Nicotine measurement on Cambridge Filter PADs: an interlaboratory comparison to evaluate exposure by different electronic devices and traditional cigarette

Pietro Zuccarello*[1], Rosalia Emma*[2,3], Massimo Caruso[2,3], Roberta Pulvirenti[2], Sonja Rust[4], Konstantinos Poulas[5,6], Fahad Zadjali[7], Silvia Boffo[8], Vladislav Volarevic[9], Ronny Lesmana[10], Sayar R Abdulkhakov[11], Konstantinos Mesiakaris[5,6], Mohammed Al Tobi[7], Antonio Giordano[8], Aleksandar Arsenijevic[9], Melisa I Barliana[12,13], Kristina V Kitaeva[11], Riccardo Polosa[3,14], Giovanni Li Volti[2,3], Margherita Ferrante[1]

Affiliations

1. Department of Medical, Surgical Sciences and Advanced Technologies "G.F. Ingrassia", Catania, Italy.
2. Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
3. Center of Excellence for the Acceleration of Harm Reduction (CoEHAR), University of Catania, Catania, Italy.
4. ECLAT Srl, spin-off of the University of Catania, Catania, Italy
5. Institute for Research and Innovation, IRIS, Patras Science Park, Patras, Greece.
6. Laboratory of Molecular Biology and Immunology, Department of Pharmacy, University of Patras, Patras, Greece.
7. College of Medicine and Health Sciences, Department of Clinical Biochemistry, Sultan Qaboos University, Khodh, Oman.
8. Sbarro Institute for Cancer Research and Molecular Medicine, Department of Biology, College of Science and Technology, Temple University, Philadelphia, USA.
9. Center for Molecular Medicine and Stem Cell Research, Department of Microbiology and Immunology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia.
10. Department of Biomedical Science. Faculty of Medicine Universitas Padjadjaran Bandung, Indonesia.
11. Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Tatarstan, Russian Federation.
12. Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, Indonesia.
13. Centre of Excellence in Higher Education for Pharmaceutical Care Innovation, Universitas Padjadjaran, Bandung, Indonesia.
14. Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy.

* These authors contributed equally to this work

Abstract

Inter-laboratory comparison is widely used to ensure quality control among laboratories. In *in vitro* toxicology studies for tobacco harm reduction (THR), exposure system performance and laboratory proficiency along with product smoke and aerosol stream are tested for variability to assess accuracy. Here we aim to test a novel inter-laboratory setup created in a new collaborative research group using identical and small footprint systems- in order to minimize variability factors and increase reproducibility.

Seven independent laboratories from different geographical areas tested the aerosol and smoke stream and exposure system performance (LM1 and LM4E) using Cambridge Filter Pad (CFP) trapping techniques. We tested 1R6F reference cigarettes, two electronic cigarettes (Vype e-Pen and Vype e-Stick Maxx), and two tobacco heating products (IQOS and Glo™) under the appropriate ISO and/or HCI regimes. Nicotine quantification was performed by GC-FID at the laboratory of the leading center. The performance of participant laboratories was assessed by z-score values obtained from results either in relation to the mean and standard deviation of total participants or in relation to the reference leading center. Z-Scores were satisfactory when $|z| \leq 2$, questionable when $2 < |z| < 3$ and, unsatisfactory when $|z| \geq 3$. In the first evaluation, for all the tested devices, Z- scores values generated by dosimetry data ranged from -2 to +2. However, high intra-laboratory variability (RSD> 10%) was observed for almost all laboratories. In the second, data showed borderline and unsatisfactory exposure performances versus LAB-A. Particularly, Z-scores ≥ 3 were observed once for LAB-B (e-Stick exposure) and LAB-G (e-Pen exposure), twice for LAB-C (1R6F-ISO and e-Stick exposures) and LAB-E (e-Pen and e-Stick exposures), and three times for LAB-F (1R6F-HCI, e-Pen, and Glo exposures).

This study demonstrates that nicotine dosimetry is a fundamental method for quality assurance of smoke/vapor run exposure in the early stage of an interlaboratory study, allowing the identification and possibly the resolution of gaps. Extended practice sessions on exposure runs and several rounds of nicotine dosimetry testing should be planned to keep in check overall equipment and operator performance.

Introduction

The Replica Project is a multicenter study that aims to verify the results of published studies from the tobacco industry and carries the underlined mission to promote research on tobacco Harm Reduction in countries where R&D capacity is needed, including low and middle-income countries [1]. Collaborative research in this field and in *in vitro* research should not be limited to developed countries which directly impact regulatory policy worldwide. Other nationalities should be involved in this type of research and contribute to this discourse from the initial stages of methodology standardization. For this reason, we initiated a collaboration with countries where harm reduction from smoke is relevant and the need for regulatory impact at the local level is necessary.

The use of a multicenter design has been chosen as a method to improve reproducibility and to produce consistent results. In this study, selected international laboratories independently conducted research experiments using shared standard operative procedures (SOPs) in order to obtain consistent results in terms of biological effect. For this purpose, accuracy of smoke and vapor exposure runs is pivotal in supporting the biological testing of next generation nicotine and tobacco products with diverse chemical profiles. Then, interlaboratory comparisons is an important part of quality assurance programs for any multicenter study allowing: I) an assessment of interlaboratory variation; II) identification of any problems , their source, and magnitude; III) to test procedures, equipment performance and the laboratory staff proficiency; IV) to assess agreement and reliability of results [2].

The CORESTA *in vitro* Toxicology Task Force recommends the use of chemical analysis of nicotine content, as well as possibly other constituents, in order to confirm the acceptable performance of the smoking machine and for accuracy within smoking samples [3]. Nicotine is a good analyte to be evaluated as it is common among the inhalable products we want to assess, including cigarettes, electronic cigarettes (e-cig), and tobacco heating products (THPs). Moreover, nicotine quantification on Cambridge filter pads (CFPs) is a simple method to perform, and it is used in several studies as a dosimetry marker to assess *in vitro* exposure systems [4-6].

