Investigating the transfer rate of waterpipe additives to smoke as an integral part of toxicological risk assessments

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ABSTRACT

Waterpipe, also known as hookah, narghile or narghila, shisha or hubbly bubbly, is a tobacco-smoking device. Waterpipe tobacco is heated and consumed by a process of inhaling tobacco smoke, that bubbles through water before being inhaled. To date, limited studies have examined the transfer of waterpipe additives from tobacco to smoke. This study was designed to investigate the filtration ability of water in the waterpipe's bowl to define exposure to additives in waterpipe smoke, which is an essential requirement to perform toxicological risk assessments of waterpipe additives. Within this study, a standard smoking protocol (ISO 22486) was used to evaluate the transfer of > 40 additives from experimental and commercially available samples. These results are the first to provide such an extensive dataset of information showing transfer rates varying between 6% and 61% depending on the additive. Various physicochemical parameters of the additives including water solubility, partition coefficient, molecular weight, boiling point, and vapor pressure were also evaluated to seek to identify any correlation to transfer rate that may be later used to predict transfer. The amount of additive transfer from waterpipe tobacco to the smoke was found to be moderately correlated to vapor pressure (Pearson correlation coefficient = 0.33) with subsequent multivariate analysis using step-wise selection indicating 39% of the transfer rate variance can be explained collectively by the additive boiling point, molecular weight, vapor pressure and water solubility. These findings underscore the complexity of additive transfer and highlight the necessity of exposure assessment for meaningful waterpipe additive risk assessments.

1. Introduction

Waterpipe, also known as hookah, narghile or narghila, shisha or hubbly bubbly, is a tobacco-smoking device. Waterpipe tobacco is heated and inhaled after bubbling through the water in the waterpipe bowl. To date, no comprehensive studies have determined the transfer of waterpipe ingredients from tobacco to waterpipe smoke. This study was designed to investigate the filtration ability of water in the waterpipe's bowl to define exposure to additives in waterpipe smoke, which is an essential requirement to perform toxicological risk assessments of waterpipe additives.

When cigarettes are smoked, the tobacco is burnt, and additives may be combusted or distill largely unchanged into the mainstream and side stream smoke at a ratio of 20/80 [4]. In contrast, waterpipe tobacco is heated giving a limited 'side stream' smoke. As there is an increased likelihood of additives distilling directly into waterpipe smoke intact with the only barrier to consumer exposure being possible filtration by the water in the bowl, waterpipe tobacco consumers may be exposed to higher levels of neat additives.

As an initial step in our Product Stewardship risk assessment process, potential additives are screened to ensure they are not Carcinogenic, Mutagenic, Reproductive (CMR*) toxicants or Respiratory sensitizers. Additives that fall into any of these hazard categories are rejected and not used, (see below, Fig. 1.).

However, if an additive passes this screening phase, a full assessment is then conducted. A key step in any risk assessment process is to estimate exposure and as such, this testing is essential to know the precise amount of additive that transfers to waterpipe smoke under conditions of use. In the absence of such information, a default value of 100% transfer would be assumed.

Considering the inhalation route of exposure, the transfer information, documented consumer behavior and the toxicological properties of

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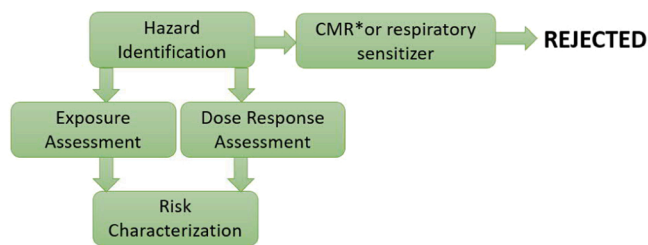
Fig. 1. Waterpipe additive risk assessments the process.

Table 1
Documented smoking procedure.

Parameter	Setting
Puff duration	2.6 s
Puff volume	530 ml
Puff frequency	3 puffs/ min
Total number of puffs	175
Puff profile	Rectangular
Water volume in waterpipe bowl	750 ml
Depth of immersion of smoke column	30 mm
Length of hose 1	100 cm

the respective additive, all information is then subsequently combined to complete a full risk characterization.

In this study we quantified the transfer of > 40 additives from waterpipe tobacco to waterpipe smoke under conditions of use in addition to investigating possible correlations between transfer rates and the physicochemical properties of the additives.

