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Insights from analysis for harmful and potentially harmful constituents (HPHCs) in tobacco products

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ABSTRACT

A total of 20 commercial cigarette and 16 commercial smokeless tobacco products were assayed for 96 compounds listed as harmful and potentially harmful constituents (HPHCs) by the US Food and Drug Administration. For each product, a single lot was used for all testing. Both International Organization for Standardization and Health Canada smoking regimens were used for cigarette testing. For those HPHCs detected, measured levels were consistent with levels reported in the literature, however substantial assay variability (measured as average relative standard deviation) was found for most results. Using an abbreviated list of HPHCs, statistically significant differences for most of these HPHCs occurred when results were obtained 4–6 months apart (i.e., temporal variability). The assay variability and temporal variability demonstrate the need for standardized analytical methods with defined repeatability and reproducibility for each HPHC using certified reference standards. Temporal variability also means that simple conventional comparisons, such as two-sample *t*-tests, are inappropriate for comparing products tested at different points in time from the same laboratory or from different laboratories. Until capable laboratories use standardized assays with established repeatability, reproducibility, and certified reference standards, the resulting HPHC data will be unreliable for product comparisons or other decision making in regulatory science.

1. Introduction

The US Food and Drug Administration (FDA) has established a list of 93 "harmful and potentially harmful constituents" (HPHCs) for tobacco products (FDA, 2012a). Currently, tobacco product manufacturers are required to report to FDA the levels of an abbreviated HPHC list by brand and sub brand (18 HPHCs for cigarette smoke and 9 HPHCs for smokeless tobacco products) (FDA, 2012b). The purpose of this reporting is to allow FDA to "publish in a format that is understandable and not misleading to a lay

Abbreviations: 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin; 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-furan; 1,2,3,4,7,8,9-HpCDF, 1,2,3,4,7,8,9-heptachlorodibenzo-*p*-furan; 1,2,3,4,7,8-HxCDD, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin; 1,2,3,4,7,8-HxCDF, 1,2,3,4,7,8-hexachlorodibenzo-*p*-furan; 1,2,3,6,7,8-HxCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin; 1,2,3,6,7,8-HxCDF, 1,2,3,6,7,8-hexachlorodibenzo-*p*-furan; 1,2,3,7,8,9-HxCDD, 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin; 1,2,3,7,8,9-HxCDF, 1,2,3,7,8,9-hexachlorodibenzo-*p*-furan; 1,2,3,7,8-PeCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin; 1,2,3,7,8-PeCDF, 1,2,3,7,8-pentachlorodibenzo-*p*-furan; 2,3,4,6,7,8-HxCDF, 2,3,4,6,7,8-hexachlorodibenzo-*p*-furan; 2,3,4,7,8-PeCDF, 2,3,4,7,8-pentachlorodibenzo-*p*-furan; 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-TCDF, 2,3,7,8-tetrachlorodibenzo-*p*-furan; A-α-C, 2-amino-9*H*-pyrido[2,3-*b*]indole; CDC, Centers for Disease Control and Prevention; CORESTA, Cooperation Centre for Scientific Research Relative to Tobacco; CRP2, CORESTA Reference Product 2; FDA, US Food and Drug Administration; GC/MS, gas chromatography–mass spectrometry; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole; HPHC, harmful and potentially harmful constituent; HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectroscopy; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; ISO, International Organization for Standardization; LC–MS/MS, liquid chromatography–mass spectroscopy–mass spectroscopy; LOD, limit of detection; LOQ, limit of quantification; MEA-α-C, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; MCS, mainstream cigarette smoke; GC/MS/MS, gas chromatograph–mass spectroscopy–mass spectroscopy; NDEA, *N*-nitrosodiethylamine; NDELA, *N*-nitrosodiethanolamine; NDMA, *N*-nitrosodimethylamine; NMEA, *N*-nitrosomethylethylamine; NMOR, *N*-nitrosomorpholine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; NSAR, *N*-nitrososarcosine; OCDD, octachlorodibenzo-*p*-dioxin; OCDF, octachlorodibenzo-*p*-furan; PAHs, polyaromatic hydrocarbons; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; total TCDD, total tetrachlorodibenzo-*p*-dioxin; total-HpCDD, total heptachlorodibenzo-*p*-dioxin; total-HpCDF, total heptachlorodibenzo-*p*-furan; total-HxCDD, total hexachlorodibenzo-*p*-dioxin; total-HxCDF, total hexachlorodibenzo-*p*-furan; total-PeCDD, total pentachlorodibenzo-*p*-dioxin; total-PeCDF, total pentachlorodibenzo-*p*-furan; total-TCDF, total tetrachlorodibenzo-*p*-furan; TPM, total particulate matter; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 1-methyl-3-amino-5*H*-pyrido[4,3-*b*]indole.

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person, and place on public display (in a manner determined by the Secretary) the list..." (Family Smoking Prevention and Tobacco Control Act, 2009, sec. 904). In addition, FDA has encouraged tobacco product manufacturers to include HPHC data in new product applications, although FDA has not been explicit in how it intends to use HPHC data when evaluating new tobacco products (FDA, 2011a,b,c). For both the purpose of consumer communication and potentially for the purpose of new tobacco product application evaluation, it is critical to understand and consider the limitations of tobacco product constituent analysis resulting from all sources of variability.

