Biological Test procedure for fresh generated smoke and aerosols


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1. Introduction

Smoke / aerosol contain particulate matter / droplets in the solid phase as well as volatile substances in the gas phase. Both fractions are of potential toxicological relevance and should be considered when assessing biological activity of fresh smoke / aerosol mixtures. Rapid ageing of the mixtures lead to problems regarding trapping and effective delivery of both fractions to the cells. Therefore, dedicated exposure systems have been developed allowing the exposure of biological in vitro systems to freshly generated smoke / aerosols. All the presented methods are ISO17025 accredited in the ITL-Lab in Hamburg/Germany.

2. Materials and Methods

2.1 Tested products (TP)

(a) Reference Cigarette 3R4F, Burley, Virginia and E-cigarettes with liquids containing 2.4% nicotine (w/w)

(b) 96 MWP - Nunclon® Delta Surface (#168136)

(c) 24 MWP - FALCON #353047 assembled with INSERTs MD24 0,4 (#NUNC 40620)

(d) IVM assay - Hamster Chinese lung cells (V79, ECACC 86041102)

(e) Ames test – Salmonella typhimurium TA100 and TA98 (MOLTOX)

2.2 Smoke / aerosol generation

(a) Fresh smoke was generated under ISO 3308 (30ml/2a/60s) bell-shape puff profile

(b) Aerosol was generated under CRM N°1 (55mL/3a/30s) square wave puff profile

2.3 Cell exposure containers

(a) 96 MWP - Nunclon® Delta Surface (#168136)

(b) FALCON #353047 assembled with INSERTs MD24 0,4 (#NUNC 40620)

2.4 Exposed cells and bacteria

(a) V79 hamster lung cells

(b) Salmonella typhimurium

2.5 In vitro exposure systems

(a) Liquid Interface (ALI) exposure - Smoke Aerosol Exposure In Vitro System (SAEIVS) designed to expose cells in multwell plates (MWP) and inserts / transwells [1].

(b) Bubbling Exposure – Smoke / aerosol bubbling system designed for direct bacteria exposure in 10 ml of PBS. 150 µl of the bacteria suspension is sampled between the smoke runs for analysis in Ames test.

Neutral Red Uptake assay with BEAS-2B cells

The cytotoxicity of the products is tested by exposure of BEAS2B cells on 25 µl of collagen I matrix in 96 MWP. The collagen layer guarantees stable exposure conditions over an extended time period sufficient for testing of several hundred of puffs. The BEAS2B cells are incubated over 3-4 cell divisions (65 hours) after the exposure covered by serum free medium. The low cell density allow constant growth of the cells over the whole incubation time.

Micronucleus assay with V79 cells

The genotoxicity testing is performed with V79 hamster lung cells grown and exposed on inserts placed in 24 MWP. After 20 hours incubation time the increased number of MN-cells was counted.

3. ALI Exposure system

SAEIVS is designed to expose cells in MWP under ALI conditions. The system has been proven for its efficiency regarding biological effects especially induced by the particles / droplets present in the aerosol. Two to three exposure chambers with separate dilution systems allow parallel exposure to the same smoke / aerosol and their gas vapour phase in different dilution levels per each plate. Furthermore, the separate chambers enable testing of the same product in 2 different in vitro assays in different MWP.

4. Bubbling Exposure system

Three-port adapter allow to bubble the smoke of up to 3 cigarettes successively through the impinger. After each puff a puff of air follows.

This test procedure is optimized for Ames testing with smoke/aerosol and their gas phase components in particular.

Ames test with S. typhimurium

Mutagenicity testing is realized by bubbling 10-fold concentrated S typhimurium suspension in PBS directly with freshly generated smoke/aerosol. The strain TA100 shows sensitivity to mutagenic substances in both the gas phase and in the particulate matter / droplets. The strain TA98 shows only a weak response with the gas vapour phase.

Bacteria for the Ames test were taken after defined number of puffs.

5. Summary

- SAEIVS enables in vitro testing of aerosols generated from different product categories encompassing tobacco products and e-cigarette devices. Parallel testing in NRU and IVM assay as well as whole aerosols and their gas phase by using different dilution factors were performed.

- NRU assay - BEAS-2B cells seeded on a collagen-I matrix in a 96 MWP allow prolonged exposure to smoke and aerosol under ALI conditions.

- IVM assay – V79 cells seeded on inserts or transwells in a 24 MWP allow exposure to smoke/aerosol under ALI conditions.

- The sensitivity of fresh smoke/aerosol in vitro test systems towards gas phase components is of crucial importance.

For more details regarding the testing strategy of TPM, e-liquids and NITMs of Imperial Tobacco please refer to poster P284.

References