CFPs are glass fiber filters able to retain at least 99.9 % of all particles contained in tobacco smoke or e-cig aerosol (Total Particulate Matter or TPM) [7, 8], and they are efficient in trapping nicotine at source for both cigarette smoke (at least 99.9%) and e-cig aerosol (> 98%) [9]. In a previous study, we investigated different storing conditions able to guarantee the preservation and stability of the nicotine trapped on CFPs. Results from that study showed that different exposure regimes and products can affect the preservation of nicotine titer on CFPs while sample storage at -80 °C prevented the loss of nicotine for at least 30 days [10]. Based on these results, we centralized shipment of

nicotine dosimetry samples to our chemistry lab which performed both extraction and chemical analysis so as to minimize bias and variability of results due to nicotine extraction procedures and distinct analyzers.

In this study, we performed an interlaboratory comparison of seven independent laboratories located in different geographical areas by nicotine quantification at source in order to verify repeatability and reproducibility, to describe the pattern of variation, and to identify laboratories producing discrepant results. Particularly, we assessed the nicotine yield in CFP using the same test products intended for our *in vitro* research [1], including reference cigarette 1R6F, two electronic cigarettes (Vype e-Pen and Vype e-Stick Maxx), and two THPs (IQOS 3 Duo and Glo™ Pro).

Material and methods

Design and Harmonization

The international partners involved in the REPLICA project were recruited by using online questionnaires as described by Caruso and colleagues (1). Seven laboratories participated in this study: Italy (LAB-A; leading center), Greece (LAB-B), Oman (LAB-C), USA (LAB-D), Serbia (LAB-E), Indonesia (LAB-F), and Russia (LAB-G). The leading laboratory with expertise on smoke and vapor exposure was the LAB-A. In the first phase of harmonization, resources, checklist templates and evaluation procedures were supplied to the team for workshop sessions and hands-on training held in the leading center, then followed by on-site calibration and system maintenance training provided by a certified service engineer and ultimately on-site laboratory capacity compliance and personnel expertise assessments by the leading scientists. Additional on-site training and planned cross border researcher exchange could not be carried out due to unforeseen events (pandemic insurgence). Moreover, detailed SOPs on smoke/vapor CFP exposure method and lab environmental conditions (ISO3308) were reviewed and harmonized across all centers according to the Center for Open Science transparency and openness promotion guidelines (https://www.cos.io/initiatives/top-guidelines). Equipment set up was also harmonized to minimize variability. Precheck lists, schedule and shipment guidelines were made available on our cloud platform as well as written guidelines and datasheet templates used for data collection and technical data recording related to critical protocol steps and environmental status reporting. Partner sample shipments were initially planned to occur during the same time frame. The feasibility was stunted due to university closings and outbreaks and lead time was extended on a case-to-case basis. Individual folders on our dedicated cloud platform were assigned to each laboratory in order to collect data, maintain privacy and prevent bias. To further minimize the variability, all the participating laboratories used the same exposure equipment, the same consumables and key products with the

same lot number, except for 1R6F reference cigarettes which were separately bought from each laboratory to avoid custom issues. Authorization to change to alternative products was required.

Test products and CFP exposure

The following products were used for CFP exposure: 1R6F reference cigarettes (University of Kentucky), Vype ePen3 with "Master Blend" flavored variant containing 18 mg/mL nicotine (British American Tobacco), Vype eStick Maxx with "Toasted Tobacco" flavored variant containing 18 mg/mL nicotine (Nicoventures Trading Ltd), IQOS 3 Duo with HeetSticks "Sienna Selection" * flavoring (Philip Morris International), and Glo™ Pro with Neosticks "Ultramarine" flavoring (British American Tobacco). Borgwaldt LM1 smoking machine and LM4E vaping machines (Borgwaldt KC, Hamburg – Germany) were used to expose CFPs to smoke and vapor respectively.

Reference cigarettes 1R6F were smoked to the length of the filter overwrap +8 mm using both ISO regime (35 mL puff volume, drawn over 2 s, once every minute with ventilation holes unblocked) and HCI regime (55 mL puff volume, drawn over 2 s, once every 30 s with ventilation holes blocked). The smoke generated by each cigarette was captured in line on a 44 mm diameter CFP (Figure 1). Vype ePen3 was vaped following a modified HCI regime (55 mL puff volume, drawn over 2 s, once every 30 s with square shape profile) plus 1 s of pre-activation, for 15 puffs per CFP. Vype eStick Maxx was vaped under CRM81 regime (55 mL puff volume, drawn over 3 s, once every 30 s with square shape profile) for 15 puffs per CFP. Both Heetsticks and Neosticks were puffed following the HCI regimen but with filter vents unblocked for respectively 12 and 8 puffs. The aerosol generated by each e-cig and THP was captured in line on a 44 mm diameter CFP (Figure 2).

Six replicates of each exposure were performed by each laboratory. Each exposed CFP was weighed before and after smoke/vapor exposure in order to quantify the total particulate matter (TPM) and the aerosol mass (AM).