Scientists and public health researchers have measured levels of chemical constituents to compare tobacco products for decades (Adams et al., 1987; Connolly et al., 2005; Ding et al., 2006; Gendreau and Vitaro, 2005; Hammond and O'Connor, 2008). Several large studies have demonstrated that measurements of tobacco and tobacco smoke constituents are not consistent as a result of assay, inter-laboratory, and temporal variability (Chepiga et al., 2000; Counts et al., 2004, 2005, 2006; Gaworski et al., 2011a; Morton and Laffoon, 2008; Oldham et al., 2012; Roemer et al., 2004). Estimates of assay and intra-laboratory variability can be made by comparing smoke chemistry analytical results for reference cigarettes that are manufactured as a single batch at a single point in time. For example, Gaworski et al. (2011a) included analyses of 52 mainstream cigarette smoke (MCS) constituents from the 1R4F and 2R4F reference cigarettes measured 107 times over a seven-year period at two different laboratories. The study found a general 10–15% relative standard deviation for the 39 of 52 smoke constituents that could be quantified, and it found significant differences between laboratories for some constituent measurements. It also included analyses of a control cigarette manufactured to the same specification 50 times over a seven-year period, which allowed assessment of temporal variability in MCS constituents. The authors reported variability to be greater for the control cigarette than for the reference cigarettes due to year-to-year variation in the tobacco crops used to manufacture the control cigarette. Recently, Purkis et al. (2012) summarized technical challenges and possible limitations for measurement of MCS constituents based on a review of Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) sponsored collaborative studies. Their review highlighted data variability issues (e.g., product variability; within and between laboratory variability) and the need for standardized analytical methodology. These studies clearly demonstrate that the inherent variability associated with constituent measurement in tobacco and tobacco smoke must be considered when comparing measured constituent levels between samples.

The purpose of the current study was to evaluate the average relative standard deviation for all chemical constituents on a draft list of 96 HPHCs from MCS and smokeless tobacco products, which FDA published in 2011 (FDA, 2011d). The study samples were commercial cigarettes and smokeless tobacco products manufactured by Philip Morris USA and U.S. Smokeless Tobacco Company. In addition, samples from the same manufacturing lot were analyzed a second time for the constituents listed on an abbreviated list of HPHCs corresponding to those included in FDA's draft guidance for constituent reporting (FDA, 2012b). This second analysis allowed assessment of short-term temporal analytical variability. These analyses confirm previous studies demonstrating the inherent variability in tobacco and tobacco smoke constituent measurement and extend these findings to the entire FDA draft HPHC list (FDA, 2011d).

2. Materials and methods

Three contract laboratories analyzed tobacco products for all 96 HPHCs (Table 1) on the draft HPHC list (FDA, 2011d). The chlorinated dioxins/furans measured in this study, which were listed as a single entity on the draft HPHC list (FDA, 2011d), are listed in Table 2. All three laboratories were ISO 17025 accredited, and all analytical methods for determination of HPHCs were on the laboratories' ISO scope of accreditation at the time of testing. The three laboratories used were Arista Laboratories (Richmond, VA; Laboratory 1), Enthalpy Analytical, Inc. (Durham, NC; Laboratory 2), and Labstat International ULC (Ontario, Canada; Laboratory 3). Laboratories 1 and 2 were accredited by the American Association for Laboratory Accreditation, whereas Laboratory 3 was accredited by the Standards Council of Canada. Whether the HPHC was analyzed in cigarettes, smokeless tobacco products, or both, was based upon recommendations from the Tobacco Product Constituents Subcommittee of the Tobacco Products Scientific Advisory Committee (TPSAC, 2010).

2.1. Tobacco products

Twenty commercial cigarette and 16 commercial smokeless tobacco products (Table 3) were assayed for all applicable HPHCs on the 96-item draft list (FDA, 2011d). The 3R4F reference cigarette (University of Kentucky) was used as the reference product for MCS constituents, and the CORESTA Reference Product 2 (CRP2; North Carolina State University) was used as the reference for smokeless tobacco constituents. In order to minimize analytical variability, all products were submitted to a single laboratory for a given method.

Based upon previous experience in measuring MCS constituents (Counts et al., 2004, 2005; Morton and Laffoon, 2008; Gaworski et al., 2011b), three replicate analyses (each analysis may require multiple cigarettes) were chosen as sufficient to provide an estimate of each HPHC. The only exception was carbonyls in smoke, for which five replicates were used. The FDA draft guidance for reporting HPHCs (FDA, 2012b), which was published after this study started, recommended that seven replicates be used for each HPHC except smoke nicotine and carbon monoxide, for which twenty replicates were recommended. Therefore, to obtain the recommended number of replicates, for each tobacco product in this study a second set of samples was analyzed for the abbreviated list of HPHCs (FDA, 2012b) at the same three laboratories. For each tobacco product, a single manufacturing lot was used for all testing.

2.2. Smoking regimens

MCS was generated under both ISO (2000b) and Health Canada (1999a) machine smoking regimens using commercially available linear and rotary smoking machines. Cigarettes were conditioned before smoking in accordance with ISO 3402 (ISO, 1999). MCS was generated and collected in basic accordance with ISO 3308 (ISO, 2000b) and ISO 4387 (ISO, 2000c). Deviations from the ISO standards were made when necessary in order to accommodate Health Canada smoking and in order to incorporate smoke traps for volatile or gas phase HPHCs.

2.3. HPHCs analytical assays for mainstream cigarette smoke

Official methods (Health Canada; Centers for Disease Control and Prevention [CDC]) are cited, and other internally validated methods are briefly described for general understanding.

2.3.1. Nicotine and carbon monoxide in mainstream cigarette smoke

Nicotine and carbon monoxide were determined using Health Canada Official Method T-115 (Health Canada, 1999a). Since this method also measures water and calculates tar, these data were also collected.