TOXICITY ASSESSMENT OF E-CIGARETTE LIQUIDS AND THEIR VAPORS ON HUMAN VASCULAR ENDOTHELIAL CELLS

Thomas Jakschitz[1], Christian Doppler[1,2], David Bernhard[2], Günther K. Bonn[1]

[1] ADSI - Austrian Drug Screening Institute GmbH, Innsbruck, Austria
[2] Cardiac Surgery Research Laboratory, University Clinic for Cardiac Surgery, Medical University of Innsbruck, Innsbruck, Austria

Abstract

The present study was conducted to provide data on toxicity of e-cigarette liquid (e-liquid) vapors and to compare e-liquid vapor toxicity to the toxicity of conventional cigarette smoke. Using an adapted Borgwaldt RM 1/G-R58.02 smoking machine, the hydrophilic fraction of e-liquid vapor was collected and condensed. The human umbilical vein endothelial cells (HUVEC) were incubated with various concentrations of codensate. Finally, the resazurin and LDH release assay were used to determine the toxicity and cell death in the HUVEC cell cultures.

Identification and quantification of harmful substances

The increasing popularity of e-cigarettes is based on the claim that they are a less harmful alternative to conventional tobacco cigarettes. ADSI developed biological and analytical test systems to measure the hazardous potential and toxic effects of e-cigarette liquids (e-liquids) on the health of consumers. The correlation of biological and analytical data generated at ADSI allows for the identification and quantification of harmful substances in e-liquids. This knowledge is used for the development of new generation of e-liquid products that are safer and healthier than e-liquids currently on the market that were never tested for their hazardous potential on health.

Quality control and monitoring of regulatory thresholds

GC-MS / LC-MS → Identification
GC-FID / LC-MS → Quantification
PTR-MS → Inhalation/exhalation studies
ICP-MS / ICP-OES → Metals
Immortalized cell systems → Toxicity and cell death
↳ Human umbilical vein endothelial cells (HUVEC)

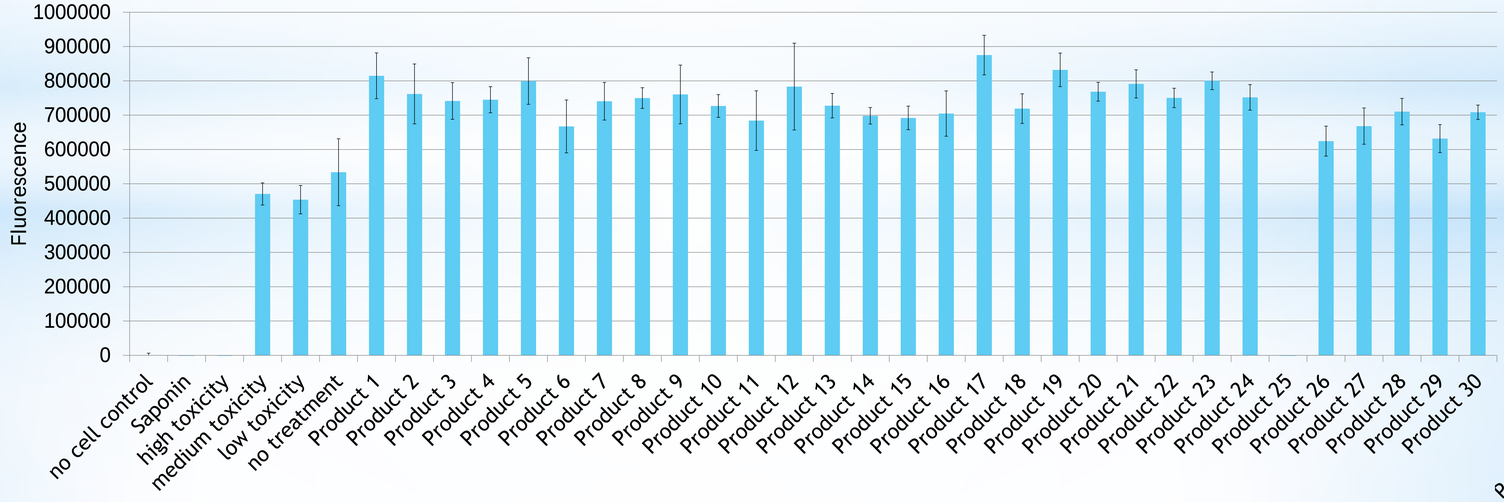
Generation of condensate samples

A modified Borgwaldt RM 1/G-R58.02 smoking machine was used to produce 70 ml aerosol in 2.5 s with a 30 s interval between two consecutive puffs. The e-cigarettes (iStick 30W mounting a JUSTFOG™ Coil Cylinder for 2043™ Clearomizer set at 8.4 W for PG/VG 50/50 samples were triggered directly by the smoking machine. After 5 puffs, the aerosol generated in the 6th puff was collected in a 20 ml glass vial containing 1 ml of PBS and sealed with a septum.

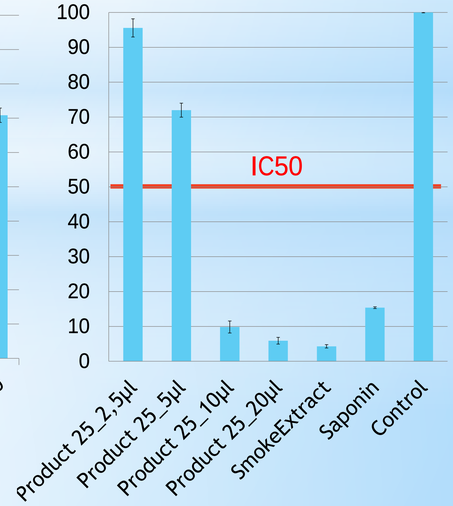
Sample preparation

120 µl of e-Liquid were diluted with 5880 µl EGM2-G in a 50 ml falcon tube and extensively mixed, followed by sterile filtration via a 0.2 µm syringe filter. 750µl sample were used per well, the measurements were performed as biological and technical triplicates.

Resazurin Assay



Toxicity Titration



Results

Conventional cigarette smoke extract showed the most severe impact on HUVEC cells. However, some e-liquid condensates showed surprisingly high cytotoxicity, which was comparable to conventional strong high-nicotine cigarettes. The vapors generated from different e-liquids using the same e-cigarette showed massive differences, pointing to some flavors as an important source of toxicity. We detected a high variability in the acute cytotoxicity of e-cigarette vapors depending on the e-liquid and the e-cigarettes used. Using the LDH release assay, no e-liquid condensate was able to induce immediate cell death of HUVEC cell cultures. Nicotine concentration does not seem to significantly influence the e-liquid vapor toxicity.

Literature

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Contact

ADSI - Austrian Drug Screening Institute GmbH
Dr. Laco Kacani
Innrain 66a
6020 Innsbruck, Austria
Tel: +4351250736307
Mobile: +4369919203322
laco.kacani@adsi.ac.at