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An inter-machine comparison of tobacco smoke particle deposition *in vitro* from six independent smoke exposure systems

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ABSTRACT

There are several whole smoke exposure systems used to assess the biological and toxicological impact of tobacco smoke *in vitro*. One such system is the Vitrocell® VC 10 Smoking Robot and exposure module. Using quartz crystal microbalances (QCMs) installed into the module, we were able to assess tobacco smoke particle deposition in real-time. We compared regional deposition across the module positions and doses delivered by six VC 10s in four independent laboratories: two in the UK, one in Germany and one in China.

Gauge *R&r* analysis was applied to the total data package from the six VC 10s. As a percentage of the total, reproducibility (between all six VC 10s) and repeatability (error within an individual VC 10) accounted for 0.3% and 7.4% respectively. Thus Gauge *R&r* was 7.7%, less than 10% overall and considered statistically fit for purpose.

The dose–responses obtained from the six machines across the four different locations demonstrated excellent agreement. There were little to no positional differences across the module at all airflows as determined by ANOVA (except for one machine and at three airflows only). These results support the on-going characterisation of the VC 10 exposure system and suitability for tobacco smoke exposure *in vitro*.

1. Background

Tobacco smoke generated by machine smoking is commonly used for tobacco product assessment *in vitro,* for modelling disease processes and for toxicological assessment. The World Health Organisation (WHO) upholds that machine testing of combustible tobacco products cannot accurately estimate human exposure, and should not be used to support claims of reduced exposure or risk (World Health Organisation, 2007). However, the WHO does support the use of machine smoke emission data for product hazard assessment, to characterise cigarette emissions for product design and regulatory purposes (World Health Organisation, 2007).

Smoking machines generate, dilute and deliver mainstream tobacco smoke (also known as whole smoke) to an exposure chamber/module containing a biological system, usually supported at the air–liquid interface (ALI). There are many types of smoking machines and exposure chambers available for the testing of whole smoke at the ALI. Some are small bespoke laboratory set-ups whereas others are commercially available systems utilised by the tobacco industry and other well-known inhalation toxicology research groups (Thorne and Adamson, 2013). Whichever system is utilised, there is a clear need to characterise the capabilities, limitations, dilution principles, smoke losses and exact dose delivery

Abbreviations: ALI, air–liquid interface; ANOVA, analysis of variance; ISO, International Organisation for Standardisation; QC, quality control; QCM, quartz crystal microbalance; R&r, reproducibility (*R*) and repeatability (*r*); SD, standard deviation; VC 10, Vitrocell® VC 10 Smoking Robot; WHO, World Health Organisation.

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of these machines, which will lend credence to biological data obtained from smoke exposures. In addition, good understanding and characterisation of the machine, and ultimately full validation, should facilitate the endorsement of the machine for the generation of biological data.

Method validation is the process of demonstrating that an analytical method is suitable for its intended use, and involves conducting a variety of studies to evaluate method performance under defined criteria (Thompson et al., 1999). Method validation studies may involve a single laboratory (intra-laboratory) or multiple laboratories (inter-laboratory). Organisations such as the Association of Analytical Communities (AOAC) and US Environmental Protection Agency (EPA) provide methods that are validated through inter-laboratory studies, and parameters which may be assessed in method validation include precision defined by reproducibility (*R*) and repeatability (*r*) and bias (Ellison et al., 2009). Thus, inter-laboratory studies/cross-machine comparisons enable confidence to be gained in a machine or laboratory set-up and can facilitate the standardisation of experimental testing protocols. As such, data generated from method validation studies or standardised protocols could provide information for future regulation or testing standards.

Currently, there are no defined *regulatory* protocols for tobacco whole smoke exposure systems, but product testing protocols for assays such as Ames bacterial mutagenicity and Neutral Red Uptake (NRU) cytotoxicity are being developed to support *in vitro* toxicity testing and disease model development. One such smoking machine/exposure system used for the testing of tobacco whole smoke is the Vitrocell® VC 10 Smoking Robot and mammalian exposure module (Vitrocell® 6 CF Stainless) both of which have been previously described (Adamson et al., 2013; Klein et al., 2013; Nara et al., 2013; Okuwa et al., 2010). Additionally, quartz crystal microbalance (QCM) technology can be employed to accurately assess deposited particle mass within the exposure module. QCMs enable a greater understanding of particle dose as mass per surface area, rather than simply a diluting airflow and sampling vacuum flow rate applied to the exposure module (Adamson et al., 2013; Paur et al., 2011; Bakand and Hayes, 2010; Lenz et al., 2009). To assess deposition, QCMs are installed in the exposure module in place of the biological system, giving real-time, *in situ* gravimetric data on particle deposition, in the nanogram range (Fig. 1).

