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November 13, 2003

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Dockets Management Branch
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

Re: Docket Number 03P-0365, Comments

Dear Sir or Madam:

We herewith submit comments in opposition to the Citizen Petition 2003P-0365 filed by Jerussi Consulting on August 11, 2003.

Jerussi Consulting requests that the Food and Drug Administration make a determination that the Galderma Laboratories, L.P. product Solagé® Topical Solution, containing mequinol 2% and tretinoin 0.01% (NDA #20-922), can be formulated as a topical solution with the substitution of hydroquinone 4% for the mequinol 2% and filed as an Abbreviated New Drug Application referencing Solagé® Topical Solution as the Reference Listed Drug.

Jerussi Consulting supports its request by citing a list of products approved and marketed in the United States, containing either tretinoin 0.01% or hydroquinone 4%. Only one of those products listed by Jerussi Consulting, Tri-Luma® Cream, contains a combination of tretinoin and hydroquinone; however, these ingredients are also in association with a corticosteroid, fluocinolone acetonide, and their respective concentrations are 0.05%, 4% and 0.01%.

We wish to highlight significant safety concerns that may be raised by: 1) substituting hydroquinone 4% for mequinol 2%; 2) combining mequinol with tretinoin; and 3) combining tretinoin with hydroquinone. Clinical data suggests that mequinol and hydroquinone are different compounds from an efficacy and a safety point of view. Further data suggests that a combination of hydroquinone and tretinoin may show different efficacy and safety profiles than each of these ingredients taken alone, due to possible interactions in the skin.

2003P-0365

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I. Substitution of hydroquinone 4% for mequinol 2%

Hydroquinone and mequinol (4-Hydroxyanisole) are both skin bleaching agents. However, the data presented below indicates that mequinol 2% and hydroquinone 4% do not have the same safety and efficacy profiles. More precisely:

1. **Mequinol and hydroquinone have different toxicological profiles when administered systemically.**

   The safety of mequinol and hydroquinone was assessed in reviews of the extensive literature available on these ingredients (***Ref. 1*** and ***Ref. 2***, respectively) and some key toxicological differences between mequinol and hydroquinone are summarized below.

   The acute oral toxicity of both products is as follows:

   - Mequinol LD_{50}: 1,630 mg/kg (sex not specified)
   - Hydroquinone LD_{50}: 743 and 627 mg/kg for males and females respectively

   This is indicative of the differences toxicity, which was confirmed after repeated exposure.

   Following subchronic and chronic administration by the oral route, mequinol was not very toxic, showing a reduction in food consumption and body weight gains up to dose levels of 5% in the diet (equivalent to 50,000 ppm). High dose levels (in the range of 1,000 mg/kg/day) were tested in several species (including rodents and non-rodents) without a clear toxicity. The only notable finding was the increased incidence of fore-stomach tumours in rats (males and females) in carcinogenicity studies. Histopathologically, these tumours were classified as papilloma and single cell carcinoma. It has to be emphasized that tumours occurred only at a very high dose level of mequinol, corresponding to 1,000 mg/kg/day for a 2-year duration.

   Hydroquinone was tested by the oral route in several species at much lower dose levels (often in the range of 1,000-4,000 ppm). More toxicity was observed with histopathological changes in the liver and the kidney and macroscopic observation revealing severe inflammations (gastro-intestinal tract). In carcinogenicity studies, hydroquinone was tested in the rat by the oral route, and the only convincing evidence of tumour formation was for renal adenomas in male rats treated with 50 mg/kg/day in drinking water.

2. **When administered alone, mequinol and hydroquinone are not equivalent regarding local safety. Preclinical and clinical data suggest that hydroquinone 4% is more irritating than mequinol 2%.**

   Mequinol and hydroquinone were tested for skin sensitization in the Magnusson and Kligman guinea pig test (maximization protocol using injection with the Freund complete adjuvant). Mequinol was injected during the induction phase as a 6.2% solution whereas hydroquinone was injected at 2% in a separate study. Under these conditions, mequinol produced a moderate skin sensitization whereas hydroquinone was classified as an extreme skin sensitizer (100% of animals having a positive response). This can be related to the extensive information showing the immunotoxicity of hydroquinone, which is absent for mequinol. (***Ref. 1*** and ***Ref. 2***).

