Application of Cigarette Smoke Characterisation Based on Optical Aerosol Spectrometry. Dynamics and Comparisons with Tar Values.

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Abstract:

Introduction. Cigarette smoking causes devastating disease worldwide. Current cigarette classification is based on standardised tar mass values obtained from smoking-machines. However, their ability to predict disease is poor, and these mass values are primarily determined by larger particles. The aim of our study is to investigate in how far claimed tar values also reflect smaller tar particles in cigarette smoke.

Methods. We developed a method to measure size-resolved particle distributions based on experimentally selecting conditions that revealed the least variety within different smoking regimes, puff numbers, diluted and undiluted ageing times, and filter taping. Next, we analysed three cigarettes types with different tar values. Cigarettes were smoked by a Cerulean SM-450 smoking machine, and subsequently smoke samples were diluted and collected in Tedlar® bags and measured for size-resolved particle distributions by a universal optical aerosol spectrometer.

Results. Our method involved a smoking regime according to ISO 3308, the sixth puff, and no delayed ageing. We attained valid size-resolved particle distributions between 250 and 1,000 nm. The results revealed similar total particle counts across different cigarette types, though with different size-resolved particle distributions. In particular, smaller particles in lower tar cigarettes were underestimated.

Conclusion. We developed a method to investigate submicron size-resolved particle distributions in cigarette smoke in order to compare cigarettes with different tar values. Our study suggests that mass-based tar values are a poor reflection of smaller particles in mainstream cigarette smoke, and hence supports the opinion that current tar values are a poor predictor of disease-risk and therefore that they are deceptive to smokers.

Keywords: Cigarette, nicotine, optical spectrometry, particle count, particle mass, size-resolved particle distribution, smoke, smoke characterization, smoke dynamics, tar.

1. INTRODUCTION

Cigarette smoking, including passive smoking, is a vital health hazard worldwide, mainly related to devastating diseases such as lung cancer, cardiovascular disease and chronic obstructive pulmonary disease (COPD) [1, 2]. Worldwide, the percentage of smokers seems to have stabilised at 20-30% [1, 3]. All cigarette ingredients – mainly dried tobacco leaves combined with a selection of over 1,000 additives – contribute to the complex mixture of smoke constituents. Approximately 4,800 potentially hazardous constituents have been identified to date [4]. 'Tar' refers to the particulate matter which is trapped using Cambridge filters that collect 99.9% of particles >100 nm [5].

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Tar values have been used to predict smoke exposure and subsequent disease risk, as these are assumed to be associated with all hazardous constituents [6, 7] and therefore, proportionately, to disease. This mass-based value is determined using smoking machines in standardised testing methods that have not been developed to predict individual exposure to cigarette smoke and subsequent disease [8-10]. Indeed, individual exposure can be substantially modified, resulting in a final smoke exposure from low-tar cigarettes that is almost equivalent to full blend cigarettes [11-14]. Furthermore, while some hazardous constituents have been reported to be disproportionately high in (ultra)low-tar cigarettes [15], no fractional risk attributions have been defined that link specific constituents to the development of disease [16, 17]. The relation between tar values and disease is far from absolute. Low-tar and low-nicotine cigarettes appeared to worsen outcomes in embryogenesis compared to regular-tar cigarettes [18], and a relation between tar values and disease was lacking or even inversed for tar values <21 mg per cigarette [19,

