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Estimation of mouth level exposure to smoke constituents of cigarettes with different tar levels using filter analysis

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ABSTRACT

A nicotine part-filter method can be applied to estimate smokers' mouth level exposure (MLE) to smoke constituents. The objectives of this study were (1) to generate calibration curves for 47 smoke constituents, (2) to estimate MLE to selected smoke constituents using Japanese smokers of commercially available cigarettes covering a wide range of International Organization for Standardization tar yields (1–21 mg/cigarette), and (3) to investigate relationships between MLE estimates and various machine-smoking yields. Five cigarette brands were machine-smoked under 7 different smoking regimes and smoke constituents and nicotine content in part-filters were measured. Calibration curves were then generated. Spent cigarette filters were collected from a target of 50 smokers for each of the 15 brands and a total of 780 filters were obtained. Nicotine content in part-filters was then measured and MLE to each smoke constituent was estimated. Strong correlations were identified between nicotine content in part-filters and 41 out of the 47 smoke constituent yields. Estimates of MLE to acetaldehyde, acrolein, 1,3-butadiene, benzene, benzo[a]pyrene, carbon monoxide, and tar showed significant negative correlations with corresponding constituent yields per mg nicotine under the Health Canada Intense smoking regime, whereas significant positive correlations were observed for *N*-nitrosonornicotine and (4-methylnitrosoamino)-1-(3-pyridyl)-1-butanone.

1. Introduction

Individual smokers exhibit a wide range of puffing behaviors (US Department of Health and Human Services, 1988). Therefore, the level of exposure in individual smokers to mainstream smoke constituents varies considerably, even for users of the same brand of cigarettes. Many attempts have been made to estimate human smoke exposure from cigarettes (Benowitz, 2001; Pritchard and Robinson, 1996; Scherer, 1999; Stephen et al., 1989).

Filter analysis using spent cigarette filters is a noninvasive method to estimate human smoke exposure (e.g., Baker et al., 1998; Polzin et al., 2009; Shepperd et al., 2006; St. Charles et al., 2006, 2009; Watson et al., 2004). Filter analysis is based on the premise that the amount of a given constituent passing through a filter is proportional to the amount retained in a spent cigarette filter. In the late 1960s and early 1970s, filter analysis involved the use of whole filters (Ashton and Watson, 1970; Forbes et al., 1976; Schulz and Seehofer, 1978). One problem with the whole filter method applied to cigarettes with ventilated filters is that the filtration efficiency for constituents such as nicotine is influenced by the flow rate through the filter. St. Charles (2001) and Shepperd et al. (2006) improved the methodology by using the 10-mm mouth end section of the filter (part-filter), where filtration is not susceptible to flow rate. St. Charles et al. (2009) reported this part-filter methodology in detail. Natural smoking behavior can be evaluated using this method, wherein spent cigarette filters from smokers are used to estimate the amount of smoke entering the smoker's mouth, referred to as the mouth level exposure (MLE). A smoker's MLE to a given constituent can be estimated from the amount of the constituent retained in spent cigarette filters from smokers by utilizing calibration curves from several smoking regimes reflecting various human yield levels. Other studies reported a high correlation between MLE estimated by filter analysis and

Abbreviations: CO, carbon monoxide; FTC, Federal Trade Commission; GC, gas chromatography; GC–MS, GC with mass spectrometry; HCI, Health Canada Intense; HPLC, high performance liquid chromatography; HPLC-FLD, HPLC with fluorescence detection; HSD, honestly significant difference; ISO, International Organization for Standardization; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MLE, Mouth Level Exposure; NNN, *N*-nitrosonornicotine; NNK, (4-methylnitrosoamino)-1-(3-pyridyl)-1-butanone; TobReg, Tobacco Product Regulation; TPM, Total Particulate Matter; WHO, World Health Organization.

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biomarkers of exposure (Morin et al., 2011; Shepperd et al., 2009; St. Charles et al., 2006). In a literature review, Pauly et al. (2009) concluded that cigarette filters may have utility as proxy measures of MLE in clinical trials.

Various researchers have estimated MLE to nicotine and nicotine-free dry particulate matter or tar using filter analysis. Nelson et al. (2011) studied MLE to nicotine and tar in 1330 smokers of 26 brand styles of US cigarettes, covering a wide range of machine-generated yields in the United States. Similarly, Mariner et al. (2011) studied MLE to nicotine and tar in 5703 smokers of 106 brand styles of cigarettes, including a wide range of machine-generated yields in 8 countries. In addition, the influence of cigarette design parameters on MLE has been studied. Ashley et al. (2011a) compared cigarettes with typical circumferences of 25 and 17 mm to evaluate their influence on MLE to nicotine and tar, and Ashley et al. (2012) studied cigarettes differing in the type and level of applied menthol. MLE to nicotine and tar using filter analysis has also been used to assess changes in smoking behavior before and after regulations were enforced. For example, Coté et al. (2011) used MLE estimates to assess the impact of changes in cigarettes to comply with ignition propensity regulations in Canada, and Ashley et al. (2011b) assessed the impact of a regulation preventing indoor smoking in public places in Scotland.