   In an animal model of skin depigmentation, the Yucatan miniature swine, hydroquinone applied at 5% in a propylene glycol vehicle was significantly more irritating than mequinol applied at the same concentration, in the same vehicle. This macroscopic observation was confirmed by histology (***Nair and Tramposch, 1991, Ref. 3***).

   The irritation potentials of seven formulations, including two depigmenting solutions, consisting of a combination of mequinol 2% and tretinoin 0.01%, their vehicle, each of the active ingredients taken alone, hydroquinone 3% and sodium lauryl sulfate 0.5% (as a positive control) were compared in healthy volunteers, in a 21-day cumulative irritation test. The results showed that hydroquinone 3% was rated with the highest irritation score (mean = 3.43 in a scale from 0 to 4) whereas mequinol was the best tolerated active ingredient of the group (irritation score = 1.61), slightly, but significantly, above the vehicle rated at 0.63 (***Solagé® Topical Solution Phase I study DE118-019-001, Ref. 4***).

   Finally, the higher irritation potential of hydroquinone 3% compared with mequinol 2% was confirmed by the related Adverse Events recorded in the Solagé® Phase II study in subjects with lentigines (either mequinol 2% alone or a marketed solution containing hydroquinone 3% were used as comparators in this study). In this study, related AEs were reported by 14% of the subjects treated with mequinol 2% alone and 30% of the subjects treated with hydroquinone 3% alone (***Solagé® Topical Solution Phase II study DE132-002, Ref. 5***).

   These studies demonstrate that hydroquinone has a significantly higher sensitizing potential than mequinol and suggest that hydroquinone 4% more irritating than mequinol 2%.

3. **Some pharmacological data suggest that hydroquinone and mequinol may have different modes of action regarding depigmentation.**

   In the previously mentioned study using Yucatan miniature swine, depimentation induced by mequinol was reversible whereas depigmentation induced by hydroquinone was not reversible 12 months after cessation of treatment (***Nair and Tramposch, 1991, Ref. 3***) suggesting that the modes of action of mequinol and hydroquinone are different.

4. **Clinical data suggest that hydroquinone and mequinol may have different levels of efficacy in depigmentation.**

   The results of the Solagé® Topical Solution Phase II study suggest that the efficacy levels of mequinol 2% and hydroquinone 3% in the topical treatment of lentigines are different (***Solagé® Topical Solution Phase II study DE132-002, Ref. 5***).

II. Combining mequinol with tretinoin

Combining mequinol and tretinoin may lead to interactions affecting the skin absorption and local tolerance of both active ingredients. It is unknown whether similar interactions may also occur when combining hydroquinone and tretinoin and we contend that this uncertainty raises a concern regarding extrapolation of the safety data supporting approval of either hydroquinone 4% or tretinoin 0.01% alone to a fixed combination of both ingredients.

When applied *in vitro* to human cadaver skin under infinite doses, penetration of mequinol through the skin is increased in the presence of tretinoin applied either simultaneously or as a skin pre-treatment (***Solagé® Topical Solution study AU-ST-91001, Ref. 6***). This observation was reproduced *in vivo* in rats and rabbits in which topical administration of a combination of tretinoin and mequinol resulted in a higher systemic exposure with mequinol than administration of the same amount of mequinol alone (***Solagé® Topical Solution toxicokinetic study 930740039, Ref. 7*** and ***Solagé® Topical Solution toxicokinetic study 930740056, Ref. 8***, respectively). This effect could not be reproduced using *in vitro* application of a finite dose, perhaps because the amount of available tretinoin was insufficient (***Solagé® Topical Solution study PARAB-92005, Ref. 9***).

Conversely, an *in vitro* study using finite doses showed that mequinol could reduce the flux of tretinoin through the skin (***Solagé® Topical Solution study PARAB-PV-92032, Ref. 10***).

Therefore, interactions between mequinol and tretinoin may affect penetration of the drugs through the skin, and therefore, the safety profile of the combination as compared to the individual drugs is not equivalent.

This safety concern seems to be confirmed by a 21-day cumulative irritation test in humans which showed that one of the depigmenting solutions, containing a combination of mequinol 2% and tretinoin 0.01%, had a significantly higher irritation potential (mean score = 2.63) than each of the active ingredients taken individually (mean scores = 1.61 and 2.36 for mequinol and tretinoin, respectively) (***Solagé® Topical Solution Phase I study DE118-019-001, Ref. 4***).

