



Investigation of multiple whole smoke dosimetry techniques using a VITROCELL® VC10® smoke exposure system

Brian M. Keyser^{a,*}, Robert Leverette^{a,*}, Michael Hollings^b, Adam Seymour^b, Lesley Reeve^b, Wanda Fields^a

^a RAI Services Company, Scientific & Regulatory Affairs, 401 North Main Street, Winston-Salem, NC, 27101, USA¹

^b Covance Laboratories Ltd., North Yorkshire, UK

ARTICLE INFO

Keywords:

Whole smoke
Smoke exposure system
Dosimetry
Aerosol
Photometers

ABSTRACT

The Vitrocell® VC10® smoke exposure system offers multiple platforms for air liquid interface (ALI) and air agar interface (AAI) exposure that mimic *in vivo* conditions for assessing toxicological impact of whole smoke using *in vitro* assays. The aim of this study was to investigate and compare multiple dosimetry techniques that may be employed during combustible cigarette whole smoke exposure using the Vitrocell® VC10® smoking robot. The following techniques were assessed: (1) quartz crystal microbalances (QCMs), (2) aerosol photometers (using area under curve, AUC), and (3) fluorescence of anhydrous dimethyl sulfoxide (DMSO)-captured smoke constituents.

Results showed that each of the dosimetry techniques was able to distinguish different levels of whole smoke airflow in a concentration-related manner. When compared to each other, the three techniques showed a high level of consistency and all were considered efficient tools in quantifying dose during an exposure, although higher variation was observed at the higher airflows tested. Overall, the dosimetry tools investigated here provide effective measures of the whole smoke concentrations tested during the exposure.

1. Introduction

Although nonclinical assessments have historically been conducted as a component of academic, public health community and tobacco industry research on a variety of tobacco products, both the testing methods [1] and the regulatory environment (e.g [2]) continue to evolve. Regulatory requirements and guidance for use of data generated from nonclinical methods to assess potential health effects of tobacco and related products have been implemented [3–6].

Testing for cytotoxicity and genotoxicity of complex aerosols, such as cigarette whole smoke, provides many challenges and is less well-defined than the established or proposed methods for testing of liquids and solids or extracts in the screening of chemicals [7–11], medical devices [12], or pharmaceuticals [13]. Although cigarette whole smoke is a mixture of more than 7000 chemicals [14], present in both the vapor phase component and the particulate fraction, historically, tobacco products have been tested in cytotoxicity and genotoxicity assays *in vitro* using partitioned exposures to either total particulate matter (TPM) or gas vapor phase (GVP) test materials. To address this, a great

deal of focus has been placed on the development of tobacco mainstream smoke exposure systems [15–18], which capture both phases of tobacco smoke together and presents a more relevant test compound for the assessment of human health risk.

More recently, there has been the introduction of *in vitro* smoking machines, paired with exposure modules that allow exposure of cells to whole smoke aerosol at the air-liquid interface (ALI). One example is a Vitrocell® exposure system which uses a constant flow of compressed air to dilute mainstream cigarette smoke. A sample of this diluted smoke is pulled, by vacuum, into the exposure module where it is delivered to individual exposure chambers [19]. The flow rate of the diluting air can then be adjusted to alter the concentration of smoke delivered to the cells. Determining the delivered test dose of any single component for comparative purposes, much less the whole smoke mixture, is particularly challenging. However, dosimetry is an important tool to allow identification of aerosol concentrations tested during whole smoke exposure and may be used to facilitate comparison of biological responses attained using different whole smoke exposure systems, which may dilute and deliver smoke to the exposed cells in different ways.

* Corresponding authors.

E-mail addresses: keyserb@RJRT.com (B.M. Keyser), leverer@RJRT.com (R. Leverette).

¹ RAI Services Company performs regulatory compliance services for Reynolds American Inc.'s ("RAI") subsidiary companies. RAI is an indirect, wholly owned subsidiary of British American Tobacco p.l.c.

<https://doi.org/10.1016/j.toxrep.2019.10.011>

Received 17 July 2019; Received in revised form 8 October 2019; Accepted 10 October 2019

Available online 04 November 2019

2214-7500/ © 2019 RAI Services Company. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The aim of this study was to investigate the effectiveness of three dosimetry techniques, QCMs, photometers, and DMSO captured/trapped smoke constituents, during whole smoke exposure using a Vitrocell® VC10® smoking robot and associated modules, with two different smoking regimens over a range of airflows (dilutions). QCMs and photometers have been used in the assessment of aerosol concentrations in general, and smoke exposure in particular [20–32]. Exposure and dose measurements calculated for *in vitro* cigarette smoke exposure systems could allow comparisons between data from different test systems and support the potential biological significance of the results obtained [33]. However, the extrapolation of *in vitro* dosimetry data to actual doses achieved *in vivo* remains challenging [34].

The three dosimetry tools assessed in this study used different approaches. One dosimetry approach used QCMs to calculate a change in mass over a fixed area by monitoring the change in frequency of a quartz crystal resonator. As mass is deposited onto the crystal, the oscillation frequency of the resonator decreases, allowing calculation of the deposited mass based on the measurable change in frequency. Since frequency measurements are readily made to high precision, the minimum mass densities that can be measured range from 1×10^{-17} kg to 1×10^{-11} kg depending on the experimental conditions (Mecea, 2006).

A second dosimetry approach used aerosol photometers to characterize the particle density of aerosols in a system by measuring the amount of light that is scattered as a laser passes through the aerosol stream. As the aerosol enters the scattering chamber, a laser diode emits light (wavelength 680 nm) through the sample, and the scattered light is detected by an offset photodetector and reported as voltage over time. The signal is amplified to an output voltage between 0 and 5 V (V). The photometers are connected to the Vitrocell® VC10® smoking robot system at an appropriate point to allow the aerosol to pass in-line through the photometer. The signal is independent of the flow direction and flow velocity through the photometer. Since cigarette smoke contains a mixture of chemical constituents that exist in gaseous and particulate phases that are often shifting between the two phases, the photometers cannot be “calibrated” to a given set point and are therefore ‘harmonized’ for combustible cigarettes. The photometers were ‘harmonized’ to mainstream smoke (HCI smoking regimen, airflow dilution rate 1.5 L/min, applied vacuum 5 mL/min) by connection end to end, attachment to a single dilution bar, and adjustment so that the voltage output from all four photometers was comparable at approximately 4.0 V. Once the voltage output from the photometers was comparable, the photometers were deemed to be harmonized.