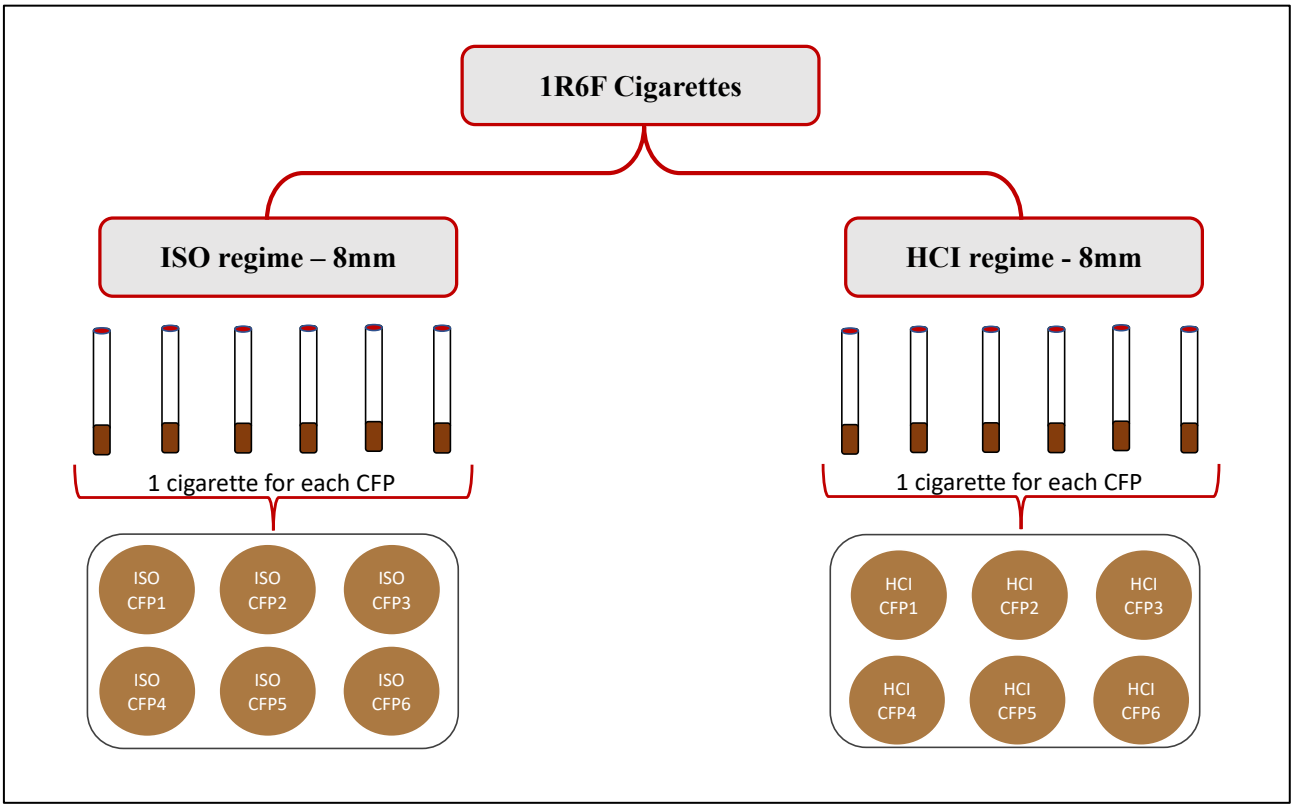

Figure 1. Schematic representation of Cambridge Filter Pad (CFP) exposure with 1R6F reference cigarettes. ISO: International Organization for Standardization; HCI: Health Canada Intense.

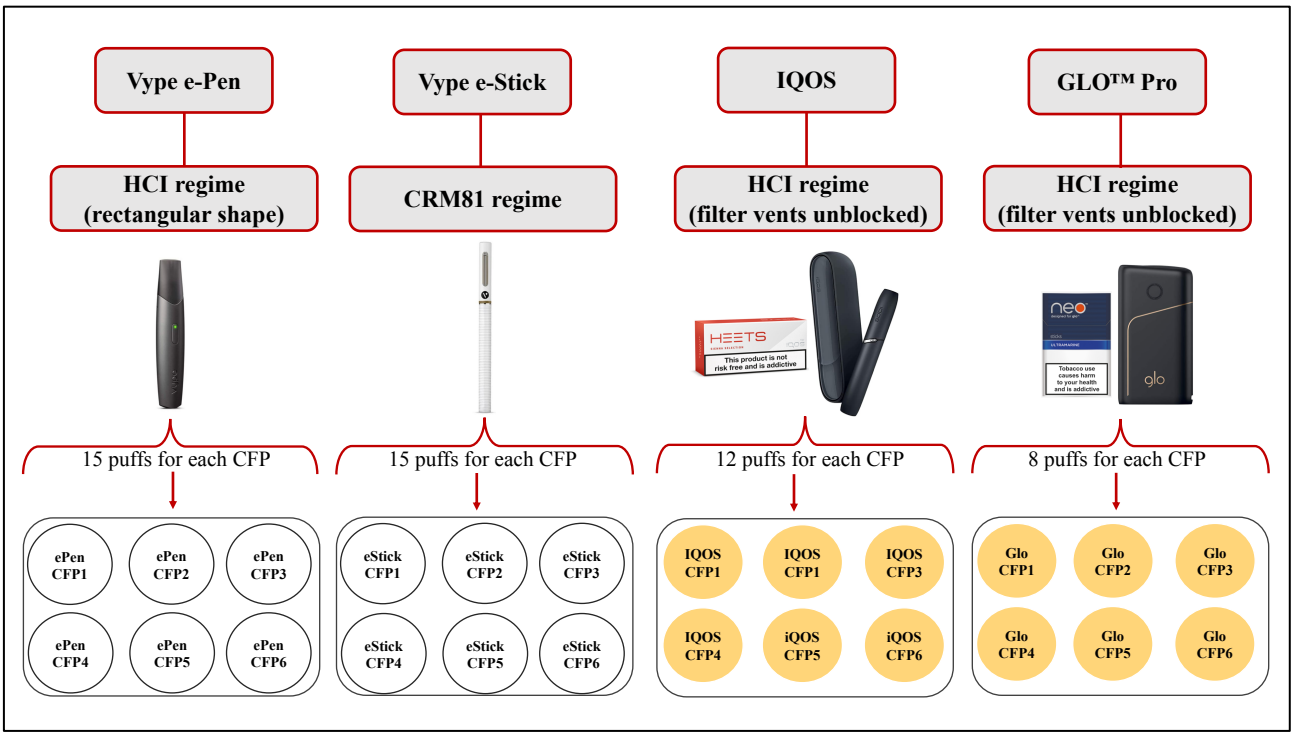

Figure 2. Schematic representation of Cambridge Filter Pad (CFP) exposure with electronic cigarettes (Vype ePen and Vype eStick Maax) and tobacco heating products (THPs - IQOS and Glo). CRM81: Coresta Recommended Method n.81; HCI: Health Canada Intense.