As such, the skin absorption and safety profiles of mequinol and tretinoin are different when they are combined than when they are applied alone. Research of potentially similar effects should also be performed when combining tretinoin with hydroquinone.

III. Combining hydroquinone with tretinoin

As shown above, there is data indicating that combining mequinol and tretinoin may lead to interactions which affect the skin absorption and local tolerance of both active ingredients. It is unknown whether similar interactions may also occur when combining hydroquinone and tretinoin and raises a concern regarding extrapolation of the safety data supporting approval of either hydroquinone 4% or tretinoin 0.01% to a fixed combination of both ingredients.

1. **The safety data reported for Tri-Luma® Cream cannot be taken to support the safety of a combination of hydroquinone and tretinoin because Tri-Luma® Cream also contains a corticosteroid which may improve the local tolerance of the other two active ingredients.**

   A Phase I irritation study showed that the complete formulation, containing the combination of tretinoin 0.05%, hydroquinone 4% and fluocinolone acetonide 0.01% was less irritating that the combination of tretinoin 0.05% and hydroquinone 4% in the same vehicle (***Tri-Luma® Cream study 36, Ref. 11***). This difference can be attributed to the anti-inflammatory effect of the corticosteroid included in the complete formula.

   This observation is also supported by the lower number of related Adverse Events reported in the Phase III studies with the complete formulation of Tri-Luma® Cream compared to the combination of tretinoin 0.05% and hydroquinone 4% (***Tri-Luma® Cream: Integrated Summary of Safety, Ref. 12***).

2. Safety concerns that are raised by a combination of tretinoin with hydroquinone.

As discussed above, a combination of tretinoin and hydroquinone cannot be considered equivalent with the combination of tretinoin and mequinol in Solagé® Topical Solution. It also cannot be considered equivalent with Tri-Luma® Cream because this product contains a corticosteroid (fluocinolone acetonide) in addition to tretinoin and hydroquinone, which may minimize skin irritation. Therefore, based on the safety assessments published for mequinol and hydroquinone (***Ref. 1*** and ***ref. 2***, respectively), the safety of the proposed new combination should be addressed as regards the following points:

- Immunotoxicity: Hydroquinone has been classified as an extreme skin sensitizer (see above).

- Phototoxicity and photosensitization: Photosensitization properties for mequinol were tested alone or in the finished product Solagé® Topical Solution. To the best of our knowledge, phototoxicity and photosensitization data are not available for hydroquinone.

- Carcinogenicity: Although the published results for hydroquinone and tretinoin do not seem to raise a particular concern for the human safety when these molecules are considered alone, a combined effect in the skin cannot be excluded due to the fact that they have very different irritation properties.

- Teratogenicity and reproduction: Both mequinol and hydroquinone were tested for teratogenicity and effects on reproduction. Results indicated that these two molecules do not raise a human safety concern. However, tretinoin is known to induce teratogenicity. Skin irritation induced by hydroquinone may significantly increase the exposure to tretinoin after a dermal application of the combined product.

- Photo(co)carcinogenicity: The photo(co)carcinogenenicity potential of Solagé® Topical Solution has been evaluated. This study gives information for the safe use of the product because Solagé® Topical Solution contains tretinoin that has been reported to increase the rate of skin tumours with a combined UV exposure. As hydroquinone is more irritating, a modification of penetration depth of UV radiations cannot be excluded which could increase the tretinoin effects in a combination using tretinoin and hydroquinone.

IV. Conclusion

A complete development program is needed to support the safety and efficacy of any new product combining hydroquinone and tretinoin as a skin bleaching agent, because such a combination drug cannot be considered equivalent with the Solagé® Topical Solution combination of tretinoin and mequinol.

The preclinical package should, at a minimum, include immunotoxicity, carcinogenicity, phototoxicity and photosensitization, photo(co)carcinogenicity, teratology, fertility and reproduction toxicity studies.

The clinical package should include Phase I studies (irritation and contact sensitization), a Phase II dose ranging study to justify the concentration of each active ingredient incorporated in the combination, and a pharmacokinetic study to evaluate the interactions between tretinoin and hydroquinone. Finally Phase III studies would be needed, as for any new product, to confirm the safety and efficacy of the new combination including a long term study to support the long term safety of the combination.