Recently, the applicability of MLE to smoke constituents other than nicotine and tar using filter analysis has been studied. If MLE can be applied to other smoke constituents, MLE provides more valuable information on assessment of exposure to cigarette smoke, and thus MLE estimation using filter analysis may be a more valuable approach as proxy measures of MLE in clinical trials. Polzin et al. (2009) reported that MLE to *N*-nitrosonornicotine (NNN) and (4-methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) can be estimated from solanesol retained in spent cigarette filters. Morin et al. (2011), Shepperd et al. (2009), and Shepperd et al. (2011) reported that MLE to NNK, acrolein, and pyrene can be estimated from nicotine retained in spent cigarette filters. These studies were conducted in Germany and Canada. Ding et al. (2012) reported that MLE to benzo[a]pyrene can be estimated from benzo[a]pyrene retained in spent cigarette filters. In another study estimating the retention of selected smoke constituents in the respiratory tract, MLE to NNK, NNN, carbon monoxide (CO), isoprene, acetaldehyde, and ethylene was estimated from solanesol retained in spent cigarette filters (Feng et al., 2007). In a series of similar retention studies by Moldoveanu et al. (2007, 2008a,b,c), MLE to carbonyl compounds, polycyclic aromatic hydrocarbons, hydroxybenzenes, benzene, and toluene was estimated from nicotine retained in spent cigarette filters.

Shepperd et al. (2009) suggested that mean MLE estimates and exposure biomarkers for nicotine, NNK, pyrene, and acrolein measured in smokers of low International Organization for Standardization (ISO) tar brands were generally lower than those measured in smokers of high ISO tar brands. This implies that tar yields obtained by ISO machine smoking may be used to rank mean MLE estimates of cigarette brands. The World Health Organization (WHO) Study Group on Tobacco Product Regulation (TobReg) proposed in its 2008 report, to measure the levels of selected smoke constituents using the Health Canada Intense (HCI) smoking regime, to report them on a constituent yield per mg nicotine, and to reduce the levels progressively over time (WHO, 2008). TobReg points out that its proposal is only an interim step in the regulation of tobacco products, before the development of approaches to assess differences in actual exposure, harm, or risk from different cigarette brands. The ultimate goal, according to TobReg, is to quantify the actual exposure of smokers by measuring biomarkers in blood, urine, and saliva. TobReg does not provide information on the relationships between smoke constituent yields per mg nicotine under the HCI smoking regime and biomarkers of exposure. Few studies have examined the ranking of mean MLE estimates of cigarette brands using various machine-smoking yields. Our group estimated MLE to 36 smoke constituents using 2 commercial cigarette brands sold in the Japanese market and examined the relationship between mean MLE estimates and various machine-smoking yields (Bito et al., 2012).

The objectives of our study were: (1) to generate calibration curves for the 47 mainstream smoke constituents using a nicotine part-filter method; (2) to estimate MLE to 10 selected smoke constituents using Japanese smokers of commercially available cigarettes covering a wide range of ISO tar yields; and (3) to investigate relationships between estimates of MLE to smoke constituents and various machine-smoking yields.

2. Materials and methods

2.1. Test cigarettes and target constituents

Fifteen commercial cigarette brands sold in Japan with a range of ISO tar yields were selected. Market share, blend type, and cigarette size were considered in the selection process. Five cigarette brands were selected from each of 3 ranges of ISO tar yields: 1–3, 5–9, and 10–21 mg/cigarette. The specifications of all tested cigarette brands are shown in Table 1. The cigarette brands used in our study accounted for a 41.5% share of the Japanese market according to data from the Tobacco Institute of Japan for the year 2011. The cigarette brands were sampled at a warehouse in July 2012, and cigarettes from each brand were confirmed to have the same batch code and to be sourced from the same batch. Five cigarette brands (Nos. 1, 7, 11, 13, and 15) of the 15 were used to generate calibration curves for the 47 smoke constituents, which were selected on the basis of regulations in Canada (Canada, 2000) and Brazil (Brazil, 2001). They are listed in Table 2. The 15 cigarette brands were used to generate calibration curves for NNN, NNK, acetaldehyde, acrolein, 1,3-butadiene, benzene, benzo[a]pyrene, and CO, which were listed by the WHO TobReg as priority constituents to be reduced (WHO, 2008) as well as nicotine and tar, supply to subjects and machine-smoking under ISO and HCI smoking regimes.

2.2. Analysis of selected constituents in smoke and cut filler

All constituents were analyzed at JT Laboratories. The calibration smoking regimes utilized by St. Charles et al. (2009) were used in our study (Table 3). Unsmoked filters were used as blanks (regime 0). Cigarettes were smoked at pack moisture because subjects smoked cigarettes without conditioning (St. Charles et al., 2009). Twenty-port Cerulean SM450RH linear smoking machines were used for smoking except for the analysis of the oxides of nitrogen, for which one-port Borgwaldt LM1 PLUS smoking machine was used. The number of cigarettes per 44-mm Cambridge filter pad was set from 1 to 5 such that the total particulate matter (TPM) collected on the pad would not exceed 150 mg. For analysis of carbonyls, 1 or 2 cigarettes was used per replicate. For the analysis of oxides of nitrogen, 1 cigarette was used per replicate. Three replicates were included in each analysis for each smoking regime and cigarette brand, and 5 replicates were included in the analysis of oxides of nitrogen. The system used for trapping the smoke routinely generated under ISO and HCI smoking regimes at JT Laboratories was modified to fit the smoking regimes used in our study. A summary of the trapping system is provided in Table 4.

Methods for analysis of smoke constituents were as follows. Tar, nicotine, and CO in mainstream smoke were analyzed according to the standard methods (Health Canada, 1999; ISO 10362-1, 1999; ISO 4387, 2008; ISO 8454, 2009; ISO 10315, 2011). TPM was