The third dosimetry tool we investigated was assessment of deposition of total particulate matter (TPM) using a liquid trap and appropriate solvent. In the current work, TPM was assessed via fluorescence measurements of DMSO-captured smoke constituents. Smoke constituents fluoresce blue (485 nm) when exposed to longwave ultraviolet radiation (355 nm) [35–37]. This property was used to allow the quantification of DMSO-captured smoke constituents from a standard curve via a fluorescence intensity assay.

2. Materials and methods

The dosimetry methods that employed quartz crystal microbalances (QCM), photometers and DMSO-capture were investigated using a Vitrocell® VC10® smoking robot, 12 mm (12-well) and 24 mm (6-well) mammalian exposure modules (Vitrocell®) and 3R4F cigarettes (Fig. 1). Whole smoke exposures were conducted using two smoking regimes: International Organization for Standardization (ISO) and Health Canada Intense (HCI) smoking regimes [38,39] (described below).

2.1. Cigarettes

3R4F reference cigarettes were obtained from the University of Kentucky, Kentucky, USA. Prior to smoking, cigarettes were

conditioned for at least 48 h and no more than 10 days at 22 ± 1 °C and $60 \pm 3\%$ relative humidity, according to the ISO guideline 3402:1999 [40].

2.2. Vitrocell® VC10® smoking robot

The Vitrocell® VC10® smoking robot, dilution system and exposure modules were obtained from Vitrocell® Systems GmbH, Waldkirch, Germany. The VC10® is a rotary style smoking machine which puffs one cigarette at a time, with a single piston that delivers tobacco smoke into an airflow dilution system, as previously described [41]. Smoke dilution is achieved by mixing the mainstream cigarette smoke with a continuous and controlled flow of compressed air within a stainless-steel dilution bar. A subsample of this diluted smoke is then pulled via vacuum into the stainless steel exposure modules. Different concentrations of smoke can be achieved by altering either the diluting airflow rate (L/min) or the vacuum rate (mL/min). The vacuum flow was maintained at a fixed rate of 5 mL/min for all experiments with a range of different diluting airflow (L/min) to adjust smoke exposure concentrations.

In order to ensure that the smoking robot and associated equipment required for whole smoke exposure were functioning as required, the system was qualified through protocols for installation, operational and performance qualification [41].

2.3. QCM

QCM were obtained from Vitrocell Systems GmbH, Germany, installed into the exposure module according to the Vitrocell® QCM operating/service manual and allowed to stabilize for a period of up to 20 min prior to the start of exposure.

2.4. Photometers

Photometers were obtained from Vitrocell® Systems GmbH, Germany, harmonized to mainstream smoke generated using the HCI smoking regimen and diluted at an airflow rate of 0.25 L/min. Harmonization was performed by connecting the four photometers end to end with a minimal amount of tubing. They were then attached to a single dilution bar at the port furthest away from the diluting air inlets, with a 5 mL/min vacuum applied. All other sample ports on the dilution bar were sealed. Using a 0.25 L/min airflow each photometer potentiometer was adjusted so that the voltage output from all four photometers was comparable at approximately 4.0 V. However, this initial harmonization run resulted in a voltage output greater than 5.0 V for subsequent measurements of whole smoke at 0.25 L/min, meaning that the results exceeded the maximal output voltage yielding a clipped signal. Further harmonization runs showed that a 1.5 L/min diluting airflow was optimal for harmonizing at approximately 4.0 V. Therefore, the initial and subsequent harmonization were conducted at 1.5 L/min. Photometers were connected to a PC via the VC Photometer Control Box and data was collected using VC Photometer software. Harmonization of photometers was checked at least every 6 experiments and at the end of the investigation.

2.5. DMSO data capture

Standard curves for colorimetric assessments (Excitation at 355 nm / Emission at 485 nm) were determined from 3R4F pad-collected TPM extracted in DMSO generated under ISO and HCI smoking conditions. The TPM samples were serially diluted (2-fold dilutions) in DMSO from 5 mg/mL to 0.0391 mg/mL. Additional standard curves were generated initially from 1.25 mg/mL to 2.4 µg/mL and finally from 512 µg/mL to 0.25 µg/mL. For sample analysis of the DMSO-trapped material, triplicate 100 µL aliquots from each of the two Transwells® containing DMSO were plated into 96 well plates, along with triplicate 100 µL aliquots of

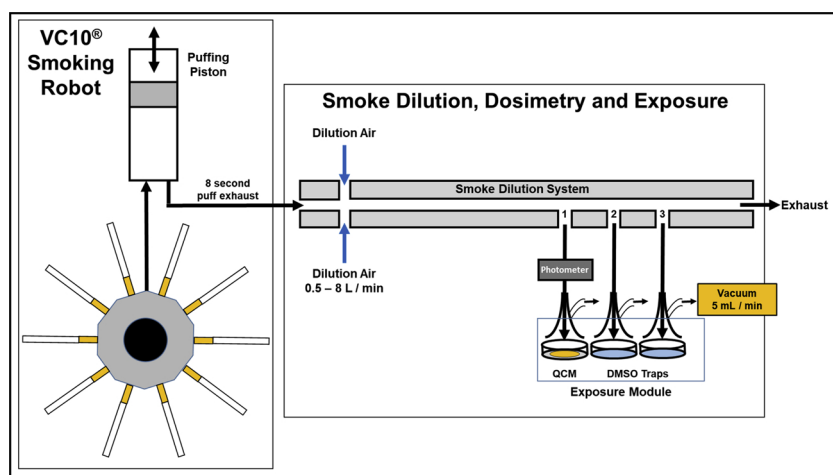


Fig. 1. Schematic representation of the Vitrocell® VC10® smoking robot, smoke dilution and exposure system. VC10 robot was used to generate smoke from 3R4F Kentucky Reference cigarettes. Freshly generated smoke was delivered to the smoke dilution and exposure system via the puffing piston with an 8 s puff exhaust. Dilution air (0.5–8 L/min) was used to dilute the smoke, and a 5 mL/min vacuum flow rate was used to deliver the diluted smoke to the exposure module (positions 1–3). Deposition of smoke particulate was determined by photometer and QCM located at position 1, and DMSO-capture at positions 2 and 3 within the smoke exposure module.

Table 1

QCM deposition data for 6-well and 12-well modules using ISO and HCI smoking regimen.