For the foregoing reasons, we respectfully request that the Citizen Petition 2003P-0365 be denied.

If you need any additional information on our position or have any questions, do not hesitate to contact me directly at (817) 961-5336.

Regards,

Paul Clark
Vice President, Regulatory Affairs

Enc.

Summary of Referenced Studies

Ref. 1: **Final Report on the Safety Assessment of p-Hydroxyanisole.**
J. Am. Coll. Toxicol., 1985, 4(5): 31 – 63

Ref. 2: **Addendum to the Final Report on the Safety Assessment of Hydroquinone.**
J. Am. Coll. Toxicol., 1994, 13(3): 167 - 230

Ref. 3: **X. Nair, K.M. Tramposch. The Yucatan miniature swine as an *in vivo* model for screening skin depigmentation.**
J. Dermatol. Science 1991; 2: 428-433.

The Yucatan miniature pig is a naturally occurring bred of swine with light brown to dark brown skin that is used to screen depigmenting activity by topical products. In this model, hydroquinone (HQ) and mequinol (= 4-Hydroxyanisole, 4HA) were tested at the same concentration (5%) in a propylene glycol / ethanol (50:50) vehicle applied twice daily, 7 days a week for 90 days.

Signs of local irritation were graded weekly on a scale from 1 (mildly irritating with scaling and erythema) to 4 (severely irritating with dark redness, eschar and bleeding). HQ and 4HA showed a very different irritation potential: HQ produced a severe local irritation (grade 4) following 49 days of treatment whereas 4HA induced only a mild irritation (grade 1) following 70 days of treatment. This difference was confirmed in skin biopsies which showed hyperplasia of the epidermal layer and a moderate degree of diffuse inflammatory cell infiltrate in the upper dermis of skins treated with HQ whereas no epidermal hyperplasia and a minimal degree of inflammatory cell infiltrate were apparent in skins treated with 4HA.

The depigmenting activity of HQ 5% and 4HA 5% was evaluated clinically, using a visual score ranging from 1 (complete depigmentation) to 4 (no visible depigmentation, i.e. normal). Similar efficacy was observed with both products, either clinically, with a complete depigmentation (grade 1) achieved at the same time, i.e. 77 days, or histologically, with an absence of pigment apparent in the epidermis or at the epidermal-dermal junction layer. However, after twelve months of no treatment, the HQ-treated sites remained completely depigmented whereas the sites depigmented with 4HA had markedly decreased in size with the repigmentation moving inward from the outer edge of the site.

Conclusion: In the Yucatan miniature pig, HQ at a concentration of 5% was significantly more irritating than 4HA at the same concentration.

The differences in reversibility of depigmentation within 12 months post-treatment suggest different modes of action for HQ and 4HA.

Ref. 4: **Solagé® Topical Solution Phase I study DE118-019-001: "Human Dermal Safety Study. 21-Day Cumulative Irritation Test. An open-label non-randomized intra-subject comparison of cumulative irritation potential"**

The objective of this study was to determine the cumulative irritation potential of two depigmenting formulations close to Solagé® Topical Solution, containing a combination of 4HA 2% and tretinoin 0.01% (= Retinoic Acid, RA). These formulations were compared to either 4HA 2% alone, RA 0.01% alone, a marketed solution containing HQ 3%, sodium lauryl sulphate (SLS) 0.5% (as a positive control) or a vehicle.

The products were applied to the back of 31 healthy subjects under occlusion daily, for 21 days. Each patch was removed after 24 hours and fresh patches were then reapplied to the same test sites. Skin irritation was evaluated daily, 5 minutes after patch removal, using a severity scale from 0 (no sign of irritation) to 4 (erythema with oedema and blistering). The results are reported in **Table 1** below.

The products were significantly different regarding the irritation induced: HQ 3% was severely irritating (mean score = 3.43), SLS 0.5% (positive control) was very irritating (mean score = 2.75), the two depigmenting solutions (mean scores = 2.63 and 2.55) and the RA solution (mean score = 2.36) were irritating. 4HA (mean score = 1.61) and vehicle solution (mean score = 0.63) were mildly irritating. One of the depigmenting solutions (W1133-M-07-A) was more irritating than either 4HA or RA alone. These differences were statistically significant ($p < 0.001$).

