skin penetration of several model compounds.

The experimental data presented on the skin penetration study are inadequate for proper evaluation.

AHA and UV sensitivity

In a previous evaluation, it was established that high concentrations of AHA (10 % at a low pH) could increase the skin's sensitivity to the sun. The SCCNFP proposed that a NOAEL for MED or SBCs production should be identified and serve as the decisive concentration for consumer information (e.g., potential sun alert statement).

From the results obtained, it can be deduced that AHA application does increase UV damage to the skin. A skin treatment with a glycolic formulation at pH 3.5, in the concentration range 2.5-4.0 %, may induce a decrease in MED and/or an increase in SBC production.
It was not possible from the data available to define a NOEL.

The SCCNFP maintains its previous opinion (SCCNFP/0370/00, 28 June 2000) because of the inadequate nature of the data submitted for evaluation.

6. References

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