2. Materials and methods

2.1. Determination of additive analytes in waterpipe tobacco

2.5–5 g of commercially available or experimental waterpipe tobacco blends were weighed into a 250 ml Erlenmeyer flask.

The extraction solvent depended on the physicochemical properties of the analytes. A procedure successful for many analytes was the following: The sample was extracted with 25 ml methanol under ultrasound for 5 min. After the addition of 50 ml of ethyl acetate the sample was shaken on a laboratory shaker at 200 rpm for 30 min

In some cases, solid phase extraction was applied for purification of the extracts. Sample extract was then passed through a conditioned Strata-X SPE cartridge (500 mg), the eluate was collected and subsequently dried with sodium sulfate. For highly concentrated analytes the extract was diluted with extraction solvent / suitable solvent. Aliquots of the eluate were spiked at three levels of analyte standard solutions for quantitation by standard addition [3]; (European Commission; SANTE/

11312/2021) and analyzed by gas chromatography coupled to mass spectrometry using electron ionization (GC-EI-MS).

2.2. Determination of additive analytes in waterpipe mainstream smoke

Waterpipe smoking samples were conditioned at 22 ± 2 °C [10] in a closed container for at least 12 h prior to smoking [11]. 10.00 ± 0.05 g of waterpipe tobacco was weighed into the waterpipe head and covered with perforated aluminum foil. The exact weight of the waterpipe tobacco was used for the calculations. The samples were smoked using a laboratory waterpipe (Borgwaldt) and a shisha smoking machine (Linear shisha smoking machine, Borgwaldt) following the procedure described in [12], with one exception. In order to achieve a more realistic simulation of a typical smoking session [15,16], one piece of quick lighting waterpipe charcoal was used for heating the tobacco instead of an electrical heating device. The smoking conditions are outlined in Table 1.

The mainstream smoke was collected on 92 mm glass fiber filter pads followed by up to three impingers, filled with solvent (see Fig. 2.). The type of solvent, its volume and temperature of the traps was dependent on the additive to be analyzed. A $1 + 2$ (v:v) mixture of methanol and ethyl acetate cooled to ≤ 10 °C with a slurry of crushed ice and sodium chloride was successfully applied for many additives. Preliminary experiments were conducted ahead of the main experiments to demonstrate a trapping efficiency of > 90%.

Filter pads were exchanged as necessary to avoid exceeding 600 mg of Total Collected Matter (TCM) per filter, using up to 10 filter pads per smoking session. The TCM was determined by differential weighing of the filter pads (ISO/TS 22487, 2019).

For all studies, the collected quantity of TCM per smoking run was reported with the Relative Standard Deviation (RSD) only deemed acceptable at ∼ 20%. Additionally, although standardized instrumentation and equipment were used, the experimental procedure was further optimized as much as possible by:

- Using the same operators.
- Adopting a unified procedure for charcoal lighting.
- Using a unified procedure on how tight or loose the waterpipe tobacco was placed into the head.
- Using the same ratio of fibers and liquid part of the waterpipe tobacco.
- Using the same lot of charcoal.

2.2.1. Sample preparation

Filter pads were collected in a 1000 ml glass bottle. After the smoking session, the hoses connecting the filter and impingers (see Fig. 2.) were each rinsed with 10 ml of solvent. The impinger and rinsing solutions were added to the filter pads followed by 120 ml of solvent
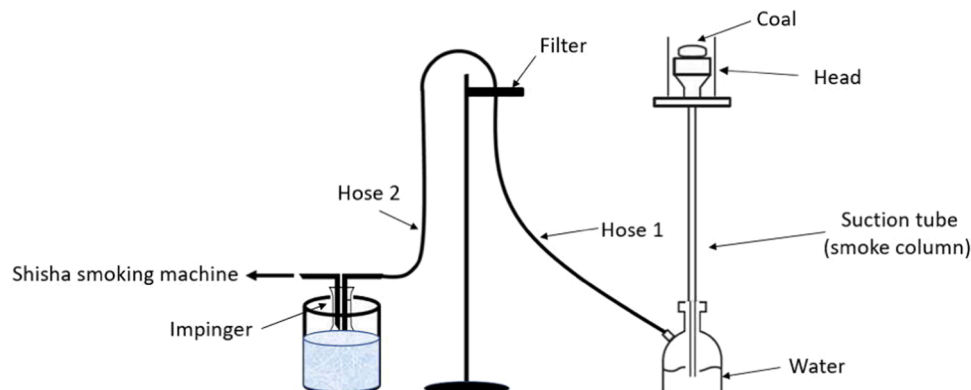

Fig. 2. Analyte Trapping System. Simplified schematic of trapping system: Waterpipe bowl – Hose 1 – Filter – Hose 2 – Impinger – Pump.