Concentration (L/min)	QCM Deposition ($\mu\text{g}/\text{cm}^2$)		
	Mean	SD	% CV
3R4F HCI regime 6-well (24 mm) modules			
8	1.86	0.408	22.0
6	3.10	0.714	23.0
4	8.39	1.32	15.8
2	21.5	1.35	6.28
3R4F HCI regime 12-well (12 mm) modules			
8	0.884	0.288	32.6
6	1.65	0.352	21.3
4	3.98	0.153	3.85
2	11.7	2.20	18.7
3R4F ISO regime 6-well (24 mm) modules			
8	0.246 ^a	0.0483 ^a	19.6 ^a
4	1.80	0.303	16.9
2	5.09	0.258	5.06
1	10.9	1.67	15.3
3R4F ISO regime 12-well (12 mm) modules			
8	0.186 ^a	0.0883 ^a	47.6 ^a
4	0.949 ^a	0.117 ^a	12.3 ^a
2	2.71 ^a	0.205 ^a	7.56 ^a
1	6.09 ^a	0.233 ^a	3.82 ^a

Data based on $n = 4$ independent experiments unless otherwise indicated;

^a $n = 3$; exposure duration of all ISO and HCI airflows were 64 and 44 min, respectively.

the controls (duplicate wells of DMSO exposed at the ALI to an air flow of 0.2 L/minute). Analysis was performed using a VERSAman™ (Molecular Devices, San Jose, CA) or other similar plate reader.

2.6. Vitrocell® VC10® setup and whole-smoke exposure

The Vitrocell® VC10® smoking robot was used to generate mainstream cigarette whole smoke from 3R4F reference cigarettes. Cigarettes were conditioned (> 48 h, < 10 days at 22 ± 1 °C and 60 ± 3 % relative humidity) then smoked according to ISO guidelines [40,38] or the Heath Canada Intense (HCI) smoking regime (Health Canada, 1999) with the following parameters:

- Puff volume: 35 mL (ISO), 55 mL (HCI)
- Puff duration: 2 s
- Puff frequency: 60 s (ISO), 30 s (HCI)
- Puff profile: Bell shaped
- Vent blocking (HCI only): 100%

Table 2

Photometer AUC deposition data for 6-well and 12-well modules using ISO and HCI smoking regimen.

Concentration (L/min)	Photometer AUC (Σ)		
	Mean	SD	% CV
3R4F HCI regime 6-well (24 mm) modules			
8	276	39.6	14.4
6	475	104	21.9
4	807	187	23.1
2	1910	166	8.69
3R4F HCI regime 12-well (12 mm) modules			
8	339	90.1	26.6
6	522	119	22.8
4	866	149	17.2
2	2310	584	25.3
3R4F ISO regime 6-well (24 mm) modules			
8	91.2 ^a	11.8	12.9
4	321	31.1	9.67
2	827	109	13.1
1	1800	210	11.7
3R4F ISO regime 12-well (12 mm) modules			
8	91.0 ^a	21.1	23.1
4	330 ^a	21.8	6.61
2	865 ^a	130	15.1
1	1670 ^a	172	10.3

Data based on $n = 4$ independent experiments unless otherwise indicated;

^a $n = 3$.

2.7. Exposure parameters

- Puff exhaust duration: 8 s
- Length of exposure: 64 min (ISO), 44 min (HCI)
- Number of puffs per exposure: 64 from 8 cigarettes (ISO), 88 from 8 cigarettes (HCI)

2.8. Dosimetry experiments for whole smoke exposures

In each whole smoke exposure experiment, the three dosimetry tools were set up in each exposure module as follows. The first chamber in each module contained a QCM and an in-line photometer at position 1 and two wells containing DMSO at positions 2 and 3 (Fig. 1). Air controls were tested in triplicate in a separate exposure module during the experiment. Experiments were conducted in 6-well or in 12-well modules, with a variety of different configurations to allow comparison of the different dosimetry techniques.

Table 3
DMSO-trapped fluorescence deposition data for 6-well and 12-well modules using ISO and HCl smoking regimen.

Concentration (L/min)	DMSO Fluorescence TPM equivalent (µg/mL)		
	Mean	SD	% CV
3R4F HCl regime 6-well (24 mm) modules			
8	11.7	0.796	6.81
6	21.6	3.39	15.7
4	42.0	4.09	9.75
2	116	15.6	13.4
3R4F HCl regime 12-well (12 mm) modules			
8	37.6	12.5	33.1
6	63.3	7.01	11.1
4	128	10.8	8.41
2	348	13.3	3.83
3R4F ISO regime 6-well (24 mm) modules			
8	2.30 ^a	0.996	43.3
4	8.89	3.59	40.4
2	30.5	6.02	19.7
1	61.1	19.9	32.6
3R4F ISO regime 12-well (12 mm) modules			
8	5.99 ^a	2.54	42.5
4	27.4 ^a	5.84	21.3
2	79.2 ^a	25.7	32.5
1	186 ^a	43.9	23.5

Data based on n = 4 independent experiments unless otherwise indicated;
^a n = 3.

2.9. Data analysis

QCM data output was collected as both comma separated value (CSV) and virtual compact disk (VCD) file formats detailing the deposition on each balance for each airflow exposure. Data output from the photometers (once per second) was collected as CSV and VCD files detailing the voltage reading for each airflow, allowing an AUC (area under the curve) to be calculated for each exposure. For measurements of DMSO-captured material, standard curves were used to convert relative fluorescence units at 485 nm wavelength (RFU₄₈₅), respectively, into corresponding TPM concentrations.

Pad-collected 3R4F TPM in DMSO (both ISO and HCl regimes) was analyzed to generate DMSO-captured matter standard curves. The TPM samples were serially diluted (2-fold dilutions) in DMSO from 5 mg/mL to 0.0391 mg/mL. Additional standard curves were generated initially from 1.25 mg/mL to 2.4 µg/mL and finally from 512 µg/mL to 0.25 µg/mL. The Ex₃₅₅ and Em₄₈₅ readings were taken on these dilutions and used to prepare the standard curves.

The results obtained with the three dosimetry methods were analyzed in pairwise comparisons for consistency, based on calculated correlation coefficient (r²) values.

