

Wednesday, January 20, 2021

Cytotoxicity Test



دانشگاه علوم پزشکی و خدمات
بهداشتی درمانی شیراز
دانشکده داروسازی

To the Company: "Aria Rahavard Behnoud"

Hereby I report the results of cytotoxicity test for the "Non-Alcoholic Hand Sanitizer Solution" provided by your company. Full descriptive data are provided within appendix figure. To carry out this test, the Organization for Economic Cooperation and Development (OECD) *In Vitro* Skin Irritation test Guideline 439 was used.

Description of the method:

Human blood was obtained from 3 healthy male volunteers between 20 and 30 years of age (non-smokers and not under any medications), after their consent. The lymphocytes were obtained by centrifugation. Cell viability was checked by Trypan blue exclusion method and was found to be approximately 95%. Lymphocytes were incubated for 5 days at 37 °C in RPMI media with different concentrations of Test substance (25, 50, 100, 150 and 200 µg/ml, equivalent to 250, 500, 1000, 1500 and 2000 ppm). MTT assay was performed to assess the effect on mitochondrial succinate dehydrogenase activity. Cells with treatment concentrations of Test substance (0, 25, 50, 100, 150 and 200 µg/ml) were seeded onto 96-well culture plates (at 1×10^5 cells per well). At the end of exposure, cell culture medium was discarded, and cells washed with PBS twice to remove excess test substance and media. The cells were resuspended in fresh media and were treated with 0.5 mg/ml solution of 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA; 100 µl/well) at 37 °C for 3 h. The number of viable cells was determined by uptake of MTT. Optical density (OD) was read at 570 nm. The interference of excipients in the assays was eliminated by maintaining sample blanks for all the concentrations tested. The OD values obtained from the sample blanks were deducted from the OD values obtained after the assays. The values obtained thereafter have been expressed as percentage compared to control. All experiments were performed at least in triplicate on three separate occasions. Data are presented as mean \pm SD (Figure 1).

Amir Baghaei

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Expert opinion: Through measurement of the activity of the mitochondrial dehydrogenases for the "Non-Alcoholic Hand Sanitizer Solution" by the MTT assay **Cytotoxicity was not evident** in MTT and results show an Inhibitory Concentration-50 (IC50) above 2000 ppm ($\mu\text{g/ml}$).

Reference: OECD (2010), OECD GUIDELINE FOR THE TESTING OF CHEMICALS, In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method.

Acknowledgment: This letter is attached with a further page containing 1 figure. This study was accomplished upon request of "Arya Rahavard Behnoud" Company who financially supported the study.

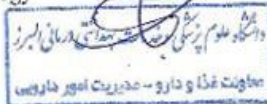
Approved by Amir Baghaei

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Faculty of Pharmacy

Alborz University of Medical Sciences



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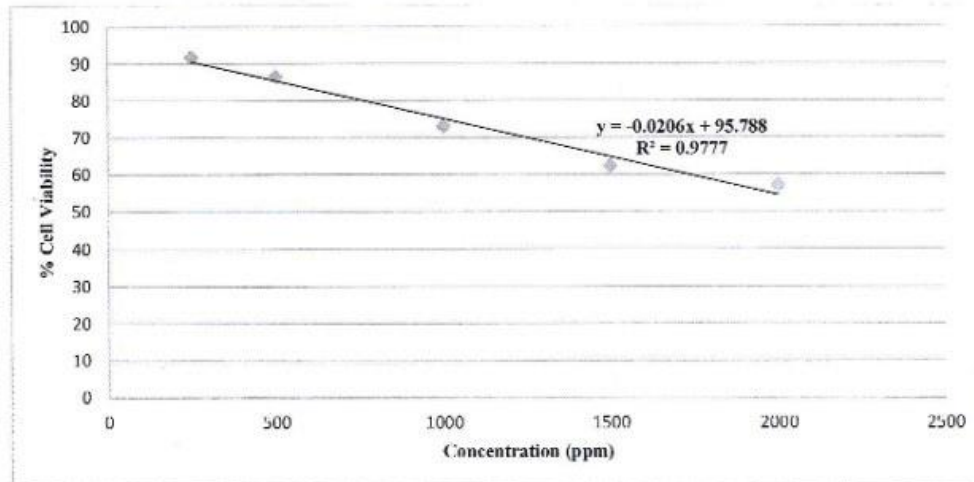


Figure 1. MTT cell viability assay, cytotoxicity induced in test substance treated human lymphocyte cells.

Amir Baghaei