Characterisation of a cigarette smoking machine designed for air-liquid interface exposures: an inter-laboratory study

Adamson J[1], Kaur N[2], Lacasse M[3], Roy J-P[3], Cabral J-L[3], Errington G[1], Morin A[3], Gaça M D[1]

[1]British American Tobacco, GR&D, Southampton, SO15 8TL, UK, [2]Department of Chemistry, University of Montréal, Montréal, Québec, Canada, and [3]Imperial Tobacco Canada Ltd., Montréal, Québec, Canada

Corresponding author: jason_adamson@bat.com

INTRODUCTION

The *in vitro* biological assessment of cigarette smoke is becoming an important area of research. We have previously reported a whole smoke system using a Borgwaldt RM20S and BAT's exposure chamber **[1 - 4]**, exposing cells at the air-liquid interface to different doses of smoke. Recently we conducted an inter-laboratory study with Imperial Tobacco Canada (ITCAN) investigating the repeatability and reproducibility of the RM20S system in Montréal, Canada (4 syringe) and Southampton, UK (8 syringe). With two different operators over several days, using a methane gas standard and hydrocarbon analysis, syringe precision was investigated.

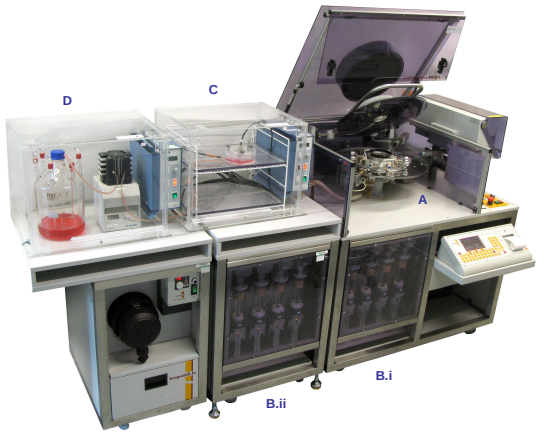

Figure 1. The Borgwaldt RM20S 8-syringe smoking machine and BAT's exposure chamber (pictured in Southampton) [1]. **A** – Cigarette smoke generation; **B.i** – integral 4-syringe unit; **B.ii** – additional 4-syringe unit to increase capacity; **C** – BAT's exposure chamber housed in an incubator at 37ºC, connected to a syringe line from the smoking machine to deliver smoke; **D** – incubator containing cell culture media reservoir at 37ºC which supplies the exposure chamber

SYSTEM SPECIFICATIONS

- 4/8 independent dilutor syringes (Canada/UK) allowing a dose response in a single run
- Smoke dilution range 1:2 - 1:4,000 (smoke:air, v/v ratio)
- ISO standard smoking regime (35ml puff over 2 seconds, once a minute)
- Exposure chamber size: 12cm x 9cm
- Chamber capacity: 3 x 24mm ø inserts, 6 x 12mm ø inserts or 8 x 6mm ø inserts
- Chamber total internal volume: ~188cm^3
- Chamber internal volume of air space (+30ml media): ~140cm^3
- Chamber internal surface area: ~200cm^2

EVALUATION OF SYRINGE PRECISION

RM20S syringes were tested using a methane gas standard and hydrocarbon analysis to assess how accurately they met their target dilutions. Three dilutions were tested 1:1000, 1:500 and 1:193 (smoke:air, v/v) and syringe output was quantified as parts per million (ppm) of methane in the total diluted gas collected. The accuracy and precision of dilution of methane for all 12 individual syringes (in the two locations) and the individual dilution levels were plotted to show syringe bias depending on dilution level, and inter-syringe variability (Fig. 2).

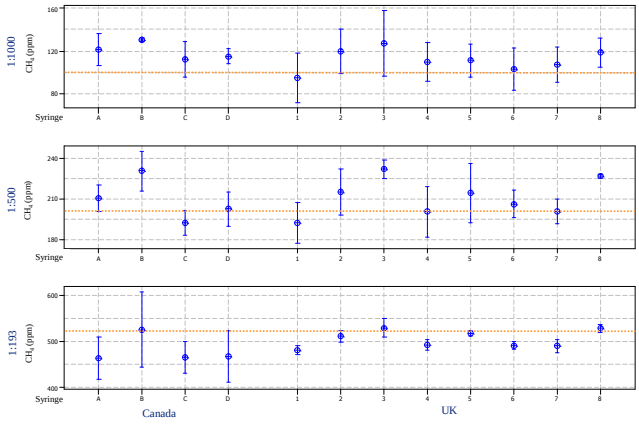

Figure 2. Dilution precision for the individual syringes in both laboratories. Top, middle and bottom panels show dilutions of 1:1000 - target 100 ppm, 1:500 - target 200 ppm, and 1:193 - target 520 ppm, respectively. Syringes A–D in the Canadian laboratory (n=3), syringes 1–8 in the UK laboratory (n = 4) **[2]**