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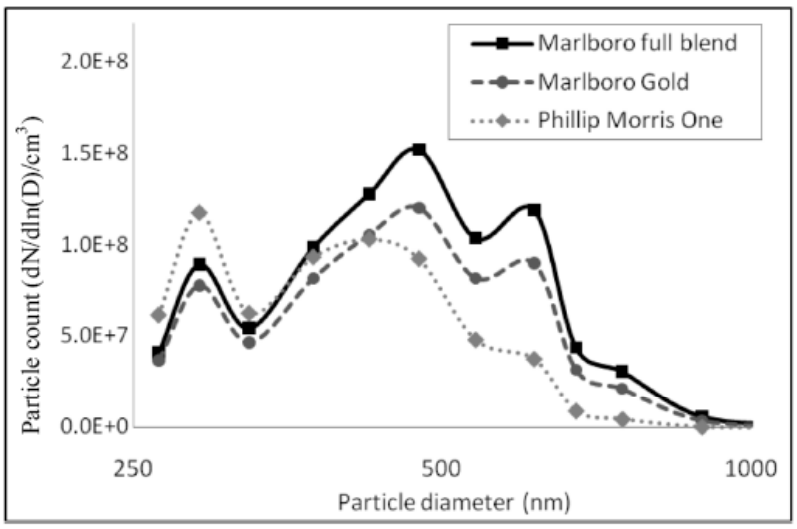

Fig. (1). Logarithmic particle size distribution for different cigarette types (6th puff, ISO 3308 smoking regime, 77,143 dilution and 45 seconds from smoking to measurement). Error bar (⊤) is standard error.

20]. Unfortunately, the ability of tar to predict disease clearly remains poor. Though there have been calls to change smoking standardisation, many countries still oblige manufacturers to inform consumers by displaying these claimed values on cigarette packs [10].

Tar values are based on particle mass, even though mass is considered to be an inferior measure of toxicity compared to particle count and surface area [21, 22]. Total particle mass is dominated by larger particles, while actual particle sizes range from 200 to 600 nm, with some additional particles between 100 and 1,000 nm [23-25]. In investigating the deceptive implications of claimed tar values, we hypothesised that mass-based tar values only fairly reflect the relatively small quantity of larger tar particles, thus providing another reason to question the reliability of cigarette standardisation based on tar values as displayed on cigarette packs. The aim of our study is to develop a valid measurement method that can be used to investigate the ability of tar yields displayed on cigarette boxes to reflect submicron tar particles in mainstream cigarette smoke.

2. METHODS

2.1. Study Design

Since smoke yields depend on cigarette design and standardised parameters including humidity, smoke generation (puffing), collection and measurement [16], we developed a valid method that measures size-resolved particle distributions based on experimentally selected conditions (supplemental Table **1**) that show the least variety among ten different smoke samples: puff sequential number (2, 4, 6, 8, 10), smoking regime (supplemental Table **2**), undiluted smoke ageing within the first holding vessel (0, 3 and 10 minutes) and diluted smoke ageing within the first holding bag (0, 3, 6, 10, and 15 minutes). To minimise variance, we conducted all initial tests using Coresta Monitor No. 6 cigarettes (CM6), which is a non-commercial maximal homogenised research cigarette from a certified batch. Smoking regimes were also tested on Phillip Morris One cigarettes (PhM1).

We first validated our method by making ten measurements of five cigarette brands, each with 10 mg tar and 0.8 mg nicotine displayed on the cigarette pack (1 mg variation in both true yields allowed): Marlboro, L&M, Camel, Pall Mall and Lucky Strike. Next, we used our method to analyse three cigarettes types with different tar values, manufactured by Philip Morris: Marlboro Red (10 mg regular-tar/full blend), Marlboro Gold (7 mg low-tar) and the high-ventilated filter cigarette Philip Morris One (1 mg ultralow-tar). For further validation, we calculated tar mass per cigarette for these three types. All cigarettes were purchased in the Netherlands in 2009.

2.2. Setup: Smoking, Sampling and Measuring (Supplemental Fig. 1)

Cigarette smoke was produced by a Cerulean SM 450 smoking machine conforming to ISO standards, with electric ignition and cigarette conditioning at 22 °C and a relative humidity of 60%. Mainstream smoke sampling was conducted using cascaded dilution in order to create both a substantial measurement volume and a measurable concentration range. We collected generated smoke per single puff in a 190 ml ellipsoidal glass holding vessel filled with filtered air and with indentations in the glass to create turbulence for homogenisation (step 1). We diluted the whole puff sample (35 or 55 ml, depending on puff volume) within 5 seconds by transporting 6 litres of filtered air through the vessel and subsequently collecting the homogenised sample in a 10 litre octagonal Tedlar® bag (step 2). This produced first dilution factors of 171 (6,000/35) and 109 (6,000/55). At 15 seconds, we sampled another 10 or 20 ml of the dilutions into another glass vessel – depending on the initial puff volume – transported 9 litres of filtered air through the vessel and collected the final dilution into a second 10 litre Tedlar® bag, thus producing final samples with dilution factors of 77,143 for 35 ml puffs and 98,182 for 55 ml puffs (step 3).