Table 1: Total Cumulative and Mean Score by Treatment
(n = 31)
Friedman's Test, $p \leq 0.001$

Treatment	Total cumulative*	Mean Score**	Tukey's***
HQ 3%	1913	3.43	A
SLS 0.5%	1532	2.75	B
Depigmenting solution W1133-M-07-A	1468	2.63	B
Depigmenting solution W1133-M-01-A	1421	2.55	BC
RA 0.01%	1317	2.36	C
4HA 2%	900	1.61	D
Vehicle	351	0.63	E

* Total Cumulative Score is the sum of all readings for all subjects for a given product.
** Mean Score is the average score for all subjects for all readings for a given product.
*** Means with different letters are significantly different.

Conclusion: Under occlusive conditions, HQ 3% was significantly more irritating than either 4HA 2% alone or combinations of 4HA 2% and RA 0.01%. HQ 3% was severely irritating whereas 4HA 2% was mildly irritating. This study provides evidence that 4HA 2% and HQ 3% cannot be considered as equivalent from a local tolerance point of view.

A combination of tretinoin and mequinol may be more irritating than each of these ingredients taken alone.

Ref. 5: **Solagé® Topical Solution Phase II study DE132-002: "A Double-Blind, Parallel Group Comparison of the Efficacy and Safety of BMS-181158/BMS-181159 Solution (4-Hydroxyanisole 2% / Tretinoin 0.01%) Versus Individual Active Agents, Vehicle and Hydroquinone 3% topical solution in the Treatment of Solar Lentigines and Related Hyperpigmented Lesions."**

The objective of this study was to evaluate the efficacy and safety of a combination of 4HA 2% and RA 0.01% as a depigmenting agent in the treatment of solar lentigines when administered topically. This combination product was compared to either 4HA 2% alone, RA 0.01% alone, a marketed solution containing HQ 3% or the combination vehicle.

These products were assigned to subjects with solar lentigines involving the dorsal forearm and the forehead or cheek area on the face in a 1:1 ratio. Treatments were applied twice daily for 16 weeks. Evaluations were continued for 24 additional weeks without treatment as a follow-up phase (i.e. the total study duration was 40 weeks). The primary efficacy parameters were the Physician's Assessment of Overall Cosmetic Effect and the Physician's Global Assessment of improvement / worsening. These parameters were evaluated on scales ranging from 0 (completely cleared) to 6 (worse) at each visit. In the Physician's Assessment of Overall Cosmetic Effect, a clinically significant result for a subject was defined as a moderate improvement or greater. Out of the 221 enrolled subjects, 216 were evaluable for safety and 194 completed the study.

A difference of 10% to 15% in percentage of efficacy was observed between 4HA 2% and HQ 3% in both primary efficacy parameters. This is only a trend but it should be mentioned that this study was not designed to achieve a statistical significance on this comparison. This data is presented in **Table 2** below.

Table 2: Efficacy Evaluations in Subjects with Moderate or Greater Clearance at the End of Treatment (Evaluable Subjects)

Treatment	Physician's Assessment of Overall Cosmetic Effect		Physician's Global Assessment	
	Arm n (%)	Face n (%)	Arm n (%)	Face n (%)
Vehicle	6 (15%)	8 (21%)	6 (15%)	10 (26%)
4HA 2%	8 (20%)	17 (43%)	8 (20%)	18 (45%)
HQ 3%	14 (35%)	21 (53%)	15 (38%)	23 (58%)

The number of subjects who experienced a treatment-related Adverse Event was higher by 16% with HQ 3% compared with 4HA 2%. The results are reported in **Table 3** below.

Table 3: Number of Subjects with Related Adverse Events

Treatment groups	Number of subjects	Number of subjects with related AEs (%)
4-HA 2%	42	6 (14%)
HQ 3%	44	13 (30%)

Conclusion: This study suggests that the efficacy levels of 4HA 2% and HQ 3% are different.

These results suggest that HQ 3% was less well tolerated than 4HA 2% in this study.

Ref. 6: **Solagé® Topical Solution study AU-ST-91001: "Effect of Tretinoin on the *in vitro* Skin Permeation of 4-Hydroxyanisole."**

The main objective of this study was to evaluate if RA could modify the skin permeation characteristics of 4HA *in vitro*, when applied either at the same time as 4HA or previously, as a pre-treatment. The products were applied to human cadaver skin in hydro-alcoholic solutions under infinite doses using Valia-Chien diffusion cells (applied volume: 3.5 ml). Analyses were performed in the receptor fluid.

