National Institute of Public Health

L 1206

Centre of Laboratories
in Public Health Protection and Promotion
Laboratories of Toxicology

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Laboratory No. 1206, accredited by CAI

Test Report No. 3/13/8

Sponsor:
Address: **Reference No.:** CTZB 187-46/2013

Test Material

Identification:

TM 3/13/8: AntiVirus

Laboratory Tests

SOP 2/3 Tests for irritation and delayed-type hypersensitivity (EN ISO 10993–10. Part 10, Articles 1, 2, 3, 4, 5, 6.2, 6.3, 6.4, 6.5, 7.5, Annex A, B.1, B.2, C, E, F)

Expertise reception date: 18.1.2013
Final report date: 6.3.2013
Number of pages: 8

Test report completed by: Hana Bendová, M.Sc., Ph.D.

Technical manager: Dagmar Jírová, M.D., Ph.D.

TEST REPORT
EVALUATION OF HUMAN SKIN IRRITATION

Testing facility: Laboratories of Toxicology (Centre of Laboratories in Public Health Protection and Promotion, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic).

Date of study: 26.2. - 1.3.2013
Test carried out by: Hana Bendová, M.Sc., Ph.D.
Study supervisor: Dagmar Jirová, M.D., Ph.D.

The test was carried out in compliance with: SOP 2/3 Test for irritation and delayed-type hypersensitivity (ČSN EN ISO 10993-10: Part 10: articles 1, 2, 3, 4, 5, 6.2, 6.3, 6.4, 6.5, 7.5 Annex A, B.1, B.2, C, E, F)

Aim of the study: Assessment of the potential of the test material to produce dermal irritation.

MATERIALS AND METHODS

TEST MATERIAL (TM):

TM 3/13/8: AntiVirus

Sponsor:

PREPARATION OF MATERIALS FOR TESTING

- **Tested material**

  TM 3/13/8: applied directly on skin (2.5 x 2.5 cm), moistened with distilled water (0.2 ml).

CONTROLS

- **Positive control**
  Sodium dodecyl sulfate (SDS) - 20% aqueous solution - applied directly on skin (0.4 ml).
- **Negative Control**
  Distilled water - applied directly on skin (0.4 ml).

PARTICIPANTS IN THE STUDY

The selection of volunteers and the test methods complied with the Declaration of Helsinki (1964) and the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS, 2002). The study was approved by the Ethical Review Committee of the National Institute of Public Health.

The volunteers were selected on the basis of inclusion and non-inclusion criteria and for this purpose filled in a special form. The volunteers were clearly informed regarding the nature of the study, timetable, constraints and possible risks. They gave their written informed consent before participation in the study was permitted. All the documentation is strictly confidential. 30 volunteers took part in the study.

Table 1 – Demographic data

Subject Number	Subject Initials	Age	Gender
1	BO	20	M
2	ŠJ	18	F
3	JD	30	M
4	PH	32	F
5	UR	49	Ř
6	JM	41	F
7	JM	18	M
8	JM	38	M
9	TA	51	M
10	SM	62	F
11	BH	49	F
12	KK	47	F
13	ML	59	F
14	DH	57	F
15	KZ	58	F
16	SD	39	M
17	SM	38	M
18	ŠV	67	M
19	RL	63	M
20	DJ	58	M
21	BT	46	F

22	DL	62	F
23	PD	52	F
24	OD	55	F
25	ŠO	60	F
26	KD	43	F
27	HK	29	F
28	VL	51	F
29	KL	54	F
30	PJ	27	F

Test procedure

- **Application of the test material**

The test material TM 3/13/8 was applied in occlusion on the upper outer arm.

Occlusive: Hill Top Chambers (containing a gauze pad, diameter 1.8 cm), HILL TOP BIOLABS, USA

- **Application of the positive control**

The positive control - 20% SDS (0.4 ml) was applied on the upper outer arm.