Table 2
Additive transfer to waterpipe smoke and associated physicochemical properties.

Name	CAS number	log K_{ow}	Molecular weight [g/mol]	Vapor pressure @ 25 °C [mm Hg]	Water solubility @ 25 °C [g/L]	Boiling point [°C]	Smoke transfer [%] *
para-Toluldehyde	104–87–0	2.26	120.2	0.2500	2.27	205	6
Citral	5392–40–5	3.45	152.2	0.0910	0.59	227	8
cis-3-Hexen-1-ol	928–96–1	1.46	100.2	1.3600	187	157	9
Phenethyl acetate	103–45–7	2.30	164.2	0.0683	0.71	233	9
Benzaldehyde	100–52–7	1.71	106.1	1.2676	6.95	179	10
Benzyl acetate	140–11–4	1.96	150.2	0.1770	3.1	213	10
L-Carvone	6485–40–1	2.71	150.2	0.1150	1.3	229	10
Methyl benzoate	93–58–3	1.83	136.2	0.3790	2.1	199	10
Ethyl benzoate	93–89–0	2.40	150.2	0.1970	0.4215	216	11
para-Methoxybenzaldehyde	123–11–5	1.76	136.1	0.0329	4.29	248	11
Menthone	89–80–5	2.93	154.3	0.2780	0.688	207	11
Hexanol	111–27–3	2.03	102.2	0.9280	5.9	158	12
Menthol	2216–51–5	3.40	156.3	0.0008	0.49	212	14
beta-Damascenone	23696–85–7	4.21	190.3	0.0382	0.19	266	15
Diphenyl oxide	101–84–8	4.13	170.2	0.0202	0.018	258	16
Glycerol	56–81–5	-1.76	92.1	0.0002	1000	290	17
Linalool	78–70–6	2.97	154.3	0.1600	1.56	197	17
Propionic acid	79–09–4	0.33	74.1	6.0400	173.6	145	18
Phenyl carbinol	100–51–6	1.10	108.1	0.0535	40	205	19
Acetanisole	100–06–1	1.74	150.2	0.0064	2.5	258	20
Eugenol	97–53–0	2.27	164.2	0.0095	2.45	248	23
Benzyl carbinol	60–12–8	1.36	122.2	0.0870	22.2	218	23
Geraniol	106–24–1	3.56	154.3	0.0300	0.1	229	26
trans-Anethole	4180–23–8	3.40	148.0	0.0690	0.111	235	26
Cinnamaldehyde	104–55–2	1.90	132.2	0.0289	2.865	250	26
Acetic acid	64–19–7	-0.17	60.1	17.200	475.9	122	27
4-(para-Hydroxyphenyl)-2-butanone	5471–51–2	1.22	164.2	0.0007	25.1	281	30
Eucalyptol	470–82–6	2.74	154.3	1.9000	3.5	176	30
alpha-Ionone	127–41–3	3.29	192.3	0.0271	0.106	250	31
Ethyl cinnamate	103–36–6	2.99	176.2	0.0033	0.178	271	35
Ethyl vanillin	121–32–4	1.58	166.2	0.0000	2.82	294	38
Isoamyl isovalerate	659–70–1	3.66	172.3	0.8860	0.045	192	38
Hexyl acetate	142–92–7	2.80	144.2	1.3200	0.51	170	39
Ethyl maltol	4940–11–8	0.63	140.1	0.0001	24.2	235	39
gamma-Decalactone	706–14–9	2.72	170.3	0.0051	0.2916	281	41
beta-Ionone	14901–07–6 79–77–6	3.84	192.3	0.0540	0.169	271	41
Ethyl caproate	123–66–0	2.80	144.2	1.5600	0.629	167	42
Isoamyl butyrate	106–27–4	3.20	158.2	1.0100	0.1178	178	46
Butyl acetate	123–86–4	1.78	116.2	11.5000	8.4	126	47
Cyclopentadecanolide	106–02–5	5.78	240.4	0.0001	0.0001484	364	47
Terpinolene	586–62–9	4.47	136.2	0.7470	0.0095	186	51
Methyl-2-methylbutyrate	868–57–5	1.73	116.2	22.5000	3.172	112	52
Isobutyl acetate	110–19–0	1.77	116.2	18.3000	3.128	112	54
Triacetin	102–76–1	0.25	218.2	0.0025	58	259	54
3-Methylbutyl acetate	123–92–2	2.25	130.2	5.6700	2	142	56
4-Acetyl-6-tert-butyl-1,1-dimethylindane	13171–00–1	5.53	244.4	0.0005	0.00392	305	56
Propylene glycol	57–55–6	-0.92	76.0	0.1300	1000	188	58
Limonene	5989–27–5	4.57	136.2	1.9800	0.0138	176	61