3. Results

3.1. QCM

QCM deposition data is presented in Table 1. Greater variability (expressed as coefficient of variance [%CV]) was observed at the higher

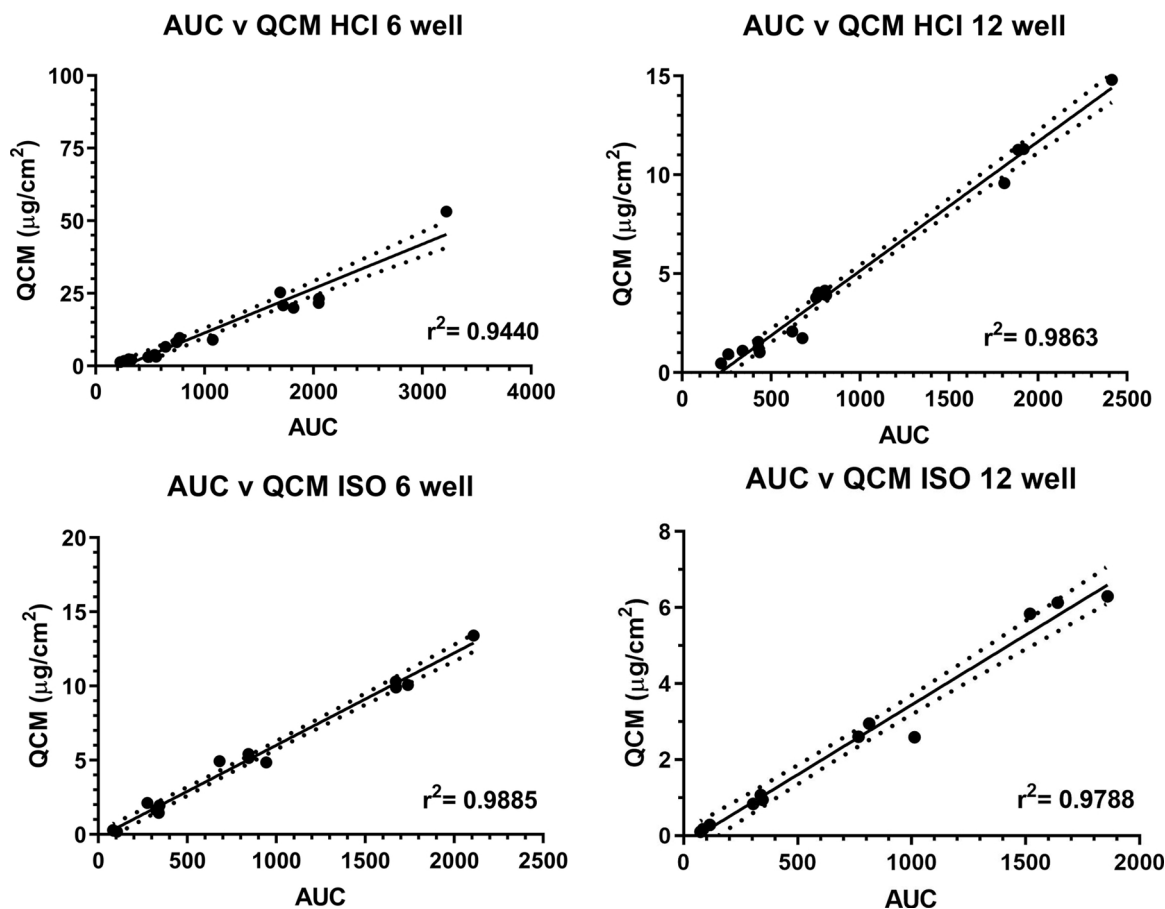


Fig. 2. Consistency in dosimetry measurements using QCM compared with aerosol photometers in 6- or 12-well test modules using HCl or ISO smoking regimen.

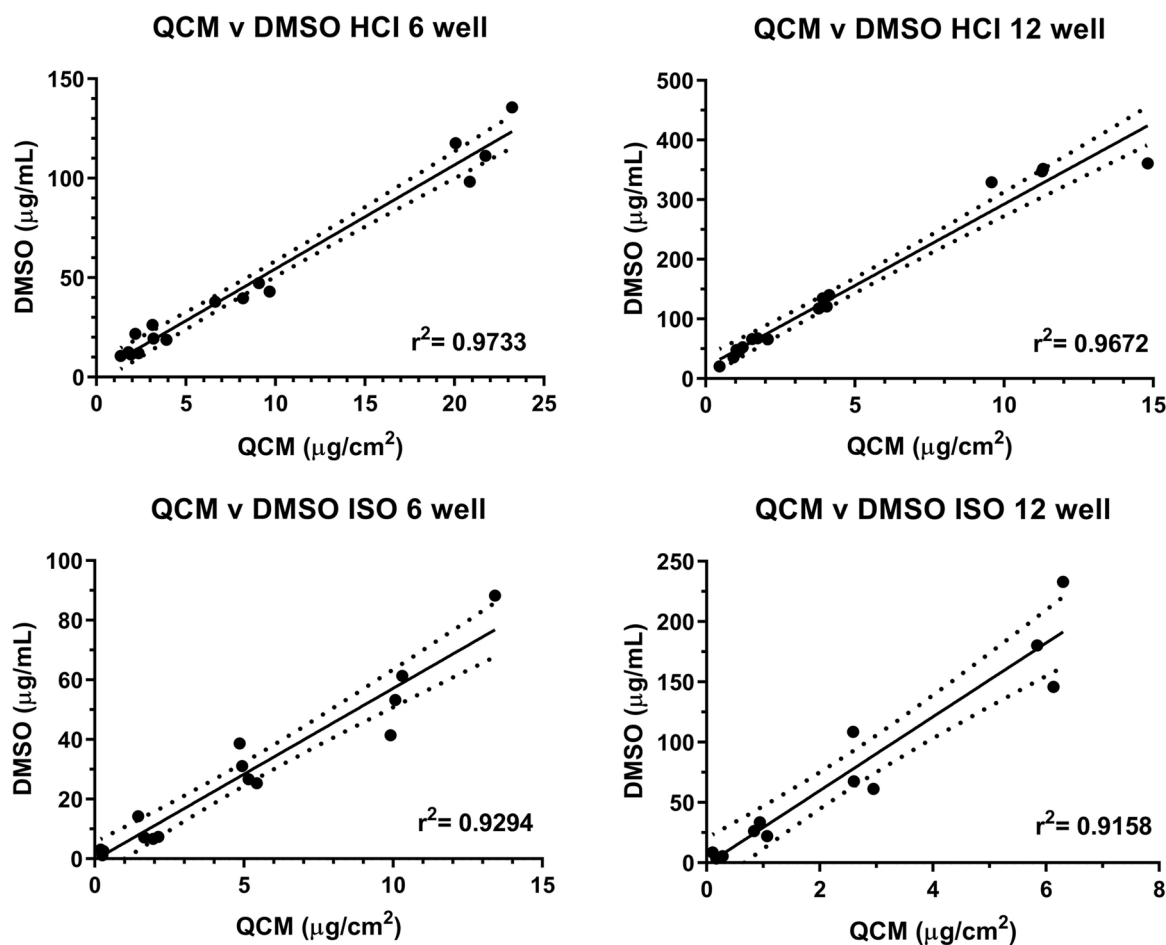


Fig. 3. Consistency in dosimetry measurements using DMSO fluorescence method compared with QCM in 6- or 12-well test modules using HCl or ISO smoking regimen.

airflows tested (8 and 6 L/min). This trend was noted using both 6-well (24 mm) and 12-well (12 mm) configurations for both ISO and HCl smoking regimens and was considered to be due to lower deposition at these airflows rather than greater experiment-to-experiment variation.