The flux of 4HA at a concentration of 2% through the skin was increased in the presence of RA at concentrations ranging from 0.005% to 0.05%. A similar increase was observed with 4HA 4% in the presence of RA 0.01%. These results are presented in **Table 4** below.

Table 4: Flux of 4-Hydroxyanisole through Human Skin *in vitro*, in the Presence of Tretinoin

4HA concentration (% w/v)	RA concentration (% w/v)	4HA flux ± s.d. ($\mu g.hr^{-1}cm^{-2}$)
2	0.00	72.58 ± 9.42
2	0.005	103.40 ± 5.95
2	0.01	96.00 ± 29.53
2	0.05	160.12 ± 48.55
4	0.00	160.86 ± 15.67
4	0.01	232.91 ± 17.52

Pretreatment of the skin with the RA-containing formulation for 16 hours also increased the flux of 4HA through the skin whereas no increase was observed following pre-treatment of the skin with vehicle. Following skin pre-treatment with RA, addition of RA to 4HA was without effect on the skin penetration of 4HA. These results are presented in **Table 5** below.

Table 5: Effect of Skin Pretreatment with Tretinoin on the Flux of 4-Hydroxyanisole through Human Skin *in vitro*

RA concentration in combination with 4HA 2% (% w/v)	Pretreatment	4HA flux ± s.d. ($\mu g.hr^{-1}cm^{-2}$)
0	With RA	110.56 ± 25.01
0.01	With RA	110.28 ± 31.19
0	None	72.58 ± 9.42
0.01	None	96.00 ± 29.53
0	With vehicle	66.27 ± 3.74

Conclusion: In this study, the flux of 4HA through the skin could be increased either by addition of RA, with a dose-related effect, or by pre-treatment of the skin with RA. The reason for the skin permeation enhancement is not known, but these results suggest that pre-treatment or long-term treatment with RA may induce a change in the characteristics of the skin resulting in an increased permeation of other compounds.

Ref. 7: **Solagé® Topical Solution toxicokinetic study 930740039: "181158/181159 Formulation: Toxicokinetics of 181158 and 181159 in Rats During a 6-month Dermal Toxicity Study (Study N° 92005)"**

The objective of this study was to evaluate the concentration of 4HA and RA in serum samples from rats included in a 6-month dermal toxicity study. The test products were applied topically, once daily. A formulation containing the combination of 4HA 2% and RA 0.01% was applied to 3 groups of 32 rats (16 males and 16 females) at a dose of 0.2, 0.6 or 2.0 mL/kg. A formulation containing only 4HA 2% was applied at a dose of 2.0 mL/kg to a fourth group, and a fifth group was treated with 2.0 mL/kg of the vehicle. The dosing was applied to the clipped skin of the rats, and the skin in the clipped area was abraded in eight male and eight female rats of each group. Blood was collected from five male and five female rats of each group at 1, 3, 6 and 24 hr after daily dermal treatment during weeks 4 and 21 of the study.

Concentrations of 4HA in serum samples of drug-treated animals increased in a dose-related manner. However, this study showed that the animals treated with the combination dosing formulation containing RA and 4HA had higher systemic exposure to 4HA than the animals treated with dosing formulation consisting of 4HA alone. In all treatment groups, serum concentrations of RA were below quantifiable limits (1.0 ng/mL) or slightly above, comparable to the concentrations of endogenous RA (about 1.3 ng/mL). No differences in systemic exposures were observed among animals with intact skin and the abraded skin. The serum AUC (0-24 hr) Values of 4HA are presented in **Table 6** below.

Table 6: Serum AUC (0 – 24 hr) Values for 4HA in Rats during Weeks 4 and 21 of a 6-month Dermal Toxicity Study (Study N° 92005)

Treatment	Dosage Volume (mL/kg)	AUC (0 – 24 hr) ($ng.hr.mL^{-1}$) Week 4	Week 21
4HA 2% + RA 0.01%	0.2	95.5	623
4HA 2% + RA 0.01%	0.6	357	675
4HA 2% + RA 0.01%	2.0	1571	2312
4HA 2%	2.0	1078	1845

Conclusion: Systemic exposure of 4HA was increased by the presence of RA in the combination formulation.

