Recent improvements in *in vitro* models need to be supported with refinements in dosimetry to enable possible future *in vivo* data extrapolation

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**1. Introduction/Context**

Based on the National Research Council (NRC) report “Toxicity Testing in 21st Century (2007)”, there is a focus on using human based *in vitro* assays as the ultimate replacement of animal studies. The use of 2D and 3D human lung cell models can be used to replicate physiologically relevant exposure to tobacco smoke and Electronic Vapour Products (EVPs) in users.

For the assessment of tobacco additives, Imperial Tobacco toxicologists routinely use consumption data collected for the different product types, product specific additive transfer rates and assume 100% deposition in the human respiratory tract. The estimated additive exposure is compared to relevant published animal data using standard uncertainty factors to calculate a Derived No Effect Level (DNEL). Estimated exposure values below the DNEL are considered tolerable. A better understanding of the dosimetry of whole smoke is required to investigate how in *in vitro* assays could be potentially extrapolated to estimate human exposure levels. Future work will focus on *in vivo* exposure at the cellular level.

An additional consideration, is that the toxicological assessment of EVPs poses a challenge for standard assay systems. Due to the low toxicity of the vapour produced by EVPs, the sensitivity of *in vitro* test systems towards vapour components is of crucial importance. The direct exposure of human cells to the freshly generated smoke/vapour at air liquid interface (ALI) seems to be the most promising approach. Here, the cells need to be kept under optimal conditions during prolonged experiments to better replicate human exposure. Our in-house system enables accurate delivery of smoke/vapour to cells for extended periods and is key for potential incorporation of *in vitro* data into human exposure/hazard assessments.

**2. Methods: Aerosol Exposure in Vitro System (AEIVS)**

**3. Results**

**Cytotoxicity of smoke and vapour to BEAS-2B cells in NRU assay**

**AEIVS – Diagram of experimental set up**

**4. 3Rs* Impact of research**

- Imperial do not commission or conduct research involving animals and would not undertake such research unless formally requested to do so by a recognised regulatory authority
- Imperial Tobacco favours human in vitro alternatives, due to ethical considerations, physiological relevance, the speed of the assay and flexibility when compared to the use of animal models (2)
- The use of human derived cells/organ/tissue systems at the air-liquid interface is considered to be representative of human exposure
- Human derived cellular systems are cost effective and provide potentially relevant insights into possible toxicity risks in humans

**5. Conclusions/Future work**

- Using AEIVS the linear dilution of smoke/vapour is both accurate and highly reproducible
- Achieves rapid delivery of whole smoke/vapour to the cells (within 10 seconds)
- The use of separated dilution pumps for both exposure chambers allows highly flexible and independent testing
- Prolonged exposure periods at ALI are possible (up to 3 hours)
- AEIVS enables a direct comparison of products and potential effects of product modifications at the same time

**References**