3.2. Photometers

AUC data from the photometers was consistent between experiments for both 6-well (24 mm) and 12-well (12 mm) configurations using the ISO smoking regimen, without any apparent influence of the higher airflows on the AUC values. Compared to the ISO regimen, the %CV for both the 6-well (24 mm) and 12-well (12 mm) configurations using the HCl smoking regimen was slightly elevated. This is likely due to the more concentrated bolus of whole smoke generated by the HCl regimen rather than experiment-to-experiment variation. Both the ISO and the HCl regimen AUC data demonstrate a concentration-related increase in measured dose (Table 2).

3.3. DMSO fluorescence

For the DMSO fluorescence data, the relative fluorescent units (RFU)₄₈₅ values were extrapolated using the standard curves for each smoking regimen to determine TPM equivalent ($\mu\text{g}/\text{mL}$) dose values (Table 3). TPM equivalent values demonstrated a concentration-related increase, with lower airflow resulting in more TPM being deposited. Low %CV in the data set indicated that there was overall uniformity between HCl experiments in 6- and 12-well modules except for the highest airflow (8 L/min) in 12-well modules. The TPM equivalent data

generated under the ISO smoking regimen was not as consistent as the HCl regimen, with %CV values ranging from 19% to 43% in both the 6-well and 12-well modules. This was not unexpected due to the lower deposition of mass seen using the ISO regimen compared to HCl data, and the greater variability noted when assessing smaller deposition values. However, the consistency between experiments did not appear to be markedly better at the lower airflows using the ISO regimen, in contrast with the improved consistency at the lower airflows observed under the HCl regimen.

3.4. Comparison of dosimetry techniques

The multiple dosimetry techniques were able to distinguish different airflows of whole smoke in a concentration-related manner. Hence, the data were further evaluated to determine the level of consistency between the different dosimetry techniques.

The dosimetry methods using photometers and QCM showed a high level of consistency with each other between experiments (r^2 values of 0.9440 to 0.9885), although slightly higher variation was observed at the higher airflows tested (Fig. 2). DMSO fluorescence of captured smoke constituents also demonstrated a good level of consistency with either QCM or photometer measurements, as shown in Figs. 3 and 4, respectively.

4. Discussion and conclusion

The Vitrocell® VC10® *in vitro* whole smoke/aerosol exposure system has been developed for the assessment of potential biological activity

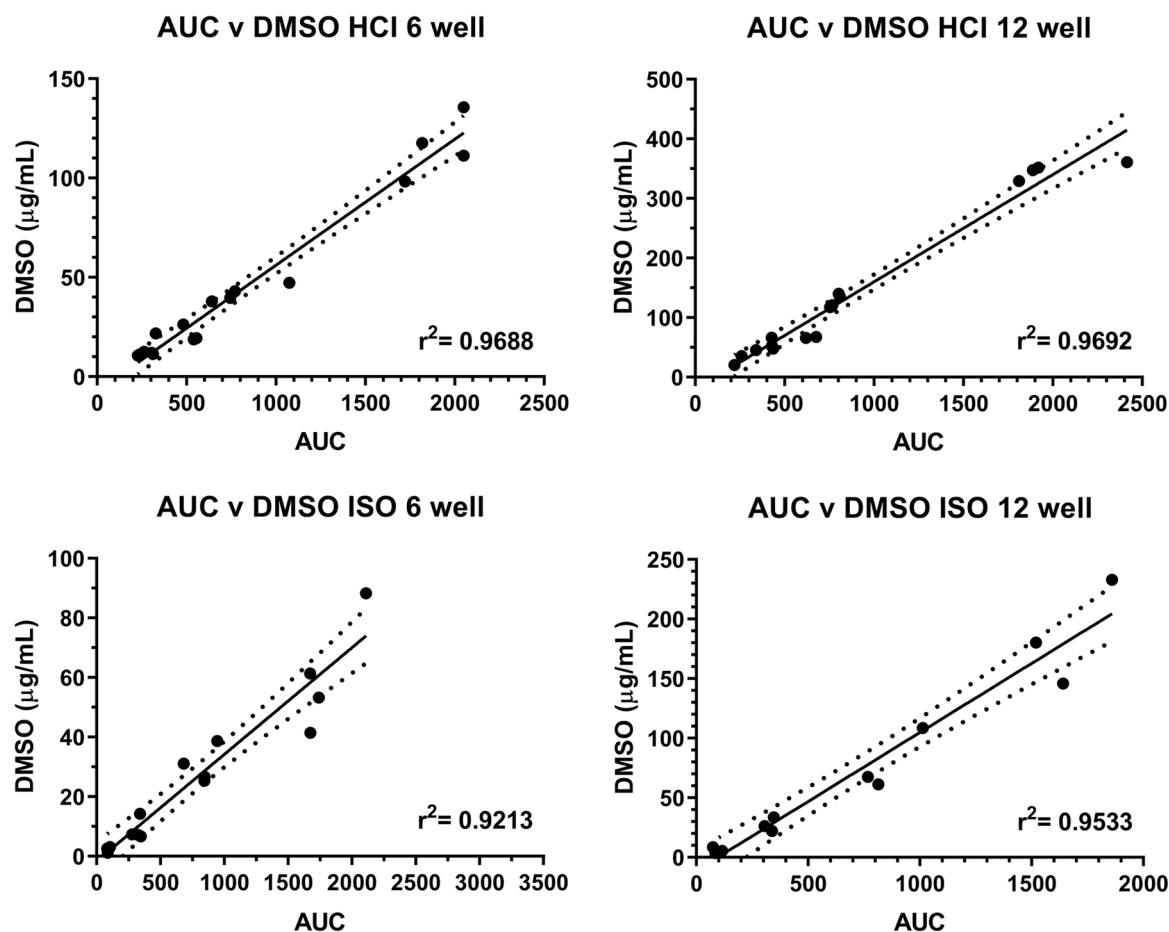


Fig. 4. Consistency in dosimetry measurements using DMSO fluorescence method compared with aerosol photometer in 6- or 12-well test modules using HCl or ISO smoking regimen.