We initiated sample measurements at 30 seconds from smoke production using a universal optical spectrometer

Table 1. Condition characteristics and statistics of size-resolved particle distributions.
All channels between 250 and 1,000 nm by 10 different measurements. TPC: mean total particle count/cm^3/puff. CMD: count mean diameter (95%-confidence interval). CoV: coefficient of variance (%). LOA: limits of agreement, 100% indicates maximum agreement, <100% underestimation and >100% overestimation. Correlation between conditions by Pearson's correlation coefficient. .[a] is redundant. [$] ANOVA: $p \leq 0.05$. * $p \leq 0.001$

	TPC	CMD (nm)	*CMD difference*[$]	CoV	LOA	Correlation
Puff #						
#2	8.9 x 10^7	459 (439-479)	*#2 vs. 8*	27%	68% - 179%	0.99*
#4	9.1 x 10^7	468 (440-496)		26%	97% - 111%	1.00*
#6	9.4 x 10^7	470 (454-487)		22%	.[a]	.[a]
#8	1.0 x 10^8	474 (459-489)		19%	83% - 99%	1.00*
#10	9.9 x 10^7	473 (454-492)		27%	80% - 111%	1.00*
Regime (CM6)						
ISO #5	8.3 x 10^7	489 (475-502)	*All except Canadians*	24%	68% - 194%	0.98*
ISO 3308	9.4 x 10^7	470 (454-487)		22%	.[a]	.[a]
Canadian w/o tape	1.3 x 10^8	430 (412-449)		32%	22% - 310%	0.86*
Canadian with tape	1.2 x 10^8	432 (416-447)		38%	25% - 297%	0.88*
Regime (PhM1)						
ISO 3308	7.2 x 10^7	400 (384-416)	*All*	17%	.[a]	.[a]
Canadian w/o tape	7.9 x 10^7	386 (368-403)		25%	62% - 147%	0.98*
Canadian with tape	1.0 x 10^8	432 (414-449)		28%	9% - 328%	0.95*
Glass ageing						
0 minutes	9.4 x 10^7	470 (454-487)	*None*	22%	.[a]	.[a]
2 minutes	2.0 x 10^7	478 (459-496)		27%	10% - 52%	0.97*
9 minutes	6.6 x 10^6	484 (462-505)		21%	4% - 16%	0.94*
Bag ageing						
0 minutes	8.5 x 10^7	478 (457-498)	*0 vs. 9 min.*	29%	.[a]	.[a]
2 minutes	8.3 x 10^7	473 (452-493)	*0 vs. 14 min.*	28%	91% - 119%	1.00*
5 minutes	7.9 x 10^7	468 (447-488)	*2 vs. 14 min.*	26%	83% - 147%	0.99*
9 minutes	7.2 x 10^7	462 (440-483)		27%	81% - 190%	0.98*
14 minutes	6.0 x 10^7	452 (426-478)		29%	77% - 304%	0.95*
Types #1						
CM6	1.2 x 10^8	432 (416-447)	*None*	38%	.[a]	.[a]
Philip Morris 1	1.0 x 10^8	432 (414-449)		28%	75% - 92%	0.99 *

(1.109 aerosol spectrometer, Grimm Aerosol Technik GmbH), with size-dependent induced laser scattering of particles between 0.25 and 32 µm at a flow rate of 1.2 l/min, (step 4). Grimm calibration procedures as at 2009 assured an accuracy of ±2%. Though we cleaned the setup between measurements through repeated vacuuming and rinsing with filtered air, we discarded the first two six-second measurements to obtain ten successive valid sample distributions from about 45 seconds of smoke production.