*Percentage transfer value derived from a minimum of duplicate analytes results on two different days
Identifiers and physicochemical values taken from NIH NLM PubChem, ChemIDPlus, EPA https://comptox.epa.gov or as available from Research Institute For Fragrance Materials (RIFM) reviews

resulting in a total volume of 300 ml. After shaking by hand for 1 min the sample was shaken on a laboratory shaker at 200 rpm for 30 min. An aliquot of the extract was filtered over a syringe filter and dried with sodium sulfate. In case of high concentrations of analytes, the extract was diluted with solvent. Aliquots of the extract were spiked at three levels of analyte standard solutions for quantitation by standard addition and analyzed by Gas Chromatography-Electron ionization-Mass spectrometry (GC-EI-MS).

2.2.2. Validation data

2.2.2.1. Selectivity.
The identity of all analytes was confirmed by comparison of the retention time in sample to the respective standard. The maximum permitted deviation between retention times was ± 0.1 min.

In addition, the relative intensity of the quantifier and qualifier ions was compared to the respective standard. The maximum permitted tolerance between ion intensity ratios was ± 30% [8].

2.2.3. Sensitivity
LOD (limit of detection) and LOQ (limit of quantification) were determined from tobacco and smoke extracts using the quantifier ion of each analyte. LOD is the analyte concentration where the signal-to-noise ratio is 3, whereas LOQ is the analyte concentration where the signal-to-noise ratio equals 10. The signal used for quantification was also used for determination of the signal-to-noise ratio.

Recoveries in tobacco were determined from the response of the analyte in samples spiked prior to extraction relative to the response of the analyte in an external standard.

The recoveries ranged from 76% to 124% for all analytes except ethyl vanillin and acetic acid, which had recoveries of 144% and 180% respectively, [9].

2.2.4. Quantification

The concentrations of all analytes were determined by standard addition at three spiking levels.

The minimum coefficient of correlation accepted in a sample was r = 0.992, typical values ranged from r = 0.997–1.000.

All analytes were determined in duplicate on two different days in waterpipe tobacco and mainstream smoke. Concentrations in smoke are given in μg/g smoked tobacco. Transfer ratios were calculated based on the respective tobacco and waterpipe smoke concentrations using the following formula:

$$\text{Transfer ratio}[\%] = \text{Concentration in smoke}\left[\tfrac{\mu g}{g}\text{ smoked tobacco}\right] \div \text{Concentration in tobacco}[(\mu g)/g] \times 100$$

2.2.5. Statistics

Statistical analysis was conducted to investigate if additive transfer from tobacco to waterpipe smoke was dependent on any of the single individual additive physicochemical properties. Further statistical evaluations were then applied using the General Linear Model (GLM) procedure method of least squares, to fit general linear models, to investigate interactions between pairs of physicochemical properties and transfer rate.

This approach was adopted as El Hourani et al. [6] had previously documented that 'if substances were both volatile and water-soluble, they are absorbed to some extent into the water, while substances that are either non-volatile or volatile but negligibly soluble are not absorbed or deposited into the water and would therefore be transferred to the waterpipe smoke'.

To conclude, a complete multivariate analysis using stepwise selection was conducted to evaluate any relationship between any of the physicochemical properties using the model below:

Transfer (Y) = All the physicochemical properties (x1…x5).