Occlusive: Hill Top Chambers (containing a gauze pad, diameter 1.8 cm), HILL TOP BIOLABS, USA

- **Application of the negative control**

The negative control - distilled water (0.4 ml) was applied on the upper outer arm.
Occlusive: Hill Top Chambers (containing a gauze pad, diameter 1.8 cm), HILL TOP BIOLABS, USA

- **Duration of exposure**

The patches were applied progressively starting with duration of 15 min and 30 min, and up to 1h, 2h, 3 h and 4h. The test substance was removed by rinsing and gentle swabbing.

- **Clinical observation and grading of skin reactions**

The reactions were assessed in the interval 0 h (immediately after patch removal), subsequently 1 - 2 h, 24 h, 48 h and 72 h after patch removal. Skin reactions were graded and recorded according to the grading given in Tab. 2.

Table 2 – Human skin irritation test, grading scale

Description of response	Grading
No reaction	0
Weakly positive reaction (usually characterized by mild erythema and/or dryness across most of the treatment site)	1
Moderately positive reaction (usually distinct erythema or dryness, possibly spreading beyond of the treatment site)	2
Strongly positive reaction (strong and often spreading erythema with oedema and/or eschar formation)	3

- **Data evaluation / interpretation**

The number of volunteers who developed a positive reaction after test material application (Tab. 3) and after positive control application (Tab. 4) was used for skin irritation evaluation.

The skin irritation potential hazard was determined by comparison of number of volunteers that produced skin reaction after test material application and number of volunteers that produced skin reaction after positive control application.

If the material produces a frequency of skin irritation in the test subjects which is substantially and significantly less than the positive control, it is not regarded as a significant skin irritant.

If the material produces a frequency of skin irritation in the test subjects which is similar to, or grater than, the positive control, it is regarded as a significant skin irritant.

Fisher´s exact test was used for the statistical treatment of the results.

RESULTS

The skin reactions are recorded in the Annex I.

ASSESSMENT OF RESULTS

Skin reactions after application of the test material TM 3/13/8 were recorded for 0 of 30 volunteers. Skin reactions after application of the positive control were recorded for 24 of 30 volunteers. The Fisher´s exact test confirmed substantially and significantly lower frequency of the skin irritation in case of the test material application than in case of the positive control.

The test material TM 3/13/8 is not regarded as a significant skin irritant.

Date of report: 6.3.2013

Principal investigator: Hana Bendová, M.Sc., Ph.D.

Study supervisor: Dagmar Jírová, M.D., Ph.D.

Annex I

Table 3 - TM 3/13/8 - observation and grading of skin reactions

Volunteer No.	Time interval / Grading				
	0h grading	1 - 2h grading	24h grading	48h grading	72h grading
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0
28	0	0	0	0	0
29	0	0	0	0	0
30	0	0	0	0	0

Table 4 - Positive control - observation and grading of skin reactions

Volunteer No.	Time interval / Grading				
	0h grading	1 - 2h grading	24h grading	48h grading	72h grading
1	1	1	2	3	3
2	0	0	0	0	0
3	1	2	2	1	1
4	2	2	3	3	3
5	1	1	2	2	2
6	1	1	1	1	1
7	0	0	0	0	0
8	1	1	2	2	2
9	1	2	3	3	3
10	1	2	2	2	1
11	1	2	3	3	3
12	0	0	1	1	1
13	2	3	3	3	3
14	1	3	2	1	1
15	2	3	2	1	1
16	2	3	3	3	3
17	1	1	1	1	0
18	1	1	2	1	1
19	1	1	1	1	1
20	2	3	3	3	3
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	1	2	2	2	1
25	1	2	3	3	3
26	0	0	0	0	0
27	0	1	2	2	1
28	1	2	2	2	1
29	1	1	1	1	1
30	1	2	3	3	3

Table 5 - Negative Control - observation and grading of skin reactions

Volunteer No.	Time interval / Grading				
	0h grading	1 - 2h grading	24h grading	48h grading	72h grading
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0
28	0	0	0	0	0
29	0	0	0	0	0
30	0	0	0	0	0