Evaluation in human of the anti-wrinkles effect of TurnOver cream

Instrumental evaluation

Checking in human of its acceptability after application under normal conditions of use subjective assessment of its cosmetic qualities and efficacy

Dr. Vigneron[1], B. Pecal[2], Paul Scannell[3], Clotilde Quelen[4]
1. Dermatologist 2. Quality Assurance Unit 3. Head Manager 4. Scientific Department

Aim and principle of the study

This study intended:

To assess the anti-wrinkles effect of the cosmetic product, under strictly controlled conditions. To assess the acceptability and to appreciate the cosmetic qualities and the efficacy of a cosmetic product after application under the normal conditions of use.
To meet the requirements of the Directive 93/35/EEC of 14/6/93 - Art 7 bis - conceming the justification of the effect claimed in the advertising media.

The anti-wrinkle effect of the product was assessed:

Objectively and quantitatively: After taking of replicas, before and after treatment and image analysis with computer (Quantirides® software)

Subjectively: By questioning of the volunteers at the end of the study.

The acceptability:

Had been assessed, day by day, by the volunteers at home and controlled after visual examination of the experimental area, by the Dermatologist investigator or by a technician, under his authority, and after questioning the volunteers.

The cosmetic qualities:

Were assessed, at the end of the study, using a target questionnaire.

Type of study

This monocentric study was performed in open.
The subject was used as own control.
The number of volunteers whose data was exploitable at the end of the study was 22.

Specific inclusion criteria

The specific inclusion criteria were the following ones:
Sex: female.
Age: between 35-70 years old.
Phototype (Fitzpatrick): I - V, having wrinkles and little wrinkles on the eye contour, lip contour and forehead areas. Particularly on the crow's feet area the importance of the wrinkles must corresponds to a value of 2 or 3, evaluated following an ordinal scale in 4 points established by the investigator centre:

0 - Absence of wrinkles and/or little wrinkles
1 - Light wrinkle condition
2 - Moderate wrinkle condition on at least one crow's feet area
3 -Moderate wrinkle condition on both crow's feet areas.

Using regularly or occasionally facial care products.

Methodology

Experimental conditions of use of the test product

The experimental conditions, defined in the protocol, were the following ones:

Experimental areas: Face

Product directions for use: Application of the product as it is, twice a day (mornings and evenings) by the volunteer herself, after cleaning the face, with a gentle massage until complete penetration.

Applications at the Institute: On Day 28, 1 hour ± 30 min. before the performance of the replica by the technician at the Institute.

Applications at home/Frequency/duration: From Day 0 to Day 28. Application, twice a day for 28 consecutive days ± 2 days.

Assessment of the efficacy

Instrumental assessment of the anti-wrinkle effect

Principle: The skin relief was appreciated quantitatively, after taking of replicas and image analysis with computer before and after 28 days of treatment.
The anti-wrinkle effect of the test product was evaluated by comparison of the results obtained before and after 28 days of treatment.

Silicone replicas and equipment: The taking of replicas was performed according to the standard procedure defined by the Investigator Centre.
After cleansing of the skin, an adhesive ring was fixed on the defined skin site.
The silicone material (Silflo®) was applied in the middle of the ring. After a defined time, necessary for the silicone to harden, the silicone was removed.
A negative replica of the skin was obtained.
The capture of images (shadows process) was performed from the replica by a CDD camera connected to a computer, the replica being illuminated by a boring light source of 35° incidence.
The image analysis was performed with the Quantirides® software (Monadernl).

Frequency of measurements: The measurements were performed on D0/T0 and on D28, 1 hour ± 30 min. after the last application of the product by the technician at the institute.

Expression and interpretation of the results: The Quantirides® software allows to calculate the following parameters:
ST: Total surface of wrinkles, expressed in mm²
N: Total number of wrinkles
LT: Total length of wrinkles, expressed in mm
PM: Mean depth of wrinkles, expressed in µm

Individual results were expressed:
A- In absolute values for each experimental times D0/T0 and D28,
B- On D28 in percentages of variation in comparison with value at D0/T0 values.