2.3. Study Outcome Parameters

We present size-resolved particle distributions per ml of produced smoke as lognormal size distributions: dN/dln(D) – dN equals particle count per volume (ml), dln(D) equals lognormal channel size range [26]. Channel sizes are presented as geometric means: (lower channel limit * upper channel limit)$^{0.5}$. We calculated particle mass/cm^3 per channel, assuming sphericity, and determined total tar mass per cigarette as follows: $\sum^{channels}(1/6\pi \cdot D_{channel\ mean}{}^3 \cdot \rho \cdot dN) \cdot$ puff volume · total number of puffs. $D_{channel\ mean}$ equals geometric mean diameter and mean tar density (ρ) was set at 1 gram/cm^3 [27].

2.4. Statistics

We first evaluated channel sample distributions by Shapiro-Wilk test and variance distributions by Levene's test. We evaluated reproducibility of all channels combined based on the mean coefficient of variance (CoV): $\sqrt{(\sum sd^2/n)} / (\sum mcpc/n)$ with sd: standard deviation, n: number of channels, mcpc: mean count per channel.

Table 2. Brand and type characteristics and statistics of size-resolved distributions.
All channels between 250 and 1,000 nm by 10 different measurements. TPC: mean total particle count/cm^3/puff. CMD: count mean diameter (95%-confidence interval). $ ANOVA: $p \leq 0.05$. CoV: coefficient of variance (%). Tar: yields per cigarette, calculated from size-resolved particle distributions. .[a] is not determined.

	TPC	CMD (nm)	*CMD Difference*[$]	CoV	Tar (mg)
Brands (10 mg tar)					
Marlboro	1.0×10^8	467 (456-478)	*Marlboro vs. Camel*	11%	.[a]
Lucky Strike	9.4×10^7	451 (429-473)		22%	.[a]
Pall Mall	1.1×10^8	460 (442-478)		26%	.[a]
Camel	1.4×10^8	449 (436-461)		13%	.[a]
L&M	1.1×10^8	452 (429-475)		23%	.[a]
Types #2					
Philip Morris One (1 mg tar)	7.2×10^7	400 (384-416)	*1 vs. 7 mg*	17%	1.5
Marlboro Gold (7 mg tar)	8.1×10^7	457 (437-476)	*1 vs. 10 mg*	26%	3.0
Marlboro Red (10 mg tar)	1.0×10^8	467 (456-478)		11%	3.9

We analysed the (level of) agreement between different sampling conditions, based on mean log-normal size distributions, using both two-tailed Pearson correlation analyses and Bland-Altman modelling. Due to proportional differences, the Bland-Altman 95% confidence interval (limits) is based on a logarithmic agreement, and includes the 95% confidence intervals of these limits. These reveal over- and underestimation for all channels jointly. [28] We performed analysis of variance (ANOVA), repeated measurements ANOVA and mixed modelling to analyse condition differences for count mean diameters, single-channel particle counts, and combined channel counts. We analysed all results using SPSS 16.0. Statistical significance was assumed for p-values < 0.05.

3. RESULTS

3.1. Methodological Conditions.

We obtained reproducible particle measurements between 250 and 1,000 nm, encompassing 11 channels: the CoV for ten runs within a single sample remained below 5%. Larger particles, if present, could not be measured reliably due to the high dilution (results were mostly 0) and were therefore discarded. The methodological conditions with the least between-sample variation and the best between-condition agreement included ISO method 3308, puff number 6 and direct collecting, diluting and measuring: total particle count (TPC) 9.4×10^7 particles/cm^3, mean count particle diameter (CMD) 470 nm and mean CoV 22% (Table **1**).