(e.g., cytotoxicity, genotoxicity) for a variety of inhaled materials including tobacco smoke [42,43]; Iskander et al., 2013; [44,45,26,46], Electronic Nicotine Delivery Systems (ENDS) [47], and airborne chemicals and pollutants [48–51]. Quartz crystal microbalances have been used as a dosimetry tool in a variety of smoke/aerosol exposure systems as a means of measuring real-time deposition of particles in smoke/aerosol at a range of dilutions [21–24,26]. Photometers are also used to measure real-time exposure to particles in smoke and other common indoor and outdoor aerosols [27–32]. Exposure and dose measurements calculated for *in vitro* cigarette smoke exposure systems may provide a platform for comparisons between the data generated using different systems and aid in interpretation of the potential biological significance of the results obtained [33]. However, the extrapolation of *in vitro* dosimetry data to actual doses achieved *in vivo* remains challenging. The challenges are associated with multiple factors in each experimental system including use of human primary cells or tissue cultures, species differences, actual exposure system, method for determination of dose, and how the human equivalent exposure concentrations are calculated [34].

The data presented here show that each of the three dosimetry techniques investigated were able to distinguish between different dilutions of whole smoke through the Vitrocell® VC10® *in vitro* exposure system in a concentration-dependent manner. Dosimetry measurements using aerosol photometers, DMSO-captured smoke constituents and QCM each showed a high level of consistency between experiments (within a single technique) and may be considered efficient tools in quantifying dose during an exposure. More variation was observed at the higher airflows (i.e., higher dilutions) using all three assessment

methods; however, this was not unexpected and may be driven by lower and potentially more variable deposition at these higher airflows [42]. Caution should therefore be applied when comparing and interpreting data generated at higher airflows. In addition, all three of these techniques showed a high degree of correlation with one another in measurement of the assessed dose (Figs. 1–3), demonstrating that the amount of smoke constituents delivered into the module as assessed by a photometer correlates with the amount deposited into a dry (QCM) or liquid (DMSO) environment. Therefore, any of these tools are appropriate for the determination of the dose from a combustible tobacco product and for comparison of doses between separate experimental occasions.

Although the general conclusion from this body of work is that any of the dosimetry methods investigated may be used for dose determination from a combustible tobacco product using the Vitrocell® system, there remain challenges associated with each of these dosimetry methods which should be considered in future applications. Specifically, QCMs are widely used for assessment of deposited material from traditional combustible tobacco products but have limitations for assessment of next generation products, especially ENDS, since the crystal can become overloaded in a short time due to the high glycerol and propylene glycol content of their aerosols [52]. DMSO-capture does not provide real-time analysis, so results are available only after the experiment has concluded, and, a standard curve must also be generated. In contrast, photometers, which do allow for real-time assessment, require a positive airflow to function properly and a specific aerosol is needed for calibration. Further research will be necessary to confirm the specific applicability of these dosimetry methods to other tobacco

products (e.g., ENDS and tobacco heating products) [53].

Overall, it is concluded that the dosimetry tools evaluated in this work provide effective measures to identify the concentrations tested during a combustible whole smoke exposure *in vitro*. Deposition measured by QCM, AUC calculations as supplied by the photometers and quantification of captured matter via DMSO provided reproducible and consistent data across all the air flows tested using ISO and HCI smoking regimens.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

None.

Acknowledgements

Sincere thanks to Dr. Stephen Sears and Dr. Wayne Spoo for their critical review of the draft manuscript and constructive editorial comments during the production of this publication. The data presented in the manuscript were generated and analyzed in studies commissioned by RAI Services Company and conducted, under contract, at Covance Laboratories Ltd. Work on the preparation of the manuscript which was contributed by Covance authors was also conducted under contract with RAI Services Company.