The means and standard deviations were calculated for each parameter and for the two experimental times.
All the volunteers included in the study were taken into account to assess the efficacy on the test product as long as they were subjected to the examinations on 0O and D28.
The interpretation of the results was absolute, referring to:

A- The percentage of "positive" volunteers on D28:
1- Volunteers for whom two (at least) parameters of the three parameters N, L_T S_T decreased with a percentage of variation in comparison with D0/T0 superior or equal to 10% : effect on the little wrinkles (effect 1).
2- Volunteers for whom the parameter P_M decreased with a percentage of variation in comparison with the value at D0/T0 superior or equal to 10%: effect on the deep wrinkles (effect 2).

B- The mean percentage of improvement of the skin relief after treatment, on D28, for the "positive" volunteers and for each parameter related to the effect 1 or 2.

C- The results of the statistical analysis of the data comparison of the values obtained on the treated site before and after treatment with the Student's "t" test for paired series.

The percentage of "reactive" volunteers and the type of effect for these volunteers are reported on the table below.

	Difference D28 / (D0/T0) in %				
Volunteer References	S_T	N	L_T	P_M	Effect type
1	-30%	-26%	-8%	-3%	1
2	-49%	-71%	-13%	-23%	1+2
3	-50%	-44%	-20%	-39%	1+2
4	49%	26%	12%	6%	/
5	-69%	-26%	-23%	-21%	1+2
6	-22%	-52%	-18%	-14%	1+2
7	16%	8%	9%	10%	/
8	-5%	-9%	-14%	-4%	/
9	-51%	-38%	-35%	-64%	1+2
10	25%	14%	40%	12%	/
11	-40%	-55%	-20%	-10%	1+2
12	-49%	-44%	-50%	-58%	1+2
13	2%	19%	4%	-9%	/
14	-35%	-27%	-12%	-28%	1+2
15	-59%	-54%	-26%	-10%	1+2
16	25%	19%	18%	-4%	/
17	-50%	-11%	-48%	-33%	1+2
18	-31%	-15%	-20%	-29%	1+2
19	-47%	-46%	-10%	-16%	1+2
20	-48%	-43%	-33%	-6%	1
21	-39%	-6%	-58%	-36%	1+2
22	-30%	-60%	-40%	-16%	1+2
			% of "positive" volunteers (Effect 1 and/or 2)		73%
			% of "positive" volunteers: Effect 1		73%
			% of "positive" volunteers: Effect 2		64%

The percentage of improvement for each of the parameters and for the "positive" volunteers are reported on the table below:

Parameters	% of improvement
S_T	44%
N	41%
L_T	28%
P_M	21%

Statistical analysis of the results

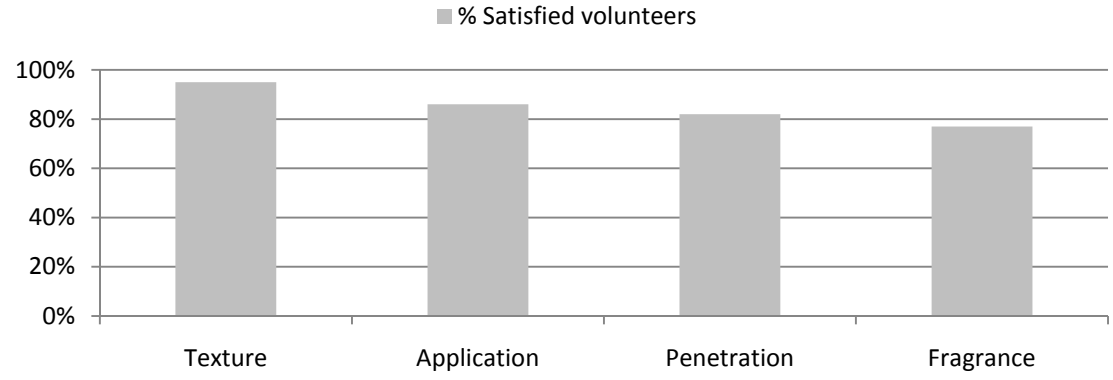
Student's "t" test For paired series	**Comparison between D28 and D0/T0 values**		
	t	P	Significance of the difference
S_T	3,99098051	0,00066387	S
N	3,73866542	0,00121151	S
L_T	2,56793791	0,01792928	S
P_M	3,93280384	0,00076281	S

The statistical analysis shows a significant decrease for all parameters.