Ref. 8: **Solagé® Topical Solution toxicokinetic study 930740056: "181158/181159 (Depigmenting) Formulation: Verification of Exposure to 181158 and 181159 in Rabbits During a Segment II Dermal Teratology Study (Study N° 92714)"**

The objective of this study was to evaluate the concentration of 4HA and RA in serum samples from pregnant rabbits included in a dermal teratology study. The test products were applied topically to the clipped skin of the rabbits, once daily on gestation days 6 to 18. A formulation containing the combination of 4HA 2% and RA 0.01% was applied to 3 groups of 20 pregnant rabbits at a dose of 0.2, 0.6 or 2.0 mL/kg. A formulation containing only 4HA 2% was applied at a dose of 2.0 mL/kg to a fourth group, a formulation containing only RA 0.01% was applied at a dose of 2.0 mL/kg to a fifth group and a sixth group was treated with 2.0 mL/kg of the vehicle. The skin in the clipped area of 10 animals in each group was abraded. Blood was collected 1hr after dosing on gestation day 18.

Concentrations of 4HA in serum samples of drug-treated animals increased in a dose-related manner and, within each group, plasma levels were higher in the animals with intact skin than in those with abraded skin. Moreover, this study showed that the animals treated with the combination of RA and 4HA had 3-fold higher systemic exposure to 4HA than the animals treated with the same dose of 4HA alone. In all treatment groups, serum concentrations of RA were below quantifiable limits (1.0 ng/mL). The plasma levels of 4HA are summarised in **Table 7**.

Table 7: Mean (sd) Concentrations of 4HA in Plasma of Rabbits Collected Approximately 1 Hr after dosing on Gestation Day 18 during a Dermal Teratology Study

Treatment	Dosage Volume (mL/kg)	Mean (s.d.) [a] Concentration (ng/mL)	
		Abraded Skin (n = 3)	Intact Skin (n = 2)
4HA 2%	2.0	203 (50)	259 (290, 228)
4HA 2% + RA 0.01%	0.2	80 (22)	124 (155, 92)
4HA 2% + RA 0.01%	0.6	192 (37)	375 (373, 377)
4HA 2% + RA 0.01%	2.0	598 (136)	793 (814, 771)
RA 0.01%	2.0	9.1 (9.1)[b]	2.6 (2.9, 2.3)[b]

[a] For Intact Skin, s.d. was not calculated because n = 2. It was replaced by the individual numbers.
[b] Plasma levels of 4HA in the animals treated with RA 0.01% are due to contamination during some aspect of collection, processing or analysis of samples.

Conclusion: Systemic exposure of 4HA was increased by the presence of RA in the combination formulation.

Ref. 9: **Solagé® Topical Solution study PARAB-92005: "*In vitro* Human Skin Permeation of 4-hydroxyanisole After Finite Dose Application of Hydroalcoholic Solution Formulations of 4-hydroxyanisole 2% With and Without Tretinoin 0.01%"**

The main objective of this study was to evaluate the permeation of 4HA across the skin and its retention in the skin after a finite dose, using *in vitro* Franz diffusion cells. The solutions used in this study were similar to those used in the previous study, but the amount applied in the diffusion cells was a finite dose (28 $\mu l/cm^2$).

Unlike in the former study, the permeation of 4HA across the skin and its retention in the skin were not significantly influenced by the presence of RA after a single dose application.

Conclusion: It was concluded that at a single finite dose, the amount of RA available to the skin may not be sufficient to elicit a skin permeation enhancement of 4HA.

Ref. 10: **Solagé® Topical Solution study PARAB-PV-92032: "*In vitro* human skin permeation of tretinoin after finite dose application of hydroalcoholic formulation of tretinoin 0.01% with and without 4-hydroxyanisole 2.0%"**

The main objective of this study was to evaluate the permeation of RA across the skin and its retention in the skin after a finite dose, using *in vitro* Franz diffusion cells, and to check if these parameters could be modified by the presence of 4HA.

Two hydro-alcoholic solutions containing either RA 0.01% alone or a combination of RA 0.01% and 4HA 2% were applied to human cadaver skin at a finite dose (21 $\mu l/cm^2$). Under these conditions, the apparent steady state flux of RA across the skin was reduced by 4HA ($p< 0.001$), but skin retention of RA was not reduced.

Conclusion: These results suggest that after multiple treatments, 4HA may possibly reduce the permeation of RA across the skin without affecting its retention.