After exposure, each CFP sample was transferred into a clean 15 ml tube labeled as described in SOP. According to our guidelines all the CFP samples were stored at -80 °C, and then shipped with a datalogger in dry ice to LAB-A for nicotine quantification within 30 days after exposure

Calibration curve, CFPs extraction and analysis

Nicotine stock solution at concentration of 100 µg/µL was prepared by weighing 1 g of nicotine at purity of 99% (Sigma Aldrich) into a 10 mL volumetric flask and diluted to volume with acetone. The solution was stored between 0 °C and 4 °C in the dark. Nicotine calibrating standard solutions were prepared at concentration levels 0, 100, 200, 500 and 1000 µg/mL in 1 mL of extraction solution consisting of propan-2-ol with heptadecane at purity of 99% (Sigma Aldrich, cod. 128503-100G) at concentration of 50 µg/L.

CFPs extraction and analysis were performed according to the previous study of Zuccarello et al. [10]. Briefly, CFP was cut into small pieces and transferred into a 15 mL plastic tube containing 10 mL of extraction solvent consisting of isopropanol (LC/MS grade, Carlo Erba) with N-decane (purity 99%, Sigma-Aldrich) (50 µg/mL) as internal standard. Tubes were shaken for 30 min by a vortex at 200 rpm. The samples were then sonicated for 5 min in an ultrasonication bath. Subsequently, 1 ml of each sample was filtered with cellulose acetate filters (mm 25; µm 0.45) and 100 µl of each extract was transferred in a vial with a conical insert for auto-sampler.

Analysis was performed by a gas chromatography Shimadzu (model GC, 2010 AF) coupled with Flame Ionization Detector. An Agilent J&W DB-HeavyWAX Intuvo GC column (30 m × 0.25 mm, 0.25 µm) was used. The GC-FID operating condition and the column oven temperature program are reported as Supplementary Materials.

Data Analysis rationale and Statistics applied

Mean and standard deviation (SD) were calculated for each product tested in the different laboratories. Precision was assessed by computing the Relative Standard Deviation (RSD%) as the percentage ratio between SD and the mean value. The performance of participant laboratories was evaluated for each tested product by z-scores calculation [11].

The z-score is calculated with Equation:

$$\text{z-score} = x_i - \mathbf{x}_{pt}/SD$$

Where:

- for the first evaluation *"Z-Scores vs All"*, x_i is the value obtained by each participant, x_{pt} and SD are respectively the mean and the standard deviation generated from the total participants;

- for the second evaluation *"Z-Scores vs Lab-A"*, x_i is the value obtained by each participant, x_{pt} and SD are respectively the mean and the standard deviation generated from the leading center, i.e. Lab-A;

The evaluation of the results was made according to EN ISO/IEC 17043:2010 [12], as follows:

- satisfactory, when $|z| \leq 2$;
- questionable, when $2 < |z| < 3$;
- unsatisfactory, when $|z| \geq 3$.

All analyses were performed by using RStudio Software (Version 1.2.5001)

Results

Z-scores were calculated on the basis of the mean and standard deviation of the results generated by the total participating laboratories. For all tested devices (1R6F under ISO and HCI regimes, e-Pen, e-Stick, IQOS, and Glo), Z- scores generated by inter laboratory sample analysis range from -2 to +2. In Table 1 and Figure 3 are reported the descriptive statistics and Z-Scores of results generated from all participating laboratories for each lab and each device. Although the Z-Scores are within the satisfactory range, the RDS% values (range from 17% to 29%) showed high intra-laboratory variability among all participants. The LAB-A and LAB-G exhibited a higher precision than the others with all RSD% ≤ 10% except for the RSD% of Glo (13%). Instead, the RSD% of all other laboratories were mostly ≥ 10%, and only in few conditions the RDS% were below the 10%.

Table 1. *Descriptive statistics and Z-Scores of CFP nicotine dosimetry results from all participating laboratories.*

		1R6F-ISO	1R6F-HCI	ePen	eStick	IQOS	Glo
ALL	Mean	482	1215	884	537	957	397
	SD	141	236	180	142	165	106
	RSD%	29	19	20	26	17	27
LAB-A	Mean	517	1251	824	548	1032	455
	SD	51	123	31	22	103	58
	RSD%	10	10	4	4	10	13
	Z-Score *vs* All	0.2476	0.15	-0.3326	0.077	0.452	0.548
LAB-B	Mean	436	1167	806	426	909	389
	SD	28	66	94	106	232	52
	RSD%	6	6	12	25	26	13
	Z-Score *vs* All	-0.331	-0.205	-0.435	-0.78	-0.290	-0.074
LAB-C	Mean	306	1164	829	481	963	305
	SD	157	214	117	49	162	55
	RSD%	51	18	14	10	17	18

	Z-Score *vs* All	-1.246	-0.219	-0.306	-0.395	0.035	-0.870
LAB-D	Mean	665	1371	898	566	917	510
	SD	133	54	119	39	185	59
	RSD%	20	4	13	7	20	12
	Z-Score *vs* All	1.291	0.662	0.081	0.206	-0.241	1.072
LAB-E	Mean	525	1299	999	664	1113	468
	SD	55	88	126	250	84	70
	RSD%	10	7	13	38	8	15
	Z-Score *vs* All	0.299	0.353	0.639	0.893	0.942	0.669
LAB-F	Mean	374	847	1140	NP	812	262
	SD	93	344	229	NP	142	120
	RSD%	25	41	20	NP	17	46
	Z-Score *vs* All	-0.7659	-1.5637	1.4236	NP	-0.8733	-1.2757
LAB-G	Mean	554	1408	691	NP	953	390
	DevSt	54	89	68	NP	80	49
	RSD%	10	6	10	NP	8	13
	Z-Score *vs* All	0.5049	0.8196	-1.0703	NP	-0.0241	-0.0695