Assessment of the cosmetic qualities and efficacy (Self-Assessment)

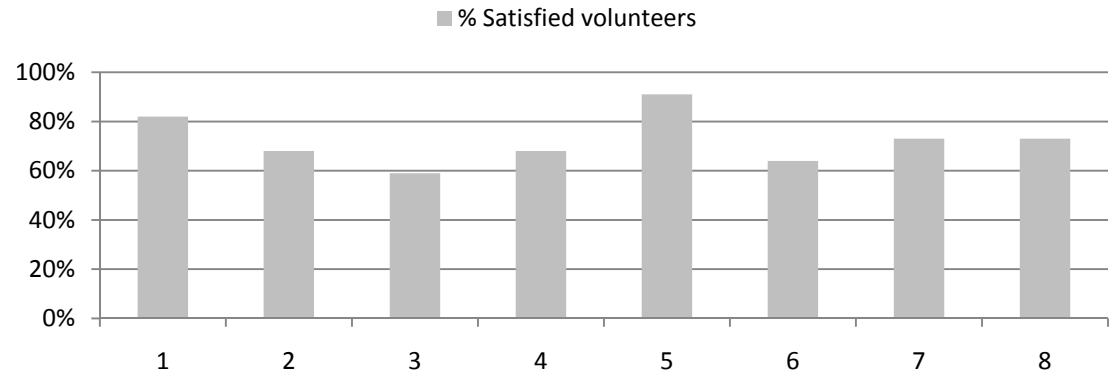
A- Cosmetic Qualities

For each item, the percentage of satisfied volunteers is reported in the graphic below:



B- Cosmetic Efficacy

For each item, the percentage of satisfied volunteers is reported on the graphics below:



Items
1. Reduction of the wrinkles
2. Younger appearance of the skin (anti-age effect)
3. Firmer skin
4. More moisturized skin
5. Nourishing effect
6. More supple and elastic skin
7. Smoother/softer skin
8. Radiant skin

Conclusion

Under the experimental conditions adopted and taking into account the evolution of the instrumental parameters defined, the scale established by the investigator centre and the subjective auto-evaluation, the product **TURNOVER** has:

1- An anti-wrinkles effect on a **73** % of the panel, working mainly on little wrinkles

2- **A very good acceptability.**

In other respects, it is very well appreciated for its cosmetic qualities and more especially for its pleasant texture and neutral fragrance and for its easy application and penetration.
The subjective evaluation of its efficacy shows that **the volunteers mainly appreciate that the product leaves the skin with a younger appearance (anti-aging effect), nourished, firmer, more supple and elastic, softer and smoother.**

Bibliography

The method chosen for assessment of the **anti-wrinkle effect** used the replica technique. Numerous publications supported this methodology, notably:

Lévêque J, EEMCG guidance for the assessment of skin topography, Jour. Gfthe European Academy of Dermatology and Venereology, 1999, 12, *pp. 103-114*

Corcuff P, Chatenay F, Brun A., Evaluation of anti-wrinkle effects on humans, Int.J Cosmet. Sei., 1985, 7,pp.117-126

Corcuff P, De Rigal J, Makki S., et al, Skin relief and aging, J Soc. Cosmet. Chem., 1983, 34, *pp. 177190*

The acceptability of the product was controlled by the doctor investigator (Dermatologist) who has an appropriate experience or by a qualified and experienced technician under his authority.
Numerous publications support this methodology, notably:

Jackson E.M. & Robillard N.F, The controlled use test in a cosmetic product safety substantation program, JToxicol.Cut. Gcular. Toxicol., 1982,20, pp. 117-132

Frances Pascher M.D., Adverse reactions to eye area cosmetics and their management, J Soc. Cosmet.
Chem., 1982, 33, *pp. 249-258*

Keswick BR., Ertel K.D., Visscher M.o., Comparison of exaggerated and normal use techniques for assessing the mildness of personal cleansers,
J Soc. Cosm. Chem., 1992, 43, *pp.* 187-193