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REPORT

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Test of Titernal W for Primary Skin Irritation in Rabbits

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I, the undersigned, hereby declare that the work described in this report was performed under my supervision, as Study Director, in accordance with OECD Guidelines for the Testing of Chemicals 404 (1992), and that the report provides a true and accurate record of the results obtained.

This is a translation of the original report, No. 203101041-004, written in Japanese.

Study Director

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1. Abstract

The test sample, Titernal W, was tested for primary skin irritation in rabbits in accordance with OECD Guidelines for the Testing of Chemicals 404 (1992).

The test sample was applied occlusively for 4 hours to the intact and abraded skin of three rabbits. As a result, no irritation response was observed in any rabbit at any of the observation time, 24, 48 or 72 hours after the application.

The primary irritation index (P.I.I.) calculated according to Federal Register (1972) was 0. Consequently, the category of the primary irritation index of the test sample was regarded as "Negligible".

2. Test sample

Titernal W

Character: Opalescent liquid with whitishsediment

3. Test period

From November 4 to 27, 2003

4. Experimental animals

Three male Japanese white rabbits, purchased from Kitayama Labes Co., Ltd., were used. They were acclimated to the laboratory conditions for more than one week to confirm no abnormalities in general condition. They were individually housed in FRP cages under the standard laboratory conditions (temperature: 22 ℃ ±2 ℃, light-dark cycle: 12/12 hours) and given tap water *ad libitum* and a certain amount of LRC4 diet [Oriental Yeast Co., Ltd.] in proportion to each animal's body weight.

5. Procedures

The back of each animal was clipped and freed of its hair about 24 hours before the test and divided into four sections (approximately 6 cm^2). Two of the test sections were abraded and the other two left intact. Abrasion was made by epidermal incision through the stratum corneum; two incisions perpendicular to the other two were made in a manner resembling a "#" pattern by use of a sterile 18-gage needle.

A 0.5-mL portion of the test solution was applied to an approximately 2 cm×3 cm gauze patch, which was held in one intact and one abraded sections on each animal with adhesive plaster (JP). To ensure the contact of the test solution with skin, the patches were covered with Blenderm surgical tape [3M Health Care Ltd.]. The other intact and abraded sections served as control.

The exposure duration was 4 hours. After the exposure, the patches were removed. All test sections were wiped with absorbent cotton wet with pure water. Readings were made, as Table 1 shows, in 1, 24, 48 and 72 hours after the removal of the patches.

According to Federal Register (1972), the primary irritation index (P.I.I.) was obtained as follows: For each animal, the scores of the readings in 1, 24 and 48 hours were added together, and the summation was divided by 6. The mean value for the three animals was calculated to obtain the P.I.I. The degree of the primary skin irritation was determined by means of P.I.I. on the criteria of ISO 10993-10 shown in Table 2.

The animals were weighed at the start and completion of the test.

6. Results (Tables 3 and 4)

No irritation response was observed in any rabbit throughout the observation period.

The P.I.I., calculated by the scores of the readings, was 0.

7. Evaluation

The test sample was tested for primary skin irritation in rabbits in accordance with OECD Guidelines for the Testing of Chemicals 404 (1992). As a result, no irritation response was observed in any rabbit throughout the observation period.

The primary irritation index (P.I.I.) calculated according to Federal Register (1972) was 0. Consequently, the category of the primary irritation index of the test sample was regarded as "Negligible".

8. References

- Federal Register (§ 191, December, 1972).
- ISO 10993-10 Biological evaluation of medical devices-Part 10: Tests for irritation and delayed-type hypersensitivity 6.3 Animal skin irritation test (2002).

Table 1. Evaluation of skin reactions

Erythema and eschar formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4*

[Total possible erythema score 4]

* Bleeding, ulcer and necrosis were classified in score 4 for injuries in depth.

Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

[Total possible edema score 4]

Table 2. Irritation response categories in rabbit

Response category	P.I.I.
Negligible	0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

Table 3. Body-weight changes (kg)

Rabbit No.	Before treatment	Completion of the test
1	3.08	3.20
2	3.28	3.32
3	2.98	3.02

Table 4. Scores of irritation response

Observation time (hour)	Rabbit No. 1		Rabbit No. 2		Rabbit No. 3	
	Intact	Abraded	Intact	Abraded	Intact	Abraded
1	0/0	0/0	0/0	0/0	0/0	0/0
24	0/0	0/0	0/0	0/0	0/0	0/0
48	0/0	0/0	0/0	0/0	0/0	0/0
72	0/0	0/0	0/0	0/0	0/0	0/0

Values are expressed as scores of Erythema·Eschar/Edema.