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REPORT

Client: Japan Spatial Hygiene Association
6-27-28, Shinjuku, Shinjuku-ku, Tokyo, 160-0022, Japan

Test sample(s): G-MIST (200-GM)

Title: Eye Irritation Test in Rabbits

Received date of test sample(s): February 18, 2020

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Signed for and on behalf of JFRL

JAPAN FOOD RESEARCH LABORATORIES

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Eye Irritation Test in Rabbits

Abstract

The test sample, G-MIST (200-GM), was tested for eye irritation potential in rabbits in accordance with OECD Guideline for the Testing of Chemicals 405 (2017). A 0.1-mL portion of the test sample was applied to one eye of three rabbits. As a result, redness of bulbar conjunctivae was seen in two animals at 1 hour after instillation. This irritation response had disappeared at the 24-hour observation. The maximum mean of the total scores calculated by Draize method was 1.3 (after 1 hour of instillation). Consequently, the test sample is considered to be a non-irritant to rabbit eyes.

1. Client

Japan Spatial Hygiene Association

2. Test sample

G-MIST (200-GM)

3. Test facility

Tama Laboratory, Japan Food Research Laboratories
6-11-10 Nagayama, Tama-shi, Tokyo 206-0025, Japan

4. Test period

From February 18, 2020 to April 06, 2020

5. Purpose

The eye irritation potential of the test sample is evaluated in rabbits according to OECD Guideline for the Testing of Chemicals 405 (2017).

6. Experimental animals

Three male Japanese white rabbits were purchased from Kitayama Labes Co., Ltd. They were acclimated to laboratory conditions for more than 1 week to confirm that there were no abnormalities in general condition. They were then individually housed in FRP cages under standard laboratory conditions (Temperature: 23 ˚C ± 3 ˚C, Light-dark cycle: 12/12 hours). Tap water was provided *ad libitum,* and a certain amount of LRC4 diet (Oriental Yeast Co., Ltd.) was provided in proportion to each animal's body weight.

7. Procedures

Both eyes of each animal were examined on the start date of the test to ensure the absence of ocular abnormalities.

Before 5 minutes of instillation, 1 to 2 drops of 0.4 % oxybuprocaine hydrochloride were instilled in both eyes for local anesthesia. Next, 0.1 mL of the test sample was instilled into the conjunctival sac of one eye of each animal. The lids were then gently held together for about 1 second. The other eye, which was not treated with the test sample, was used as a control.

The cornea, iris, and conjunctivae were observed clinically using a hand slit-lamp (× 10, Ohira Co., Ltd.) at 1, 24, 48 and 72 hours after the instillation. The ocular reactions were observed and scored according to the criteria of Draize method shown in Table 1. The corneal epithelium was examined further in detail with the use of fluorescein sodium at each observation time except for 1 hour after the instillation.

The total score was calculated for each animal according to the formula shown in Table 2, and the mean of the total scores of the three animals was obtained at each observation time. The eye irritation potential of the test sample was graded by means of the maximum mean total score on the basis of Table 3.

The animals were weighed before treatment and on the last observation date.

8. Results (Tables 4 to 8)

1) Rabbit No. 1

Neither the test eye nor the control eye showed abnormalities throughout the observation period.

No fluorescein staining was detected in either the test eye or the control eye throughout the observation period.

2) Rabbit No. 2

In the test eye, redness of bulbar conjunctivae (score 1) was seen at 1 hour after instillation. At the 24-hour observation, the reaction had disappeared. No irritant response was observed during the subsequent period.

On the other hand, the control eye showed no abnormalities throughout the observation period.

No fluorescein staining was detected in either the test eye or the control eye throughout the observation period.

3) Rabbit No. 3

In the test eye, redness of bulbar conjunctivae (score 1) was seen at 1 hour after instillation. At the 24-hour observation, the reaction had disappeared. No irritant response was observed during the subsequent period.

On the other hand, the control eye showed no abnormalities throughout the observation period.

No fluorescein staining was detected in either the test eye or the control eye throughout the observation period.

The maximum mean total score was 1.3 (at 1 hour after instillation) in the test eyes and 0 in the control eyes.

9. Conclusion

The test sample was tested for eye irritation potential in rabbits in accordance with OECD Guideline for the Testing of Chemicals 405 (2017).

As a result, redness of bulbar conjunctivae was seen in two animals at 1 hour after instillation. This irritation response had disappeared at the 24-hour observation.

The maximum mean of the total scores calculated by Draize method was 1.3 (after 1 hour of instillation).

Consequently, the test sample is considered to be a non-irritant to rabbit eyes.

10. References

- "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959). The Association of Food and Drug Officials of the United States.
- Shirasu, Y. and Hayama, T. (1985). "New Toxicity Test – Method and Evaluation," 337-339, LIC.

Table 1-1. Grades for ocular lesions

Cornea

(A) Opacity-degree of density (area most dense taken for reading)

No opacity ····· 0
Scattered or diffuse area,
details of iris clearly visible ····· 1
Easily discernible translucent areas,
details of iris slightly obscured ····· 2
Opalescent areas, no details of iris visible,
size of pupil barely discernible ····· 3
Opaque, iris invisible ····· 4

(B) Area of cornea involved

One quarter (or less) but not zero ····· 1
Greater than one quarter, but less than half ····· 2
Greater than half, but less than three quarters ····· 3
Greater than three quarters, up to whole area ····· 4

[Score = A × B × 5 Maximum possible ····· 80]

Iris

(A) Normal ····· 0
Folds above normal, congestion, swelling,
circumcorneal injection (any or all of these
or combination of any thereof) iris still reacting
to light (sluggish reaction is positive) ····· 1
No reaction to light, hemorrhage, gross destruction
(any or all of these) ····· 2