In this study, six Vitrocell® VC 10 Smoking Robots were assessed for their ability to generate a consistent smoke dose, using QCMs to quantify deposited particle matter within the exposure module (Fig. 1d). The QCMs took readings from each position in the module, the first position being proximal to smoke entering the dilution bar, the last position being distal to smoke entry (Fig. 1b). It is important to consider this arrangement, as in some instances the linear direction of smoke entry may have an effect on regional/positional deposition differences across the QCMs left to right (Deschl et al., 2011). The aim of this study was to enable the comparison of multiple VC 10s, in four independent laboratories/geographical locations, tested with an identical diluting airflow range of 0.25, 0.5, 1.0, 2.0 and 4.0 L/min. These airflows were selected for testing based on a previously published study (Adamson et al., 2013) which represented reliably detectable levels for the QCMs during a 24 min exposure. To preserve anonymity of machine, lab, group and operator, all were coded (Table 1.) as is common practice for comparison studies. The six VC 10s were located in four laboratories: two laboratories were in the United Kingdom, one was in China and one was in Germany. Regional deposition patterns across the exposure module were assessed independently at each airflow for each machine. *R*&*r* analysis was estimated for all six instruments which were collectively termed the 'measurement system'. *R*&*r* analysis determines the precision of a measurement system and is often employed to compare multiple systems in different locations or with different operators; more specifically it calculates the degree to which repeated measurements taken under the same (unchanged) experimental conditions show the same result (Measurement Systems Analysis reference manual, 2002). Reproducibility (*R*) is the closeness of agreement between measurements or observations conducted on replicate specimens (machines) in different locations by different people; it assesses the ability of the experiment or measurement to be reproduced independently. Repeatability (*r*) looks at test–retest variability; it assesses the variation in measurements made within the same system by the same operator (Kaur et al., 2010). Thus, data were compared within each machine and across all six machines. Additionally, two important variables were assessed by comparing data from VC 10s which had a significant change. The first was laboratory geography/environment, where data were acquired on the same VC 10 (serial VC 10/300412) in two different laboratories after it was moved from one to another. The second

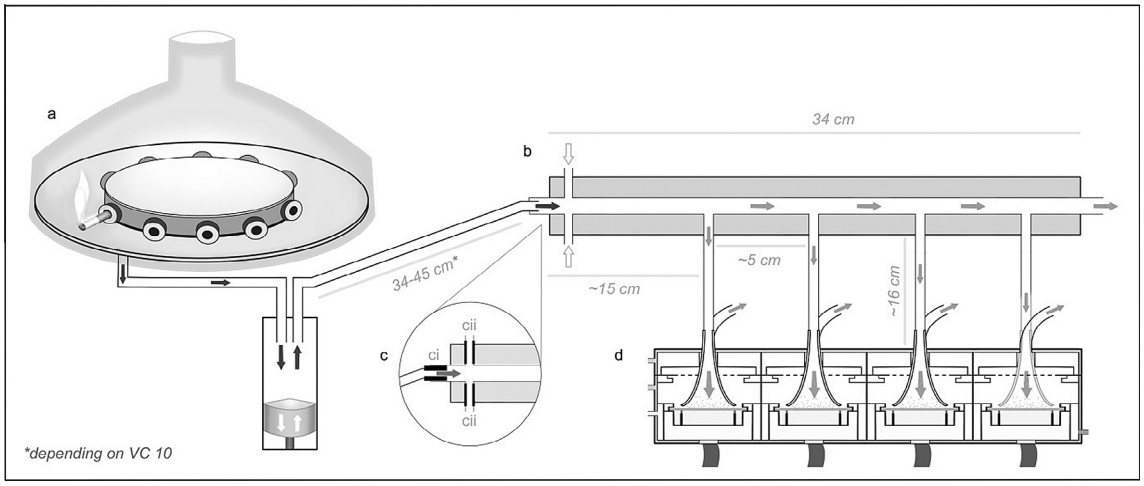

Fig. 1. A schematic cross-section of the Vitrocell® exposure system set-up (not to scale). (a) VC 10 Smoking Robot including the single piston/syringe and delivery tubing to the dilution bar; (b) smoke entry (dark arrow) to a single dilution bar where diluting air is added (white arrows). Multiple parallel bars make the dilution system; (c) each dilution bar has one smoke jet (ci) which is always 2.0 mm ∅ (in this study), and 2 identical air jets above and below the dilution bar (cii) which are either both 1.0 mm ∅ or both 0.8 mm ∅, depending on dilution airflow; and (d) mammalian 6/4 CF Stainless exposure module with QCMs installed into each of the four separate wells. Image adapted from Adamson et al. (2013).