Ref. 11: **Tri-Luma® Cream study 36: "21-day Cumulative Irritancy study"**

Tri-Luma® Cream is a cream approved in the United States for the short-term treatment of moderate to severe melasma of the face.

The objective of this study was to determine the cumulative irritation potential of Tri-Luma® Cream, containing a combination of fluocinolone acetonide (FA) 0.01%, HQ 4% and RA 0.05%, compared to either the Tri-Luma® Cream vehicle alone or the combination of HQ 4% and RA 0.05%.

The products were applied to the back or upper arms of 25 healthy subjects under occlusion, 5 days weekly, for 21 days. Each patch was removed after 24 hours and fresh patches were then reapplied to the same test sites, except during the weekends. Skin irritation was evaluated after each patch removal, using a severity scale from 0 (negative) to 4 (bullae).

The sums of the individual irritation scores show that the complete formulation (total score = 247) was more irritating than the vehicle (total score = 9), but less irritating than the combination of HQ + RA (total score = 575.5).

Conclusion: The lower level of irritation observed with the complete formulation, compared with the group treated with HQ + RA is attributed to the anti-inflammatory activity of the corticosteroid (FA).

Ref. 12: **Tri-Luma® Cream: Integrated Summary of Safety**

In the ISS of the Tri-Luma® Cream NDA, it is demonstrated that the safety profile of Tri-Luma® Cream in both Phase III efficacy and safety studies N° 28A and 28B is clearly better than that of the combination of RA 0.05% and HQ 4%. The summary of Adverse Events and the summary of the Most Common Adverse Events are presented in **Tables 8 and 9**, respectively.

The RA+HQ groups experienced the greatest level of related Adverse Events (126/158 patients, 79.75%), compared to the Tri-Luma® Cream group (102/161 patients, 63.35%).

Regarding the applications site reactions, desquamation occurred in 61.39% of patients in the RA+HQ group and in 37.89% in the Tri-Luma® Cream group. Pruritus was also much more frequent with 21.52% in the RA + HQ group compared to 11.18% in the Tri-Luma® Cream group

Table 8: Summary of Adverse Events. Studies 28A and 28B. ITT Population

	Number (%) of Patients			
	Treatment Groups			
	Tri-Luma (n = 161)	**FA + HQ (n = 161)**	**FA + RA (n = 161)**	**RA + HQ (n = 158)**
Patients with at least one adverse event	121 (75.16)	95 (59.01)	131 (81.37)	138 (87.34)
Treatment-related AE[a]	102 (63.35)	56 (34.78)	105 (65.22)	126 (79.75)
Serious AE	0 (00.00)	0 (00.00)	3 (1.86)	1 (<1.0)
Deaths	0 (00.00)	0 (00.00)	1 (<1.0)	0 (00.00)
Non-lethal AEs leading to discontinuation	0 (00.00)	1 (<1.0)	3 (1.86)	1 (<1.0)

[a] Designated as probably or possibly related to study medication by the investigator.

Table 9: Summary of Most Common Adverse Events. Studies 28A and 28B. ITT Population

	Number (%) of Patients			
	Treatment Groups			
	Tri-Luma (n = 161)	**FA + HQ (n = 161)**	**FA + RA (n = 161)**	**RA + HQ (n = 158)**
Patients with at least one adverse event	121 (75.16)	95 (59.01)	131 (81.37)	138 (87.34)
Total Adverse Events	385	192	354	426
Number of Patients with Most Common AEs[a]				
Application site:				
Desquamation	61 (37.89)	6 (3.73)	40 (24.84)	97 (61.39)
Erythema	66 (40.99)	26 (16.15)	41 (25.47)	69 (43.67)
Burning	29 (18.01)	5 (3.11)	33 (20.50)	36 (22.78)
Dryness	23 (14.29)	5 (3.11)	23 (14.29)	21 (13.29)
Pruritus	18 (11.18)	5 (3.11)	12 (7.45)	34 (21.52)
Headache NOS	16 (9.94)	17 (10.56)	13 (8.07)	13 (8.23)

[a] Events occurring in at least 10% of patients in at least one treatment group.

Conclusion: The lower frequency of Adverse Events reported with the complete formulation, compared with the group treated with HQ + RA, is attributed to the anti-inflammatory activity of the corticosteroid (FA).