

Wednesday, January 20, 2021

## Acute Inhalational Toxicity Test



دانشگاه علوم پزشکی و خدمات  
بهداشتی درماني تبریز  
دانشکده داروسازی

### To the Company: "Aria Rahavard Behnoud"

Hereby I report the results of acute inhalational toxicity tests for the "Non-Alcoholic Hand Sanitizer Solution" provided by your company. Full descriptive data are provided within appendix tables. To carry out this test, the Organization for Economic Cooperation and Development (OECD) revised acute inhalation test Guideline 403 (TG 403) was used.

### Description of the method:

Nine-week-old male and female Sprague-Dawley rats were purchased and acclimated for 1 week before starting the experiments. During the acclimation and experimental periods, the rats were housed in five mesh cages (five rats per cage each was placed in an isolated chamber) in a room with controlled temperature ( $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and humidity ( $55\% \pm 7\%$ ) with a 12-hour light/dark cycle. The rats were fed a rodent diet and filtered water ad libitum. The 10-week-old rats, weighing approximately 320 g for the males and 225 g for the females, were then divided into two groups (five rats in each group/sex): fresh-air control, test-dose group (target dose, 2000 ppm). The animals were exposed to for 4 hours and then observed for 2 weeks following OECD test guideline 403, based on acute inhalation toxicity applying good laboratory practice (GLP). During the exposure period, the animals were housed in individual wire cages. Thereafter, the animals were examined daily on weekdays for any evidence of exposure-related effects, including respiratory, dermal, behavioral, nasal, or genitourinary changes suggestive of irritation. The animals were not provided food during the 4-hour exposure period. The body weights were evaluated at the time of purchase, at the time of grouping, plus 7 and 14 days after the 4-hour inhalation exposure and before necropsy. The results with detailed data can be found in the attached tables 1-3 and figure 1.

**Expert opinion:** All animals survived inhalation exposure to test compound and gained weight through the observation period. All animals appeared active and healthy over the entire 14-day observation phase following exposure to the maximum dose (2000 ppm). There were no signs of gross toxicity, adverse pharmacologic effect, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of 14-day period, therefore no further tests were examined. Furthermore, macroscopic and histological observations of tissue samples revealed no pathological changes as compared to control group. According to

Amir Bagheri

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OECD guidelines, this compound is categorized in the **non-toxic** group. Further tests by longer duration of exposure can be done if there would be case reports in future.

**Reference:** OECD (2008) Revised Test Guideline 403. OECD Guideline for Testing of Chemicals. Acute Inhalation Toxicity Testing.

**Acknowledgment:** This letter is attached with further 4 pages containing 3 tables and 1 figure. This study was accomplished upon request of "Aria Rahavard Behnoud" Company who financially supported the study.

Approved by Amir Baghaci

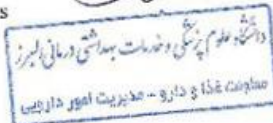
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**Table 1. Individual body weights of fresh air control group**

Animal No.	Sex	Body weight		
		Initial	Day 7	Day 14
1	M	173	178	192
2	M	165	170	183
3	M	169	174	188
4	M	215	221	239
5	M	213	219	236
6	F	209	215	232
7	F	184	190	204
8	F	203	209	225
9	F	177	182	196
10	F	201	207	223

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**Table 2. Individual body weights of test group**

Animal No.	Sex	Body weight		
		Initial	Day 7	Day 14
11	M	162	167	180
12	M	198	204	220
13	M	181	186	201
14	M	200	206	222
15	M	212	218	235
16	F	170	175	189
17	F	174	179	193
18	F	210	216	233
19	F	166	171	184
20	F	206	212	229

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**Table 3- Individual cage-side observations**

Animal No.	Findings	Day of occurrence
Males 1-5	Active and healthy	CR-14 <sup>1</sup>
Females 5-10	Active and healthy	CR-14 <sup>1</sup>
males 11-15	Active and healthy	CR-14 <sup>1</sup>
Females 16-20	Active and healthy	CR-14 <sup>1</sup>

<sup>1</sup>CR- removal from the exposure tube

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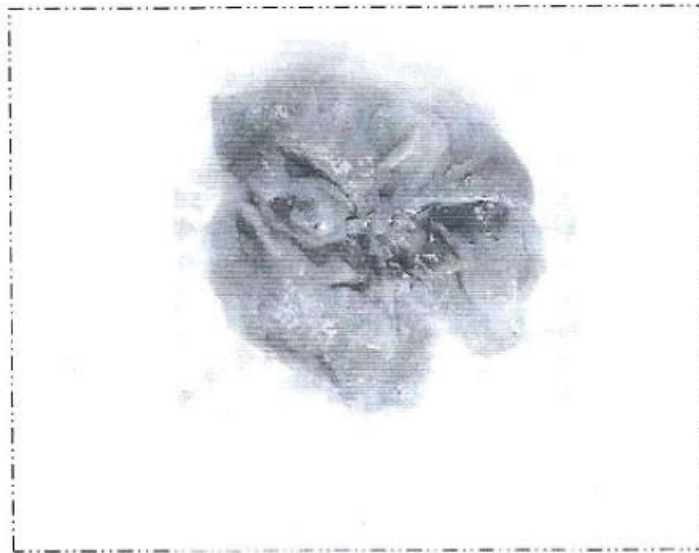


Figure 1. Macroscopic (A) image of test group lung samples showing normal lung tissue.

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