REFERENCES

[1] *Adamson, J., et al.*, 2011. Central Chemistry Journal. 5:50
[2] *Kaur, N., et al.*, 2010. Inhal Toxicol 22: 1174-83
[3] *Thorne, D., et al.*, 2009 Mut Res. 673: 3–8
[4] *Phillips, J., et al.*, 2005. Altern Lab Anim. 33(3):239-48
[5] *Measurement systems analysis, reference manual*, 3rd edition, 2002: Daimler Chrysler Corp., Ford Motor Company, General Motors Corp.
[6] *Scian, MJ., et al.*, 2009. Inhal Toxicol 21: 234-243

Despite some obvious differences between the syringes, the data were still consistent across groups **[2]**. The repeatability and linearity of the dilution range studied had an RSD of 6.3% and 9.0% for dilutions 1:193 and 1:500 respectively (and 17.3% at the highest dilution 1:1000, most likely due to the increased number of serial dilutions/stages where error can occur). Percentage error was between 4.3% - 5.1% (in dilutions <1:500) (Table 1) **[2]**.

Table 1. Overall precision figures of merit of repeatability, reproducibility, RSD, and error values from measured CH_4 for three dilution levels in the two laboratories

Dilution	Target CH_4 (ppm)	Mean CH_4 (ppm)	Repeatability (r)	Reproducibility (R)	RSD (%)	Error (%)
1:193	520	497.8	45.8	88.4	6.3	4.3
1:500	200	210.2	21.3	52.9	9.0	5.1
1:1000	100	113.1	46.4	54.7	17.3	13.1

The data from both locations were plotted together. The almost identical slopes of the combined data reflect the accuracy of CH_4 measurements across the dilution range, and were 0.86 and 0.93 for the Canadian and UK laboratories respectively, and 0.91 for the pooled data (Fig. 3).

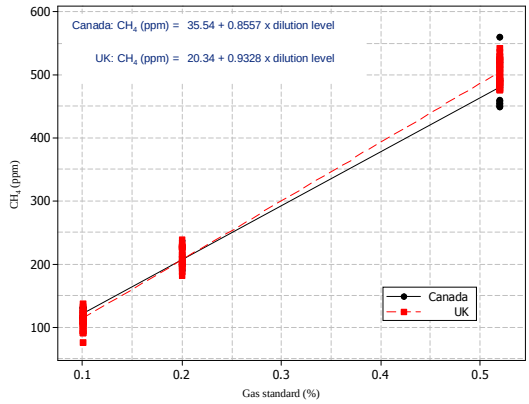

Figure 3. Correlation between quantification of collected methane (10% CH_4 reference standard) at the three dilutions tested. Data acquired from 12 syringes: A-D in Canada, n=3/syringe/dilution; 1-8 in the UK, n=4/syringe/dilution **[2]**

The values obtained from these repeatability experiments were within acceptable limits in measurement systems, within the dilution range in which we expose cell cultures to (RSD <10% and R^2 >0.95 **[5]**), indicating that the equipment can reliably generate accurate doses of cigarette smoke suitable for cell exposure and dosimetry studies.

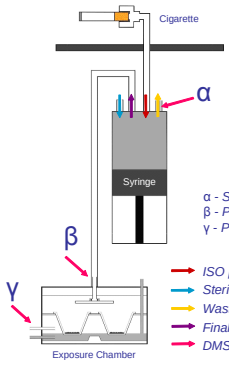

Figure 4. Diagram depicting where smoke was measured. Readings were taken on the syringe and chamber, denoted α, β and γ

Table 2. Smoke particle penetration relative to 100% smoke (10mg 3R4F cigarettes, n=3) [1]

Smoke reaching the dilution syringe	**86** ± 3.2%
Smoke reaching the exposure chamber	**53** ± 5.9%
Smoke exhausting from the chamber	**37** ± 4.9%

SYSTEM SMOKE LOSSES

Using an electrical mobility spectrometer we were able to determine the smoke loss/deposition through the UK system (Fig. 4). Smoke particle concentration was measured at three points in the system: at the syringe exhaust (α), before the exposure chamber (β) to assess the amount of smoke from generation to exposure chamber, and just after the exposure chamber (γ) to indicate the amount of smoke deposition within the chamber. Values obtained were reported relative to 100% smoke mass and indicated that 53% of particles reached the chamber (47% loss) with 16% being deposited in the chamber per 60 seconds residence time per puff (Table 2) **[1]**.

CONCLUSIONS

- These comparative values were within acceptable limits in measurement systems, indicating that the equipment can **reliably generate smoke dilutions**. Having assessed the precision of the RM20S in two different laboratories and with different operators provides us with even greater confidence in the reliability of the machine
- Further assessments of the system dosimetry and smoke losses (shown to be up to 50%) are in-line with published data from other *in vitro* smoking machines such as the Burghart Mimic Smoker-01 **[6]**
- These studies signify the RM20S to be an appropriate exposure system

www.bat-science.com

Poster presented at the CORESTA Smoke Science and Product Technology Joint Study Groups' Meeting, Graz, Austria, October 2011. Poster number: STPOST 08