References

- Behrsing, E. Hill, H. Raabe, R. Tice, S. Fitzpatrick, R. Devlin, K. Pinkerton, G. Oberdörster, C. Wright, R. Wiecezorek, M. Aufderheide, S. Steiner, T. Krebs, B. Asgharian, R. Corley, M. Oldham, J. Adamson, X. Li, I. Rahman, S. Grego, P.H. Chu, S. McCullough, R. Curren, In vitro exposure systems and dosimetry assessment tools for inhaled tobacco products: workshop proceedings, conclusions and paths forward for in vitro model use, *Altern. Lab. Anim.* 45 (3) (2017) 117–158, <https://doi.org/10.1177/026119291704500305>.
- Health Canada (2018). The Tobacco and Vaping Products Act. Health Canada. Enacted May 23, 2018. Available from <https://www.canada.ca/en/health-canada/services/health-concerns/tobacco/legislation/federal-laws/tobacco-act.html>.
- EU (2014). Directive 2014/40/EU of the European Parliament and of the Council of 3 April 2014 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products and repealing Directive 2001/37/EC. O.J. L 127, 1–38, 29.04.2014. Available from http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL_2014_127_R_000.
- FDA, Family Smoking Prevention and Tobacco Control Act, Available from U.S. Government Public Law 111-31-June 22 2009, 2009, <http://www.gpo.gov/fdsys/pkg/PLAW-111publ31/pdf/PLAW-111publ31.pdf>.
- FDA (2014). Deeming Tobacco Products to Be Subject to the Food, Drug, and Cosmetic Act, As Amended by the Family Smoking Prevention and Tobacco Control Act; Regulations Restricting the Sale and Distribution of Tobacco Products and Required Warning Statements for Tobacco Product Packages and Advertisements. DEPARTMENT OF HEALTH AND HUMAN SERVICES, Food and Drug Administration. Docket No. FDA-2014-N-0189. <http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Reports/EconomicAnalyses/UCM394933.pdf>.
- Health Canada, Regulations amending the tobacco reporting regulations. Statutory instrument registration number SOR/2005-179. June 7, 2005, *Can. Gaz.* 139 (13) (2005) 1523–1526. Available from <http://gazette.gc.ca/rp-pr/p2/2005/2005-06-29/pdf/g2-13913.pdf>. (archived).
- OECD, OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects. Test No. 471. Bacterial Reverse Mutation Test, (1997), <https://doi.org/10.1787/9789264071247-en>.
- OECD, OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects. Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests Using the Hprt and Xprt Genes, (2015), <https://doi.org/10.1787/20745788>.
- OECD, OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects. Test No. 487: In Vitro Mammalian Cell Micronucleus Test, (2016), <https://doi.org/10.1787/20745788>.
- OECD, OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects. Test No. 473: In Vitro Mammalian Chromosomal Aberration Test, (2016), <https://doi.org/10.1787/20745788>.
- OECD, OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects. Test No. 490: In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene, (2016), <https://doi.org/10.1787/20745788>.
- ISO, Biological Evaluation of Medical Devices - Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity. ISO 10993-3, Third Edition 2014-10-01, ISO Copyright Office, Case postale 56, CH-1211, Geneva 20, 2014.
- ICH (2012). Guidance for Industry. S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), June 2012. ICH. Available from <http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm074931.pdf>.
- CDC, The health consequences of smoking — 50 years of progress, A Report of the Surgeon General, US Department of Health and Human Services, 2014, <http://www.surgeongeneral.gov/library/reports/50-years-of-progress/full-report.pdf>.
- L.E. Haswell, K. Hewitt, D. Thorne, A. Richter, M. Gaça, Cigarette smoke total particulate matter increases mucous secreting cell numbers in vitro: a potential model of goblet cell hyperplasia, *Toxicol. In Vitro* 24 (2010) 981–987, <https://doi.org/10.1016/j.tiv.2009.12.019>.
- Y. Fukano, M. Ogura, K. Eguchi, M. Shibagaki, Suzuki M, Modified procedure of a direct in vitro exposure system for mammalian cells to whole cigarette smoke, *Exp. Toxicol. Pathol.* 55 (2004) 317–323, <https://doi.org/10.1078/0940-2993-00341>.
- H. Nara, Y. Fukano, T. Nishino, M. Aufderheide, Detection of the cytotoxicity of water-insoluble fraction of cigarette smoke by direct exposure to cultured cells at an air-liquid interface, *Exp. Toxicol. Pathol.* 65 (5) (2013) 683–688, <https://doi.org/10.1016/j.etp.2012.08.004>.
- D. Ritter, J. Knebel, M. Aufderheide, Comparative assessment of toxicities of mainstream smoke from commercial cigarettes, *Inhal. Toxicol.* 16 (1) (2004) 1–10, <https://doi.org/10.1080/08958370490476659>.
- J. Lin, J. Roy, J. Verreault, S. Talbot, F. Côte, R. Couture, A. Morin, An ex vivo approach to the differential parenchymal responses induced by cigarette whole smoke and its vapor phase, *Toxicology* 293 (2012) 125–131, <https://doi.org/10.1016/j.tox.2012.01.004>.
- P. Görner, D. Bemer, J.F. Fabriés, Photometer measurement of polydisperse aerosols, *J. Aerosol Sci.* 26 (8) (1995) 1281–1302.
- J. Adamson, D. Azzopardi, G. Errington, C. Dickens, J. McAughy, M.D. Gaça, Assessment of an in vitro whole cigarette smoke exposure system: the Borgwaldt RM20S 8-syringe smoking machine, *Chem. Cent. J.* 5 (2011) 50, <https://doi.org/10.1186/1752-153X-5-50>.
- J. Adamson, S. Hughes, D. Azzopardi, J. McAughy, M.D. Gaça, Real-time assessment of cigarette smoke particle deposition in vitro, *Chem. Cent. J.* 6 (2012) 98, <https://doi.org/10.1186/1752-153X-6-98>.
- J. Adamson, D. Thorne, A. Dalrymple, D. Dillon, C. Meredith, Assessment of cigarette smoke particle deposition within the Vitrocell® exposure modules using quartz crystal microbalances, *Chem. Cent. J.* 7 (50) (2013), <https://doi.org/10.1186/1752-153X-7-50> eCollection 2013.
- J. Adamson, D. Thorne, J. McAughy, D. Dillon, C. Meredith, Quantification of cigarette smoke particulate deposition in vitro using a triplicate quartz crystal microbalance exposure chamber, *BioMed Res. Int.* 2013 (2013) 685074, <https://doi.org/10.1155/2013/685074>.
- J. Adamson, D. Thorne, B. Zainuddin, A. Baxter, J. McAughy, M. Gaça, Application of dosimetry tools for the assessment of e-cigarette aerosol and cigarette smoke generated on two different in vitro exposure systems, *Chem. Cent. J.* 10 (2016) 74, <https://doi.org/10.1186/s13065-016-0221-9> Published online 2016 Dec 9.
- D. Thorne, J. Kilford, M. Hollings, A. Dalrymple, M. Ballantyne, C. Meredith, D. Dillon, The mutagenic assessment of mainstream cigarette smoke using the Ames assay: a multi-strain approach, *Mutat. Res. Genetic Tox. Environ. Mutagenesis* 782 (2015) 9–17, <https://doi.org/10.1016/j.mrgtox.2015.03.006>.
- R.T. Jiang, V. Acevedo-Bolton, K.C. Cheng, N.E. Klepeis, W.R. Ott, L.M. Hildemann, Determination of response of real-time SidePak AM510 monitor to second hand smoke, other common indoor aerosols, and outdoor aerosol, *J. Environ. Monit.* 13 (6) (2011) 1695–1702, <https://doi.org/10.1039/c0em00732c>.
- P.J. Dacunto, K.C. Cheng, V. Acevedo-Bolton, R.T. Jiang, N.E. Klepeis, J.L. Repace, W.R. Ott, L.M. Hildemann, Real-time particle monitor calibration factors and PM2.5 emission factors for multiple indoor sources, *Environ. Sci. Process. Impacts* 15 (8) (2013) 1511–1519, <https://doi.org/10.1039/c3em00209h>.
- M.M. Dahm, D.E. Evans, M.K. Schubauer-Berigan, M.E. Birch, J.A. Deddens, Occupational exposure assessment in carbon nanotube and nanofiber primary and secondary manufacturers: mobile direct-reading sampling, *Ann. Occup. Hyg.* 57 (3) (2013) 328–344, <https://doi.org/10.1093/annhyg/mes079>.
- W.A. Heitbrink, L.-M. Lo, K.H. Dunn, Exposure controls for nanomaterials at three manufacturing sites, *J. Occup. Environ. Hyg.* 12 (1) (2015) 16–28, <https://doi.org/10.1080/15459624.2014.930559>.
- K.-H. Lee, H.-J. Jung, D.-U. Park, S.-H. Ryu, B. Kim, K.-C. Ha, S. Kim, G. Yi, C. Yoon, Occupational exposure to diesel particulate matter in municipal household waste workers, *PLoS One* 10 (8) (2015) e0135229, <https://doi.org/10.1371/journal.pone.0135229>.
- S. Jones, T.R. Anthony, S. Soutan, R. Altmaier, J.H. Park, T.M. Peters, Evaluation of a low-cost aerosol sensor to assess dust concentrations in a swine building, *Ann. Occup. Hyg.* 60 (5) (2016) 597–607, <https://doi.org/10.1093/annhyg/mew009>.
- D. Thorne, J. Adamson, A review of in vitro cigarette smoke exposure systems, *Exp. Toxicol. Pathol.* 65 (7-8) (2013) 1183–1193, <https://doi.org/10.1016/j.etp.2013.06.001>.
- X. Li, In vitro toxicity testing of cigarette smoke based on the air-liquid interface exposure: a review, *Toxicol. Vitro* 36 (2016) 105–113, <https://doi.org/10.1016/j.tiv.2016.07.019>.
- Y. Wu, H. Yang, H. Liu, Z. Yang, M. Zhu, X. Pan, T. Huang, K. Zou, J. Bai, Y. Ma, J. Li, Research of herb components on scavenging harmful components and reducing cytotoxicity of cigarette smoke, *Zhongguo Zhong Yao Za Zhi* 36 (22) (2011)

