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

Test product .....: **ZUBORA TKS**
SN 9000

Concentration .....: 4%

Study commissioned by..: **Zeller+Gmelin GmbH & Co. KG**
Postfach 1365
73050 Eislingen/Fils
Germany

Test period .....: February 2007

The test was conducted on 10 female and male subjects aged 19 to 63 having healthy skin. For the duration of the test the subjects refrained from using substances and creams with active cleansing ingredients on the test areas.

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 4% in water to the marked areas of 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

By means of chromametry, brightness, reddening, and blanching effects on the skin can be assessed in a standardised manner. A color shade is defined three dimensionally by brightness, hue, and saturation. Chromametry permits the synchronous determination of these three parameters. The chromameter's measurement attachment contains a high-power xenon flash-lamp that guarantees uniform and constant illumination of the measurement area. A dual-beam procedure ensures reproducibility of standard illumination from measurement to measurement. For the colour analysis, only the vertically reflected light is conducted to six highly sensitive silicon photodiodes. The illumination system, comprised of xenon flash lamps and silicon photodiodes, is adapted by filter to the CIE's (Commission Internationale de l'Eclairage) internationally standardised spectral-response curve and detects the smallest aberrations in the spectral composition of the reflected xenon light. Chromameter CR 300 uses several measurement systems. With an eye to the task at hand, the measurements were taken exclusively in the L*a*b* colorimetry system (also designated as CIELAB system). L* represents the brightness, while a* and b* the hue and colour saturation. a* shows the position on the red-green axis and b* on the yellow-blue axis. An increase in the reddening is shown by an increase in the a* value. The values are internationally defined absolute values. Measurements were done according to the guidelines of the European Society of Contact Dermatitis (Fullerton et al., Guidelines for measurement of skin colour and erythema. Contact Dermatitis 1996: **35**; 1-10).

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

The Tewameter TM 210 (manufactured by Courage + Khazaka, Cologne) measures water evaporation on the skin surface. The basis is Fick's Second Diffusion Law (A. Fick 1985). The Law is valid only within a homogeneous diffusion zone. This is approximated by using a hollow cylinder open at both ends. The water evaporating from the skin surface escapes through the cylinder. The resulting density gradient is indirectly measured by two pairs of sensors (temperature and relative moisture) positioned in the cylinder and evaluated by microprocessor. Measurements were done according to the guidelines of the European Society of Contact Dermatitis (J. Pinnagoda et al. Guidelines for transepidermal water loss measurement. Contact Dermatitis 1990: **22**: 164-178).

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 charts:

1. Visual Evaluation

	Erythema	Scaling	Fissures
Control 1: water	0,0	0,0	0,0
Control 2: SDS (0,2 %)	2,0	0,0	0,0
ZUBORA TKS (4%)	0,0	0,0	0,0

2. TEWL Values

	Start of Test	End of Test	Δ TEWL
Control 1: water	5,3	5,7	0,4
Control 2: SDS (0,2 %)	5,6	11,3	5,7
ZUBORA TKS (4%)	5,6	6,5	0,8

3. Chromametry Values (Redness a)*

	Start of Test	End of Test	Δ a*
Control 1: water	7,96	8,22	0,26
Control 2: SDS (0,2 %)	7,80	10,62	2,82
ZUBORA TKS (4%)	7,64	8,29	0,66

Evaluation

The positive control (SDS) produced the expected irritative reactions.

The results of the positive control showed that the test procedure is suitable for determining primary irritation effects of the tested product.

On the basis of the measurements taken, the test-product

ZUBORA TKS (4%)
SN 9000

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)

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