Whereas puff numbers and diluted smoke ageing revealed only minor differences (supplemental Figs. **2** and **3**), smoking regime (supplemental Figs. **4** and **5**) and undiluted smoke ageing (supplemental Fig. **6**) appeared to be important factors determining changes in size-resolved particle distributions (Table **3**). Whereas the undiluted samples revealed a rapid TPC decline with a parallel (non-significant) increase of CMD (step 1), the diluted samples appeared rather stable during the first minutes (step 2). As expected, taping did not significantly influence CM6 smoke yields, but substantially affected the high-ventilated filter PhM1 cigarettes. Although we observed a significant increase of TPC and decrease of CMD with more intense regimes in general, PhM1 revealed an increase of CMD with taping. In addition, the taped PhM1 smoke yields were almost similar to the taped CM6 smoke yields.

3.2. Method Utilisation

Different brands of cigarettes were analysed to validate our method. This revealed that our method was fairly reproducible with CoV below 26% (Table **2**, supplemental Table **3**, and supplemental Fig. **7**). Our analyses of different cigarette types also showed the ability of tar values to reflect size-resolved particle distributions (Table **2** and Figs. **1-3**).

The final analyses to investigate the ability of tar yields to reflect size-resolved particle distributions in mainstream cigarette smoke revealed very different particle size distributions across cigarette types with different tar yields (ultra low-tar cigarettes (PhM1, 1 mg tar), low-tar (Gold, 7 mg tar) and regular-tar (Red, 10 mg tar)), while ultra low-tar cigarettes significantly exhibit the smallest CMD (Table **2** and Fig. **1**). Surprisingly, Figure **2** reveals a linear relation between particle size and the ratio between regular-tar and ultralow-tar cigarettes. The expected tenfold ratio – based on stated tar yields – was approximated for the largest particles only. Below 350 nm, ultralow-tar cigarettes have significantly higher particle counts. Figure **3** presents the total calculated size-resolved particle mass for different cigarette types.

4. DISCUSSION

4.1. Key Results

We developed a reproducible and representative method to measure size-resolved particle distributions between 250 and 1,000 nm in mainstream cigarette smoke – i.e., smoking regime ISO 3308, sixth puff and direct collecting, diluting (1:77,143) and measuring – and observed a poor ability of tar values to predict size-resolved particle distributions. In addi-

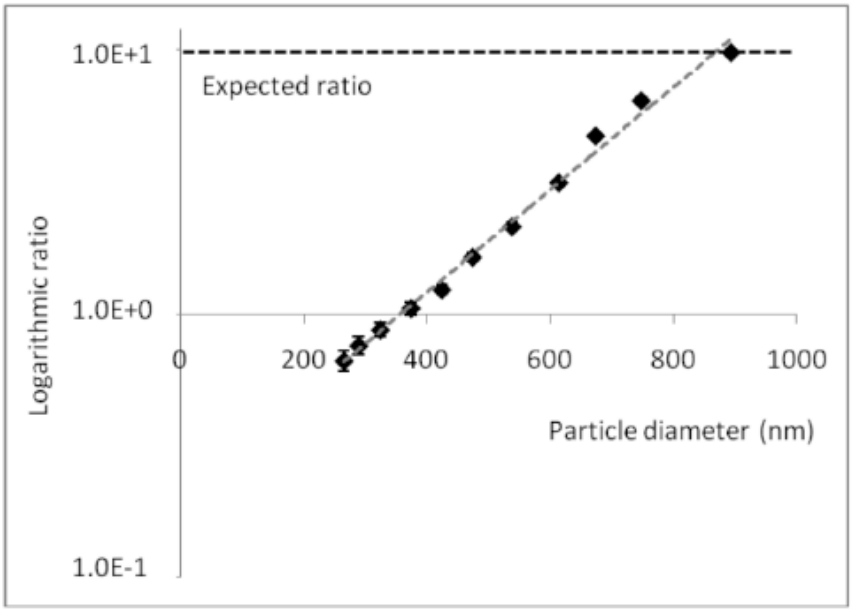

Fig. (2). Logarithmic ratio of smoke yields Marlboro full blend / Phillip Morris One ($R^2 = 0.99$, $p < 0.01$). Error bar (⊤) is standard error.