[Score = A × 5 Maximum possible ····· 10]

Table 1-2. Grades for ocular lesions (continued)

Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

- Vessels normal ····· 0
- Vessels definitely injected above normal ····· 1
- More diffuse, deeper crimson red, individual vessels not easily discernible ····· 2
- Diffuse beefy red ····· 3

(B) Chemosis

- No swelling ····· 0
- Any swelling above normal (includes nictitating membrane) ····· 1
- Obvious swelling with partial eversion of lids ····· 2
- Swelling with lids about half closed ····· 3
- Swelling with lids about half closed to completely closed ····· 4

(C) Discharge

- No discharge ····· 0
- Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) ····· 1
- Discharge with moistening of the lids and hairs just adjacent to lids ····· 2
- Discharge with moistening of the lids and hairs, and considerable area around the eye ····· 3

[Score = (A + B + C) × 2 Maximum possible ····· 20]

Table 2. Calculation method for total score

Site	Formula	Maximum possible
(1) Cornea	A × B × 5	80
(2) Iris	A × 5	10
(3) Conjunctivae	(A + B + C) × 2	20
(1) + (2) + (3) = Total score*		110

A, B, and C show the scores of (A), (B), and (C) given in Table 1, respectively.
* Calculate at each observation time.

Table 3. Evaluation of eye irritation

Maximum mean total score	Category
0 to 5.0	Non-irritant
5.1 to 15.0	Mild irritant
15.1 to 30.0	Irritant
30.1 to 60.0	Moderate irritant
60.1 to 80.0	Moderate to severe irritant
80.1 to 110.0	Severe irritant

Table 4. Body-weight changes

Rabbit No.	Before treatment	Last observation date
1	3.69	3.63
2	3.25	3.18
3	3.54	3.50

Units: kg

Table 5. Means of total scores

Rabbit No.	Total score			
	1 hour	24 hours	48 hours	72 hours
1	0 (0)	0 (0)	0 (0)	0 (0)
2	2 (0)	0 (0)	0 (0)	0 (0)
3	2 (0)	0 (0)	0 (0)	0 (0)
Mean of the total scores	1.3 (0)	0 (0)	0 (0)	0 (0)

The values in parentheses show the scores of the control eyes.

Table 6. Eye irritation scores: Rabbit No. 1

Observation site		Score			
		1 hour	24 hours	48 hours	72 hours
(1) Cornea	Opacity-degree of density (A)	0 (0)	0 (0)	0 (0)	0 (0)
	Area of cornea involved (B)	— (—)	— (—)	— (—)	— (—)
(2) Iris	(A)	0 (0)	0 (0)	0 (0)	0 (0)
(3) Conjunctivae	Redness (A)	0 (0)	0 (0)	0 (0)	0 (0)
	Chemosis (B)	0 (0)	0 (0)	0 (0)	0 (0)
	Discharge (C)	0 (0)	0 (0)	0 (0)	0 (0)
Score (1) = A × B × 5		0 (0)	0 (0)	0 (0)	0 (0)
Score (2) = A × 5		0 (0)	0 (0)	0 (0)	0 (0)
Score (3) = (A + B + C) × 2		0 (0)	0 (0)	0 (0)	0 (0)
Total score [(1) + (2) + (3)]		0 (0)	0 (0)	0 (0)	0 (0)

The values in parentheses show the scores of the control eye.
—: Not recorded.

Table 7. Eye irritation scores: Rabbit No. 2

Observation site		Score			
		1 hour	24 hours	48 hours	72 hours
(1) Cornea	Opacity-degree of density (A)	0 (0)	0 (0)	0 (0)	0 (0)
	Area of cornea involved (B)	— (—)	— (—)	— (—)	— (—)
(2) Iris	(A)	0 (0)	0 (0)	0 (0)	0 (0)
(3) Conjunctivae	Redness (A)	1 (0)	0 (0)	0 (0)	0 (0)
	Chemosis (B)	0 (0)	0 (0)	0 (0)	0 (0)
	Discharge (C)	0 (0)	0 (0)	0 (0)	0 (0)
Score (1) = A × B × 5		0 (0)	0 (0)	0 (0)	0 (0)
Score (2) = A × 5		0 (0)	0 (0)	0 (0)	0 (0)
Score (3) = (A + B + C) × 2		2 (0)	0 (0)	0 (0)	0 (0)
Total score [(1) + (2) + (3)]		2 (0)	0 (0)	0 (0)	0 (0)

The values in parentheses show the scores of the control eye.
—: Not recorded.

Table 8. Eye irritation scores: Rabbit No. 3

Observation site		Score			
		1 hour	24 hours	48 hours	72 hours
(1) Cornea	Opacity-degree of density (A)	0 (0)	0 (0)	0 (0)	0 (0)
	Area of cornea involved (B)	— (—)	— (—)	— (—)	— (—)
(2) Iris	(A)	0 (0)	0 (0)	0 (0)	0 (0)
(3) Conjunctivae	Redness (A)	1 (0)	0 (0)	0 (0)	0 (0)
	Chemosis (B)	0 (0)	0 (0)	0 (0)	0 (0)
	Discharge (C)	0 (0)	0 (0)	0 (0)	0 (0)
Score (1) = A × B × 5		0 (0)	0 (0)	0 (0)	0 (0)
Score (2) = A × 5		0 (0)	0 (0)	0 (0)	0 (0)
Score (3) = (A + B + C) × 2		2 (0)	0 (0)	0 (0)	0 (0)
Total score [(1) + (2) + (3)]		2 (0)	0 (0)	0 (0)	0 (0)

The values in parentheses show the scores of the control eye.
—: Not recorded.

End of Report