Data are reported as µg/CFP. CFP: Cambridge Filter Pad; NP: Not Performed

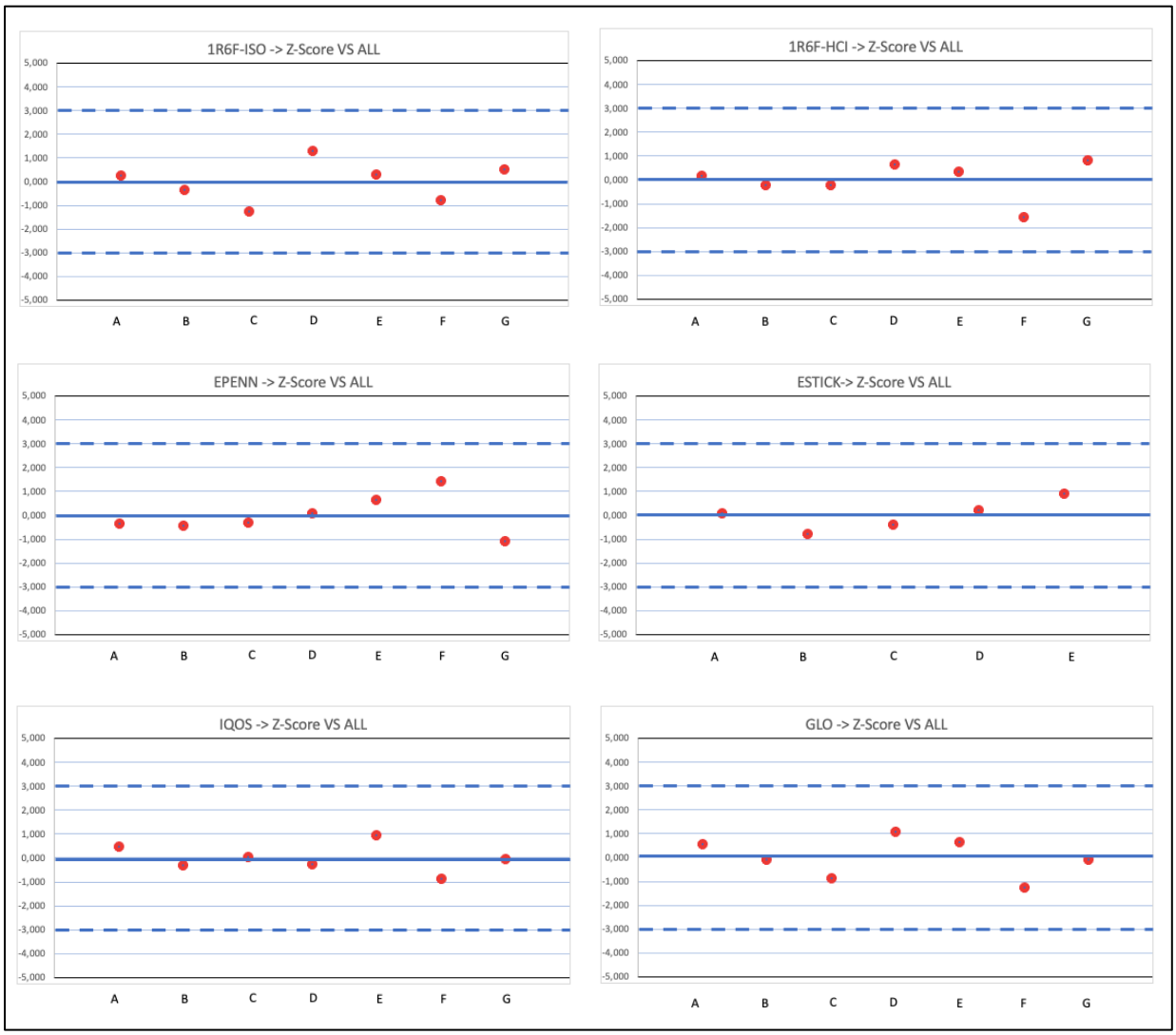

Figure 3. The z-score for the measurement of nicotine in CFPs (⁄g/CFP) after exposure with tested devises (1R6F under ISO and HCI regimes, e-Pen, e-Stick, IQOS, and Glo) generated from mean and standard deviation of 7 laboratories participating.

Further, Z-scores have been calculated based on the mean and standard deviation of reference laboratory (LAB-A) (see Table 2 and Figure 4). These results showed that a substantial number of exposure runs significantly deviated from LAB-A. As per questionable values ($2 < |z| < 3$), these was the results for 1R6F under ISO regime from LAB-D (+2.863) and LAB-F (-2.781); for e-Pen from LAB-D (+2.419); for IQOS from LAB-F (-2.124); for Glo from LAB-C (-2.600). Some laboratories were not within the acceptable interval ($|z| \geq 3$): for 1R6F under ISO regime, LAB-C (-4.099); for 1R6F under HCI regime, LAB-F (-3.293); for e-Pen, LAB-E (+5.678), LAB-F (10.266) and LAB-G (-4.313); for e-Stick, LAB-B (-5.527), LAB-C (-3.040) and LAB-E (5.254); for IQOS, LAB-F (-3,058); for Glo, LAB-C (-4,605) and LAB-F (-3.344).

Table 3. Descriptive statistics and Z-Scores of CFP nicotine dosimetry results from all participating laboratories vs LAB-A (leading center).

		1R6F-ISO	1R6F-HCI	ePen	eStick	IQOS	Glo
LAB-A	Mean	517	1251	824	548	1032	455
	SD	51	123	31	22	103	58
	RSD%	10	10	4	4	10	13
LAB-B	Mean	436	1167	806	426	909	389
	SD	28	66	94	106	232	52
	RSD%	6	6	12	25	26	13
	Z-Score *vs* LAB-A	-1.587	-0.685	-0.596	-5.527	-1.189	-1.142
LAB-C	Mean	306	1164	829	481	963	305
	SD	157	214	117	49	162	55
	RSD%	51	18	14	10	17	18
	Z-Score *vs* LAB-A	-4.099	-0.713	0.155	-3.040	-0.669	-2.600
LAB-D	Mean	665	1371	898	566	917	510
	SD	133	54	119	39	185	59
	RSD%	20	4	13	7	20	12
	Z-Score *vs* LAB-A	2.863	0.978	2.419	0.826	-1.111	0.960
LAB-E	Mean	525	1299	999	664	1113	468
	SD	55	88	126	250	84	70
	RSD%	10	7	13	38	8	15
	Z-Score *vs* LAB-A	0.142	0.384	5.678	5.254	0.784	0.220
LAB-F	Mean	374	847	1140	NP	812	262
	SD	93	344	229	NP	142	120
	RSD%	25	41	20	NP	17	46
	Z-Score *vs* LAB-A	-2.781	-3.293	10.266	NP	-2.124	-3.344
LAB-G	Mean	554	1408	691	NP	953	390
	SD	54	89	68	NP	80	49
	RSD%	10	6	10	NP	8	13
	Z-Score *vs* LAB-A	0.706	1.280	-4.313	NP	-0.763	-1.133

