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REPORT

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Test of Titernal W for Acute Oral Toxicity in Mice

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I, the undersigned, hereby declare that the work described in this report was performed under my supervision, as Study Director, and that the report provides a true and accurate record of the results obtained.

This is a translation of the original report, No. 203101041-003, written in Japanese.

Study Director

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Test of Titernal W for Acute Oral Toxicity in Mice

1. Abstract

The test sample, Titernal W, was tested for acute oral toxicity in mice.

The test sample was administered orally to male and female mice at a dose of 2,000 mg/kg b.w., and the control animals were given water for injection as vehicle control. The experimental period was 14 days. As a result, the test sample caused neither abnormalities nor death in any mice during the observation period.

Consequently, we concluded that the LD50 value of Titernal W was higher than 2,000 mg/kg b.w. in both male and female mice.

2. Test sample

Titernal W

Character: Opalescent liquid with whitishsediment

3. Test period

From October 21 to November 26, 2003

4. Preparation of test solution

The test sample was diluted with water for injection to make 100 mg/mL test solution.

5. Experimental animals

Male and female mice of the ICR strain, purchased from Japan SLC, Inc., were used. The mice were obtained at an age of five weeks and acclimated to the laboratory conditions for a week. They were housed in plastic cages (five animals per cage) under the standard laboratory conditions (temperature: 23 ℃ ± 2 ℃, light-dark cycle: 12/12 hours) and given Labo MR Stock diet [Nihon Nosan Kogyo K.K.] and tap water *ad libitum*.

6. Procedures

Five each of male and female mice were allocated to each group. The mice were not fed for about four hours prior to the administration, and then each was weighed. To the mice of the experimental group, the test sample was administered orally with a stomach tube at a dose of 2,000 mg/kg b.w. of the test sample (a dose of 20 mL/kg b.w. as the test solution). To the control group, water for injection alone was administered at a volume of 20 mL/kg b.w. in the same manner as described above.

Clinical observations were made frequently on the day of administration and once a day during the following period. The mice were each weighed weekly and the mean body weight values of the experimental and the control groups were statistically analyzed by t-test ($p=0.05$). At the completion of the test period (14 days), all mice were sacrificed for necropsy.

7. Results

1) Deaths of animals

No mice of either males or females died throughout the experimental period.

2) Clinical observations

No abnormalities were observed in either males or females throughout the experimental period.

3) Body weight changes (Tables 1 and 2)

No significant difference was detected in the body weight gain between the experimental and the control groups in either males or females.

4) Necropsy

No remarkable changes were found in any organ of either males or females.

8. Discussion

The acute oral toxicity of Titarnal W was tested in mice.

Oral administration of 2,000 mg/kg b.w. of the test sample was found to have caused neither death nor abnormalities in any mice.

Consequently, we concluded that the LD50 value of the test sample was higher than 2,000 mg/kg in both male and female mice.

9. References

· OECD Guidelines for the Testing of Chemicals 420 (2001)

Table 1. Body weight changes (Male)

Group	Body weight (g)		
	Pre-administration	7 days	14 days
Experimental group	33.2±0.8 (5)	38.4±2.0(5)	40.7±2.4 (5)
Control group	33.0±0.8 (5)	37.3±1.3 (5)	39.2±1.3 (5)

Values are mean±SD.
Values in parentheses show the number of animals.

Table 2. Body weight changes (Female)

Group	Body weight (g)		
	Pre-administration	7 days	14 days
Experimental group	26.3±0.8 (5)	28.5±1.1 (5)	31.4±1.0 (5)
Control group	26.1±0.7 (5)	28.8±1.3 (5)	31.1±1.4 (5)

Values are mean±SD.
Values in parentheses show the number of animals.