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Examination to Determine Skin Irritation in Human Subjects using a Modified Duhring Chamber Test

Test product.....: Zubora 30 Spezial
SN 9024

Concentration .....: 10% (in water)

Study commissioned by..: Zeller+Gmelin GmbH & Co. KG
Schlossstraße20
73054 Eislingen / Fils
Germany

Test period .....: October 2014

Supervisors of Study .....: Dr. med. H. Prieur, Dermatologist – Allergist
B. Nissen, Manager Derma Consult Concept

The test was conducted on 10 subjects (5 females and 5 males) aged 28 to 51 having healthy skin. From seven days prior to the start of the test and until completion of the test, the subjects refrained from using any topical preparations (leave-on and rinse-off) on the designated general test area (inner side of forearms). For cleansing, only water was allowed.

Test model

The Duhring Chamber Test to study skin tolerance is not a user test. The test model was developed to determine the irritative potential of various skin products and cosmetics.

Implementation of Test

The Duhring Chamber Test was carried out in accord with the method as outlined by P.J. Frosch and A.M. Kligman (1, 2).

The product (100 µl) was applied in a concentration of 10% in water to the marked areas on the volar side of the forearm with the aid of aluminium chambers (Finn Chambers, 12 mm diameter) containing corresponding-size filter papers. The schedule of application was as follows:

1st day ..... : 18 h application - 6 h pause

2nd, 3rd, 4th, 5th days. : 6 h application - 18 h pause

6th and 7th days ..... : pause

8th day..... : evaluation

In the case of premature termination of the test due to strong irritation, the evaluation was undertaken 24 h after the last application.
The non-skin-irritating substance water and the irritating substance sodium dodecylsulfate (SDS, 0.2%) were selected as controls.

Evaluation methods

1. The following scale was used for visual evaluation of the skin irritation:

Erythema	0	negative
	1+	very slight, point-shaped or diffuse erythema
	2+	easily visible, sharply defined erythema, moderate, uniformly shaped
	3+	medium erythema, growing in intensity
	4+	strong, fire-red erythema with edema or epidermal defect (tiny blisters, necrosis)
Scaling	0	negative
	1+	dryness
	2+	fine scaling
	3+	moderate scaling
	4+	strong scaling with large scales
Fissures	0	negative
	1+	very superficial epidermal separation, fine tears
	2+	single or several extensive fissures
	3+	deep fissures with bleeding or exudation, blistering

Upon premature termination of the applications during the study due to strong irritation, the highest rating (4-4-3) is assigned in keeping with the rules.

2. *Measurement of the degree of redness with the aid of chromametry*

Skin color is measured by Minolta Chromameter CR 400 (Minolta, Japan) using the Commission International de l´eclairage (CIE) L*a*b* color space - a standardized, device independent system to express Colors adjusted to the non-linear Color sensitivity of human eye. In the L*a*b* color space, a color is expressed in a three-dimensional coordinate system with green-red (a* - negative values are green, positive values are red), blue-yellow (b* - negative values are blue, positive values are yellow) and L* axes (lightness). During measurement, the skin surface is illuminated by a Xenon flashlight and remitted light registered and analysed by a photoreceiver. The Chromameter CR 400 is sensitive and accurate for the characterisation of skin color and measures a spot of 8mm diameter. In reddened skin an increase in the a* value can be observed.
Before each measuring series, the instrument was calibrated against a standard white tile. Each value is the average of three recordings. Chromameter used for this study: S/N C8202118.

3. *Measurement of the transepidermal water loss as a marker for damage to protective surface*

Measurements of TEWL were performed with the Tewameter TM 210 (Courage & Khazaka, Cologne, Germany). The Tewameter is a device for measurement of water evaporation on skin surfaces based on the diffusion principle discovered by A. Fick in 1885. The TEWL is calculated automatically and expressed digitally in g/m^2h. Measurements were carried out in accordance with the guidelines of the standardisation group of the European Contact Dermatitis Society (Pinnagoda et al. Contact Dermatitis 1990: **22**; 164-178). Each value is the average of three recordings. Tewameter used in this study: SN 27 022 074.

Assessment

The test substances are assessed, in comparison with the control, as

- not irritating
- slightly irritating
- moderately irritating
- strongly irritating.

Results

The results of the evaluation and the measurements are summarised in the following tables (mean values reported):

1. Visual Evaluation (mean scores)

	Erythema	Scaling	Fissures
Control 1: water	0,0	0,0	0,0
Control 2: SDS (0,2 %)	1,7	0,8	0,1
Zubora 30 Spezial (10%)	0,5	0,1	0,0

2. TEWL Values

	Start of Test	End of Test	Δ TEWL
Control 1: water	7,1	7,6	0,5
Control 2: SDS (0,2 %)	7,0	13,1	6,0
Zubora 30 Spezial (10%)	7,3	8,8	1,5

3. Chromametry Values (Redness a)*

	Start of Test	End of Test	Δ a*
Control 1: water	7,14	7,34	0,20
Control 2: SDS (0,2 %)	7,15	10,53	3,38
Zubora 30 Spezial (10%)	7,18	7,80	0,63

Evaluation

The positive control (SDS) produced the expected irritative reactions, no relevant reactions were observed on the negative control (water).

The results of the controls show that the test procedure is suitable for determining primary irritation effects of the tested product.

On the basis of the measurements taken in relation to the controls, the test-product

Zubora 30 Spezial (10%)
SN 9024

may be designated as "non-irritating".

Literature

1. P.J. Frosch, A.M. Kligman: The Duhring Chamber, Contact Dermatitis 5, 73-81 (1979)

2. P.J. Frosch, A.M. Kligman: The Soap Chamber Test, J. Am. Acad. Dermatol. 1, 35-41 (1979)

Signature:

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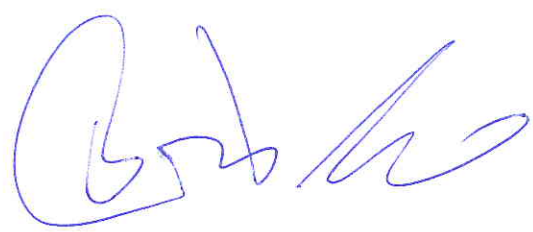

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