Data are reported as µg/CFP. CFP: Cambridge Filter Pad; NP: Not Performed

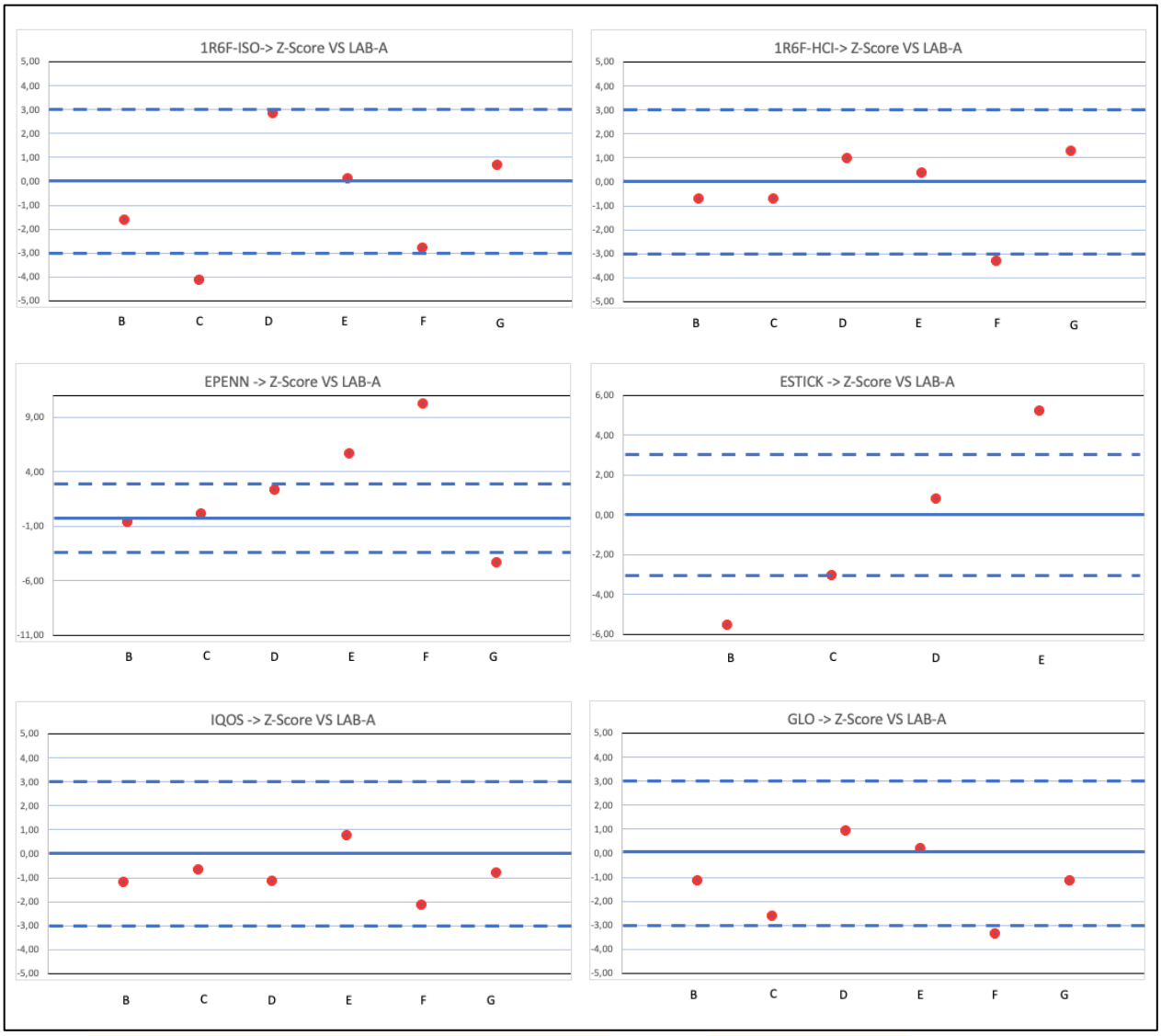

Figure 4. The z-score for the measurement of nicotine in CFPs (µg/CFP) after exposure with tested devises (1R6F under ISO and HCI regimes, e-Pen, e-Stick, IQOS, and Glo) generated from mean and standard deviation of leading center.

Discussion

Combined Nicotine dosimetry and CFP trapping techniques were used here to assess smoking and vaping exposure system performance for product testing and comparison. This was performed under an inter-laboratory setting across several geographical areas.

Aerosol and smoke generated by 1R6F reference cigarettes, and Reduced Risk Products (RRPs) such as e-Pen, e-Stick, IQOS, and Glo™ were trapped in a filter pad right behind the equipment port where test products are inserted. Data was generated by isolation of nicotine from total particulate matter (TPM) and aerosol matter (AM) through gas chromatography coupled with Flame Ionization Detector (GC-FID).

From the data generated across all labs we observed agreement when the z-scores were calculated versus the overall mean. Instead, variability was observed for some laboratories when z-scores were calculated *versus* the mean of LAB-A (leading center).