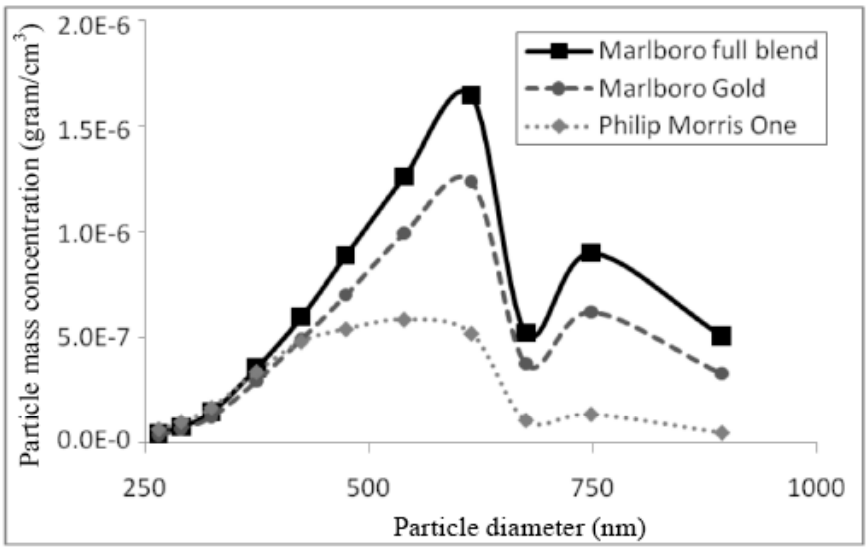

Fig. (3). Estimated size-resolved particle mass for different cigarette types (6th puff, ISO 3308 smoking regime, 77,143 dilution and 45 seconds from smoking to measurement).

tion, TPCs were similar, mainly due to underestimation of small particles in low-tar cigarettes. Blocking of filter ventilation holes, however, resulted in a similar particle distribution and particle count across cigarettes with different tar yields.

4.2. Strengths and Limitations

In order to decrease active sample sedimentation and coagulation due to fabric features and overpressure, we used inert Tedlar® bags that could contain a maximum volume of 10 litres each. The ellipsoidal vessel and octagonal bag shapes, and transportation of our samples by a relatively large flow of filtered air through the whole system diminished line or gravimetric losses and homogenised samples via turbulence. As none of the Tedlar® interior walls showed any discoloration from cigarette smoke after the experiments, diffusion, gravimetric and electrostatic losses seem negligible. Altogether, absolute sample losses were reduced to a minimum. Relative sample losses did occur however, mainly due to undiluted ageing effects, probably through coagulation. Although we minimised undiluted ageing times, this might have caused additional variation, in addition to variation due to cigarette type. This ageing process did not particularly affect larger particles, making gravitational settling unlikely. Diluted ageing caused minor irrelevant changes only, such as the decreasing CMD due mainly to gravitational losses, but did not jeopardise general reliability. Overall, variation in our method is comparable to variation in tar values (1 mg variation allowed), and mostly reflects variability among cigarettes themselves.

We characterised cigarette smoke yields as tri-modal size-resolved distributions; TPC and CMD approximately 1 x 10^8 particles/ml and 455 nm in 10 mg tar cigarettes (Table **4**). Size-resolved particle distributions have previously been measured through, for example, light scattering optical aerosol spectrometry and state-of-the-art electrical mobility spectrometers (DMS by Cambustion, SMPS+E by Grimm Aerosol Technik) [24, 29]. Compared to previous studies, our method shows good reproducibility and confirms that distributions are mainly affected by smoking regimes, filter ventilation and undiluted ageing. Studies from the 1980's exhibit the highest TPC (up to 7 x 10^9 particles/ml) in part due to higher tar yields in tested cigarettes of around 40 mg per cigarette [30-32]. More recent studies reveal 5-10 x 10^8 particles/ml [24, 25]. Nevertheless, our TPC appears low whereas our CMD appears high. Presumably, this upward shift in particle size and subsequent decrease in particle count occurred primarily due to coagulation processes before dilution and within the first second after smoke formation [33]. This shift could further have been caused by our lowest detection limit at 250 nm and by potential bias from a reduced detection efficiency at this low end of the detection range (which may have caused the tri-modal distribution). Indeed, our pilot study with simultaneous measurements from the same diluted sample using a scanning mobility particle sizer seemed to underrate the smallest particles, though appeared similar above 400 nm. However, this underrating would be proportional across different cigarettes.