The stepwise regression method fits regression models in which the choice of predictive variable (i.e. physiochemical properties) was completed via an automatic procedure. At each step, a variable was considered for addition or subtraction from the set of variables (physiochemical properties) based on their significance level (p-value). To achieve this, an Entry Significance Level (SLE) and a Stay Significance Level (SLS) was established.

Therefore, at each step, a variable was either included or excluded to establish if the variable resulted in a statistically significant improvement or deterioration in the model fit. This procedure was repeated until no further variable could be added to statistically significantly improve or be deleted without resulting in a statistically significant loss of fit. The result being a model of best fit. Model quality (using R^2) was reviewed at each step to confirm the best model.

3. Results

Representative results showed that TCM per smoking session per blend was consistent throughout these experiments indicating a certain degree of reproducibility in the process, (see Supplementary Table 1).

An overview of percentage additive transfer to smoke and associated physicochemical properties is shown in Table 2. The data shows that for > 40 additives analyzed; transfer rates ranged from between 6% and 61%. As an example of the results, limonene an additive with a molecular weight of 136 g/mol, boiling point of 176 °C and vapor pressure of 1.98 mm Hg transferred to smoke at a rate of 61%. In contrast, Eucalyptol with a molecular weight of 154 g/mol, the same boiling point of 176 °C and an almost identical vapor pressure of 1.90 mm Hg transferred to smoke at 30%. In contrast, cis-3-Hexen-1-ol, with a boiling point of 157 °C and vapor pressure of 1.36 mm Hg transferred to smoke

Table 3
Linear regression results summary.

Property	r	P value
Partition coefficient (log K_{ow})	0.14	0.34
Boiling point	0.09	–
Molecular weight	0.21	0.16
Water solubility	0.07	–
Vapor pressure	0.33	0.02

Pearson correlation coefficient (r) and p value

In contrast, statistical results from the complete multivariate analysis using stepwise selection revealed that the highest R^2 value was 0.3879 for the model:

- Transfer = Boiling point, molecular weight, vapor pressure, water solubility.

Table 4
Comparison of transfer results between published and current study.

Additive	Erythropel et al.[7] In tobacco μg/g	Erythropel et al.[7] μg/puff	Calculated % Transfer Erythropel et al.[7] *	% Transfer current study
Eugenol	1300 (n = 2)	6.1 (n = 1)	2	23
Benzaldehyde	1100	9.8	4.45	10

* Calculated using 10 g tobacco smoked and assuming a maximum of 50 puffs

at 9%.

The results of our statistical investigations to understand any relationship between individual physicochemical properties and transfer rates failed to identify strong correlations. Indeed, for two variables, the Pearson correlation coefficient (r) was low (r < 0.1), see Table 3. It should be noted that Pearson's coefficient values may range from between + 1 to − 1, where + 1 indicates a strong positive relationship, − 1 indicates a strong negative relationship, and a value of 0 indicates that no relationship exists.

The statistical output for partition coefficient (log K_{ow}) and molecular weight indicated 0.1 < r < 0.3 which could imply a weak correlation with measured transfer rates. However, the associated p-values indicate no statistically significant correlations. In contrast, the Pearson correlation coefficient (r) for vapor pressure was shown to be 0.33 with an associated p-value of 0.02, indicating a moderate correlation with transfer (0.3 < r < 0.5). This correlation was not immediately obvious from the corresponding regression plot at lower values but was visually evident at higher vapor pressure values, (see Supplementary Figure 1).

Results from further statistical evaluations between pairs of physicochemical properties and transfer rate also demonstrated no interactions for any of the additives.

i.e., 39% of the transfer rate variance can be explained by the additive boiling point, molecular weight, vapor pressure and water solubility.

4. Discussion

For the > 40 additives assessed during this study, the transfer rates ranged from 6% to 61%. It has been suggested that the water bowl could act as a filter to the smoke components including additives and that the filtration may be explained to some extent by the additive or constituent physicochemical properties, [6,7].