The first step to evaluate different exposure systems in an *in vitro* interlaboratory study is the assessment of aerosol generation accuracy. Without this step, any subsequent *in vitro* data generated with these systems cannot produce reliable data [13]. Dosimetry of nicotine can assess many aspects of smoke and vapor exposures. It can allow several test products for direct comparison, be used as a quality assurance tool during exposure, and demonstrate physiologically relevant exposure [5, 6, 13]. CFP trapping method is used to trap aerosol and smoke compounds at source avoiding loss so as to reduce variability due to equipment artifacts. To further minimize the variability and improve reproducibility of results across the board, all partners received the same exposure systems and accessories for smoking and vaping experimental set up, and the same test product lot (except to 1R6F reference cigarettes). Moreover, based on previous published data on nicotine stability in CFP, extraction and analysis have been performed by the leading center in order to avoid bias and variability. Samples were shipped and stored according to our previous study showing that freezing at -80 °C preserves nicotine stability on CFPs for at least one month [10]. Also, internal standards were included.

Two types of analysis were performed on the collected data. The first, by comparing each lab data to the average value of all laboratory results. From this analysis, all the Z-scores of each laboratory fall into the satisfactory range (from -2 to +2) suggesting a good reproducibility of exposures. The second analysis was performed by comparing data from each lab with the mean of the leading laboratory results. We used the data of LAB-A as a reference laboratory because LAB-A was more experienced in performing smoke and vapor exposures. Moreover, LAB-A showed minor variability (between 4 and 13%) in their exposure data. Indeed, comparing interlaboratory data overall, the results indicated that the precision achieved under the same conditions by the leading center further validates its role as laboratory reference. The comparison between the REPLICA partners and the leading center has shown that most laboratories were outside the parameters on one or more occasions indicating poor reproducibility. Only LAB-D presented Z score values between -3 and +3 for all test exposures and conditions. LAB-B and LAB-G underperformed only once. LAB-C and LAB-E performances were unsatisfactory for two conditions. However, LAB-F underperformed on three accounts, and the other three exposure performances showed Z-score > 2. From a product stand point, the two electronic cigarettes, e-Pen and e-Stick, gave the weakest reproducibility values across all labs, and IQOS exposures exhibited high intra-laboratory variability for four out of seven laboratories. Considering

product mouthpieces, shape and activation type, port-device interface handling along with general expertise in using smoking/vaping machines are two key points to highlight.

The present study raises some important issues regarding the difficulty of harmonizing interlaboratory performance in newly formed collaborations under limited in-person training, despite the use of the same equipment and key products, suggesting that data variability may be attributable to the exposure stage. Apart from variations of supposedly identical test products, the following factors may contribute to the variability of a test procedure: the operator, calibration of equipment, environmental conditions (temperature, humidity, air pollution, etc.), time elapsed between measurements. The comparison with the leading center undoubtedly has shown the difficulty to minimize variability and the need to further address each stage of the harmonization process. The current literature on inhalation products also addresses the same concerns [13, 14].

COVID-19 pandemic was another factor that indirectly affected the results. During the pandemic insurgence, the technical personnel of the smoking machines producer had to shorten their stay or had to support lab technicians with virtual guidance to set up the equipment. Hence, we cannot exclude possible calibration issues in those laboratories.

Partner sample shipments were initially planned to occur at the same time. The feasibility was stunted due to university closings, outbreaks, airport shutdowns and crippled logistics, therefore lead time had to be extended on a case by case basis over a 16 month period. Analyses were not performed as scheduled and some delays on the 30 day stability window may have impacted the results.

The initial data collected had early indicators of variability leading us to reassess our harmonization strategy. In this second phase we were able to pinpoint some of these issues and come up with several solutions, including remote support and assistance during exposure sessions, videos of critical steps and SOPs enriched with photos and schematic representations. Additional on-site training and planned cross border researcher exchange could not be carried out. Most of the performed on-site compliance checks and training were time limited and therefore we switched to a virtual format. The latter was implemented post initial data assessment, clearly indicating that additional training was needed. As part of a contingency plan we strengthen our virtual role and our digital platform by providing interactive lab support sessions and creating multimedia resources with lab videoclips to satisfy the gaps on visual technical resources in this niche environment – smoke and aerosol exposure systems.

Undoubtedly, although technology is an excellent tool for supporting scientific needs, it cannot be a substitute for direct interaction, especially in a newly formed international collaboration. However, the initial data received gave us time to refine our measures, improve communication and reassess

with our partners the areas that needed to be improved: instrument maintenance and cleaning, proper test product conditioning and handling, and clarification on procedural issues. Since in person training was not feasible, we resorted to optimizing guided practice through interactive lab video sessions and videoclips of key exposure processes. Because of time constraints we were not able to repeat the dosimetry. However, results of bioassays later performed are evidence of agreement within laboratories [1]. Hence, our interventions during the second phase have shown to be effective. We plan to repeat nicotine dosimetry testing prior to further research.

There are a few considerations and recommendations to this study which the authors acknowledge. Dosimetry methods (e.g nicotine quantification) should be used repeatedly when an interlaboratory study on smoke and vapor exposure is planned in order to provide quality assurance of the exposure methods. Training exercises in exposure runs should be also planned as a continuum in an interlaboratory study since time and space can be a limiting factor for laboratories with limited smoke and aerosol exposure system experience. Several rounds of nicotine dosimetry testing should be required to keep in check overall equipment and operator performance since undetected problems can arise during long-term projects. The study pinpoints the difficulty incurred, the lesson learned and the solution implemented before phasing to biological analysis. The study demonstrates that inter-laboratory temporal, geographical, cultural, professional composition may be affected by extrinsic variables overtime and that standardized harmonization strategies may need to be readdressed ad hoc and in a continuum. This work has the ambition to be used as a tool for newly formed inter-laboratory collaborations for *in vitro* exposure studies. When optimization is reached, this novel inter-laboratory set up - using identical and small footprint systems - may be a feasible solution for regions with limited R&D capacity interested in conducting new collaborative research on tobacco harm reduction and possibly impacting local regulatory policies.