Our method approximates claimed tar values, but seems to underestimate tar yields in regular tar cigarettes in particular. Since we were unable to produce valid results for particles with the most mass (>1 μm) due to massive dilution, gravitational losses or else by their absence, this might explain some of the underestimation. More specifically, particles >1 μm were only demonstrated once, which required several dilution steps [32].

4.3. Interpretation

Smoking causes devastating diseases. Currently, disease-risk is categorised based on tar values, which are a poor predictor. Both tobacco industry development and government monitoring focus on these values only, which implies an unfortunate prioritisation. Our study may help to expose the deceptive nature of current categorisation. Although regional and seasonal batch-specific variations restrict generalisation of our results, some general observations are possible. Aside from moistening, we believe our dilution cascade more or less reflects real-life two-phased smoking patterns – i.e. a short intraoral retention of approximately two seconds followed by a relatively stable dilution by inhalation. Factors such as puff number and smoking regime were fairly representative for the measured distributions.

As particle mass has a non-linear relation with particle size, particle mass would poorly reflect size-resolved particle distributions in cigarette smoke. Indeed, our findings support this thesis. Moreover, our findings suggest that particle counts and distributions < 1 μm do not change in proportion to the tar values displayed on cigarette packs. In fact, tar values severely underestimate particle counts in low and ultra low-tar cigarettes, and of smaller particles in particular. These tar measurements are affected by filter ventilation [6, 34], and our method confirms that this appears to effect a similar change in calculated mass yields. As these yields were comparable when the vent holes were blocked, filter ventilation appears to be an important determinant of particle size-distributions. However, other factors such as porous cigarette papers, expanded tobacco and reconstituted tobacco sheet may interfere. In our study, filter ventilation seemed to mainly decrease counts of larger particles and increase counts of smaller particles. It could be conjectured that this shift is due to dilution via vent holes, whether through vaporisation or reduced agglomeration. Alternatively, the decreased flow in a filter-ventilated cigarette could cause a shift towards larger particle diameters. A comparison with two studies demonstrated an overall decrease of particles with an additional shift from small to large particles due to filter ventilation [24, 25]. Anderson and colleagues did not observe this diameter shift [30]. Apparently, the decisive factor in particle shifts can be variable.

Overall, our study suggests that people who smoke low or ultra low-tar cigarettes are exposed to an excess of small particles, which are able to penetrate deeper alveolar regions, have a larger reactive surface area per mass, have a capacity for extra-pulmonary translocation and thus are potentially more toxic than larger particles [21, 22, 35-38]. Vent-blocking would expose these smokers to similar smoke yields as smoking regular-tar cigarettes. Therefore, our results cast further doubt on the reliability and value of current tar measurements with respect to smoke exposure and smoke-related disease.

CONCLUSIONS

We developed a valid, reproducible and fairly representative method to study the ability of tar values to represent particle counts of smaller particles in mainstream cigarette smoke. Our method revealed that claimed tar values poorly reflect particle counts and size-resolved distributions between 250 and 1,000 nm. In particular, smaller particles in lower tar cigarettes were underestimated. As these smaller submicron particles contribute little to mass-based tar values, but may be more toxic than larger particles, our study supports the view that current tar values are poor predictors of disease risk and are deceptive to smokers. We therefore support a change in the current characterisation and labelling of cigarette smoke.

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DECLARATION OF INTERESTS

There are no conflicts of interest of any kind related to this paper. As government official from the Dutch Food and Consumer Product Safety Authority (VWA), Walther Klerx is involved in law enforcement of tobacco products, though independent of the tobacco industry. In addition, the VWA (and Walther Klerx as technical expert) is a member of the task force tobacco laboratories of the WHO and EU and therefore obliged to comply to article 5.3 of the FCTC (to

prevent conflicts of interest with the tobacco industry of any kind).

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