- 3184–3188 [China journal of Chinese materia medica] — Abstract only used, full article available in Chinese.
- [36] G. Pohlmann, A. Preiss, K. Levsen, M. Raabe, W. Koch, Collection, validation and generation of bitumen fumes for inhalation studies in rats Part 2: collection of bitumen fumes from storage tanks, *Ann. Occup. Hyg.* 50 (8) (2006) 805–812, <https://doi.org/10.1093/annhyg/mel048>.
- [37] D.S. Douce, M.R. Clench, B. Frost, Variations in the estimation of the contribution of environmental tobacco smoke (ETS) to respirable (± 5 microns) indoor air particulates obtained by the use of different analytical methods, *J. Environ. Monit.* 3 (3) (2001) 295–301.
- [38] ISO, ISO 3308:2012. Routine Analytical Cigarette-smoking Machine – Definitions and Standard Conditions, International Standard available to purchase from (2012) http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=60404.
- [39] Health Canada, Health Canada Test Method, T-115. Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31, (1999).
- [40] ISO, ISO 3402:1999. Tobacco and Tobacco Products – Atmosphere for Conditioning and Testing, International Standard available to purchase from (1999) <https://www.iso.org/search.html?q=ISO%203402>.
- [41] W.R. Fields, K. Fowler, V. Hargreaves, L. Reeve, B. Bombick, Development, qualification, validation and application of the Neutral Red Uptake assay in Chinese Hamster ovary (CHO) cells using a VITROCELL® VC10® smoke exposure system, *Toxicol. In Vitro* 40 (2017) 144–152, <https://doi.org/10.1016/j.tiv.2017.01.001>.
- [42] J. Adamson, D. Thorne, G. Errington, W. Fields, X. Li, R. Payne, T. Krebs, A. Dalrymple, K. Fowler, D. Dillon, F. Xie, C. Meredith, An inter-machine comparison of tobacco smoke particle deposition in vitro from six independent smoke exposure systems, *Toxicol. Vitro* 28 (2014) 1320–1328, <https://doi.org/10.1016/j.tiv.2014.06.012>.
- [43] S. Ishikawa, Y. Kanemaru, H. Nara, K. Erami, Y. Nagata, Assessing the mutagenic activities of smoke from different cigarettes in direct exposure experiments using the modified Ames *Salmonella* assay, *Mutat. Res.* 803-804 (2016) 13–21, <https://doi.org/10.1016/j.mrgentox.2016.04.008>.
- [44] W.K. Schlage, A.R. Iskandar, R. Kostadinova, Y. Xiang, A. Sewer, S. Majeed, D. Kuehn, S. Frentzel, M. Talikka, M. Geetz, C. Mathis, N. Ivanov, J. Hoeng, M.C. Peitsch, In vitro systems toxicology approach to investigate the effects of repeated cigarette smoke exposure on human buccal and gingival organotypic epithelial tissue cultures, *Toxicol. Mech. Methods* 24 (7) (2014) 470–487, <https://doi.org/10.3109/15376516.2014.943441>.
- [45] D. Thorne, J. Kilford, R. Payne, J. Adamson, K. Scott, A. Dalrymple, C. Meredith, D. Dillon, Characterisation of a Vitrocell VC10 in vitro smoke exposure system using dose tools and biological analysis, *Chem. Cent. J.* 7 (2013) 146–155, <https://doi.org/10.1186/1752-153X-7-146>.
- [46] D. Thorne, D. Breheny, C. Procter, M. Gaca, Assessment of novel tobacco heating product THP1.0. Part 7: comparative *in vitro* toxicological evaluation, *Regul. Toxicol. Pharmacol.* 93 (2018) 71–83, <https://doi.org/10.1016/j.yrtph.2017.08.017>.
- [47] L. Neilson, C. Mankus, D. Thorne, G. Jackson, J. DeBay, C. Meredith, Development of an *in vitro* cytotoxicity model for aerosol exposure using 3D reconstructed human airway tissue; application for assessment of e-cigarette aerosol, *Toxicol. In Vitro* 29 (2015) 1952–1962, <https://doi.org/10.1016/j.tiv.2015.05.018>.
- [48] S.E. Anderson, L.G. Jackson, J. Franko, J.R. Wells, Evaluation of dicarbonyls generated in a simulated indoor air environment using an *in vitro* exposure system, *Toxicol. Sci.* 111 (2010) 453–461, <https://doi.org/10.1093/toxsci/kfq067>.
- [49] M. Aufderheide, J.W. Knebel, D. Ritter, An improved *in vitro* model for testing the pulmonary toxicity of complex mixtures such as cigarette smoke, *Exp. Toxicol. Pathol.* 55 (2003) 51–57, <https://doi.org/10.1078/0940-2993-00298>.
- [50] R. Gminski, T. Tang, V. Mersch-Sundermann, Cytotoxicity and genotoxicity in human lung epithelial A549 cells caused by airborne volatile organic compounds emitted from pine wood and oriented strand boards, *Toxicol. Lett.* 196 (2010) 33–41, <https://doi.org/10.1016/j.toxlet.2010.03.015>.
- [51] C. Persoz, C. Leleub, S. Acharda, M. Fasseuc, J. Menottib, P. Meneceurb, I. Momasa, F. Derouinb, N. Seta, Sequential air-liquid exposure of human respiratory cells to chemical and biological pollutants, *Toxicol. Letters* 207 (2011) 53–59, <https://doi.org/10.1016/j.toxlet.2011.07.028>.
- [52] J. Adamson, X. Li, H. Cui, D. Thorne, F. Xie, M. Gaça, Nicotine quantification *in vitro*: a consistent dosimetry marker for e-cigarette aerosol and cigarette smoke generation, *Appl. In Vitro Toxicol.* 3 (1) (2017) 1–14, <https://doi.org/10.1089/aivt.2016.0025>.
- [53] T. Jaunky, J. Adamson, S. Santopietro, A. Terry, D. Thorne, D. Breheny, C. Procter, M. Gaca, Assessment of tobacco heating product THP1.0. Part 5: *in vitro* dosimetric and cytotoxic assessment, *Regul. Toxicol. Pharmacol.* 93 (2018) 52–61, <https://doi.org/10.1016/j.yrtph.2017.09.016>.