Funding

This investigator initiated study was sponsored by ECLAT srl, a spin-off of the University of Catania, with the help of a grant from the Foundation for a Smoke-Free World Inc., a US nonprofit 501(c)(3) private foundation with a mission to end smoking in this generation. The contents, selection, and presentation of facts, as well as any opinions expressed herein are the sole responsibility of the authors and under no circumstances shall be regarded as reflecting the positions of the Foundation for a Smoke-Free World, Inc. ECLAT srl is a research based company from the University of Catania that delivers solutions to global health problems with special emphasis on harm minimization and technological innovation.

Competing Interests

Riccardo Polosa is full tenured professor of Internal Medicine at the University of Catania (Italy) and Medical Director of the Institute for Internal Medicine and Clinical Immunology at the same University. In relation to his recent work in the area of respiratory diseases, clinical immunology, and tobacco control, RP has received lecture fees and research funding from Pfizer, GlaxoSmithKline, CV Therapeutics, NeuroSearch A/S, Sandoz, MSD, Boehringer Ingelheim, Novartis, Duska Therapeutics, and Forest Laboratories. Lecture fees from a number of European EC industry and trade associations (including FIVAPE in France and FIESEL in Italy) were directly donated to vaper advocacy no-profit organizations. RP has also received grants from European Commission initiatives (U-BIOPRED and AIRPROM) and from the Integral Rheumatology & Immunology Specialists Network (IRIS) initiative. He has also served as a consultant for Pfizer, Global Health Alliance for treatment of tobacco dependence, CV Therapeutics, Boehringer Ingelheim, Novartis, Duska Therapeutics, ECITA (Electronic Cigarette Industry Trade Association, in the UK), Arbi Group Srl., Health Diplomats, and Sermo Inc. RP has served on the Medical and Scientific Advisory Board of Cordex Pharma, Inc., CV Therapeutics, Duska Therapeutics Inc, Pfizer, and PharmaCielo. RP is being paid textbook royalties from ELSEVIER. RP is also founder of the Center for Tobacco prevention and treatment (CPCT) at the University of Catania and of the Center of Excellence for the acceleration of Harm Reduction (CoEHAR) at the same University, which has received support from Foundation for a Smoke Free World to conduct 8 independent investigator-initiated research projects on harm reduction. RP currently involved in a patent application concerning an app tracker for smoking behaviour developed for ECLAT Srl. RP is also currently involved in the following pro bono activities: scientific advisor for LIAF, Lega Italiana Anti Fumo (Italian acronym for Italian Anti-Smoking League), the Consumer Advocates for Smoke-free Alternatives (CASAA) and the International Network of Nicotine Consumers Organizations (INNCO); Chair of the European Technical Committee for standardization on “Requirements and test methods for emissions of electronic cigarettes” (CEN/TC 437; WG4).

Giovanni Li Volti is currently elected Director of the Center of Excellence for the acceleration of HArm Reduction. Konstantinos Poulas has received service grants and research funding from a number of Vaping Companies. He is the Head of the Institute of Research and Innovations, which has received a grant from the Foundation for a Smoke Free World. All other authors declare no competing interests.

References

1. Caruso, M., et al., *Electronic nicotine delivery systems exhibit reduced bronchial epithelial cells toxicity compared to cigarette: the Replica Project.* Sci Rep, 2021. **11**(1): p. 24182.
2. Scott, E.M., D.D. Harkness, and G.T. Cook, *Interlaboratory Comparisons: Lessons Learned*. 1998, Department of Geosciences, The University of Arizona. p. 331 - 340.
3. Jordan, K., et al., *Rationale and Strategy for In Vitro Toxicity Testing of Combustible Tobacco Products* 2019, Cooperation Centre for Scientific Research Relative to Tobacco
4. Adamson, J., et al., *Characterisation of the borgwaldt LM4E system for in vitro exposures to undiluted aerosols from next generation tobacco and nicotine products (NGPs).* Food Chem Toxicol, 2018. **113**: p. 337-344.
5. Adamson, J., et al., *Nicotine quantification in vitro: a consistent dosimetry marker for e-cigarette aerosol and cigarette smoke generation.* Applied In Vitro Toxicology, 2017. **3**(1): p. 14-27.
6. Adamson, J., et al., *Application of dosimetry tools for the assessment of e-cigarette aerosol and cigarette smoke generated on two different in vitro exposure systems.* Chemistry Central Journal, 2016. **10**(1): p. 1-16.
7. ISO International Organization for Standardization, *ISO/DIS 24197 Vapour products — Determination of e-liquid vaporised mass and aerosol collected mass*. 2022, ISO.
8. Maskos, Z., L. Khachatryan, and B. Dellinger, *Role of the Filters in the Formation and Stabilization of Semiquinone Radicals Collected from Cigarette Smoke.* Energy Fuels, 2013. **27**(9).
9. Alderman, S.L., et al., *Particle size distribution of e-cigarette aerosols and the relationship to Cambridge filter pad collection efficiency.* Contrib. Tob. Res, 2015. **26**(4): p. 183-190.
10. Zuccarello, P., et al., *Nicotine dosimetry and stability in cambridge filter PADs (CFPs) following different smoking regime protocols and storage conditions.* Regulatory Toxicology and Pharmacology, 2021. **122**: p. 104917.
11. ISO International Organization for Standardization, *ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison*. 2015, ISO.
12. ISO International Organization for Standardization, *ISO/IEC 17043:2010 Conformity assessment — General requirements for proficiency testing*. 2010, ISO.
13. Thorne, D.A., Jason; Sticken, Edgar Trelles; Wieczorek, Roman; Behrsing, Holger; Steiner, Sandro; Majeed, Shoaib; Frentzel, Stefan; Ishikawa, Shinkichi; Ito, Shigeaki; Simms, Liam; Yoshino, Kei; Hoeng, Julia; Gaca, Marianna, *An interlaboratory in vitro aerosol exposure system reference study.* 2021.
14. Lacroix, G., et al., *Air-Liquid Interface In Vitro Models for Respiratory Toxicology Research: Consensus Workshop and Recommendations.* Appl In Vitro Toxicol, 2018. **4**(2): p. 91-106.