The amount of additive transfer from waterpipe tobacco to smoke was not found to be strongly dependent on any single physicochemical property but 39% of the transfer rate variance can be explained collectively by the additive boiling point, molecular weight, vapor pressure and water solubility. Indeed, waterpipe smoking is a complex process, dependent on several parameters e.g., heating source, temperature, charcoal type, number of charcoal pieces used [5,13], user puff profile, waterpipe size/ shape and tobacco composition [1,2,16,17]. Erythropel et al. [7] had reported that 'water solubility probably only plays a minor role for determining the "efficiency" of the filtration step'.

Table 5

Percentage transfer of menthol, geraniol, propylene glycol and glycerol to smoke from waterpipe tobacco and to cigarette mainstream smoke.

Name	CAS number	log K_{ow}	Molecular weight [g/mol]	Vapor pressure @ 25 °C [mm Hg]	Water solubility @ 25 °C [g/L]	Boiling point[°C]	Transfer Rate to Waterpipe smoke [%]	Transfer Rate to cigarette smoke [%] [18]
Menthol	2216–51–5	3.40	156.3	0.000767	0.49	212	14	11.9
Geraniol	106–24–1	3.56	154.3	0.030000	0.1	229	26	7.8
Propylene glycol	57–55–6	-0.92	76.0	0.130000	1000	188	58	1
Glycerol	56–81–5	-1.76	92.1	0.000168	1000	290	17	4.6

For the additives common in both studies, the transfer rates in this study were higher than those recently reported by Erythropel et al. (see Table 4). This may be explained by differences in methodology such as total puff number (175 and 40 – 50) and volume of water in the waterpipe bowl (750 ml and 1200 ml) for current study and Erythropel et al. respectively.

Maziak et al. [14] had previously documented high frequency flavored waterpipe tobacco users as smoking for a mean duration of 42 min with an associated mean total puff number of 111.93. As such, Erythropel et al. may have underestimated total transfer due to limiting puff number, although it should be clearly noted that no machine smoking regime can represent all human smoking behavior.

Additionally, Hauser et al. (2020), communicated on the impact of waterpipe size with respect to smoke toxicity, with experiments being conducted using a range of waterpipes with different height and bowl sizes. Height was defined as small, medium, and large (22 cm, 36 cm and 55 cm respectively) with bowl sizes ranging from 300 to 1250 ml. The results showed that stem length and insertion depth were a factor in contributing to differences in generated smoke particle number and size. Additionally, it would be reasonable to assume that the large difference in water volume between the bowls would also be a contributing factor.

In the absence of a means to reliably predict additive transfer, these results highlight a clear need to conduct transfer testing studies for additives proposed for use in waterpipe tobacco as an aid to perform toxicological risk assessments for the additives used. Additionally, and as highlighted previously in the text, heating of the tobacco plays a much more important role in waterpipe smoking than in conventional cigarettes where the tobacco is combusted. A comparison of our results with transfer rates documented for cigarette smoke demonstrates that the percentage transfer of several additives to waterpipe smoke was higher than to cigarette mainstream smoke (see Table 5).

Interestingly, Stabbert et al. reported only a 1% transfer for propylene glycol to cigarette smoke, whereas this water-soluble additive transferred up to 58% in waterpipe smoke. In contrast, transfer rates for menthol in cigarette or during waterpipe use were comparable. Consequently, additive levels in waterpipe tobacco should be based on transfer data to waterpipe smoke and not just follow the justification for additive levels as used in cigarettes.

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CRediT authorship contribution statement

Jacqueline Miller Holt: Conceptualization, Methodology, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Maurane Cherrie:** Formal analysis, Writing - review & editing, visualization **Bernhard Mayer-Helm:** Conceptualization, Methodology, Investigation, Data Curation, Resources, Review & Editing, Supervision, Project administration. **Jeremie Gafner:** Investigation, Writing - review & editing. **Michael Zierlinger**: Investigation, Methodology, Data Curation, Review & Editing,. **Carole Hirn:** Writing - Review & Editing. **Thilo Paschke:** Writing - Review & Editing. **Markus Kuba:** Investigation, Methodology, Data Curation, Review & Editing **Stefan Pummer:** Investigation, Methodology, Data Curation, Review & Editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jacqueline MILLER HOLT reports financial support was provided by JT International SA. Jacqueline MILLER HOLT reports a relationship with JT International SA that includes: employment. JT International SA (JTI) funded the project, and all authors were employees of JTI at the time of the study. JTI is a company that manufactures waterpipe tobacco.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.04.022.

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