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Mutagenic, cytotoxic, and genotoxic properties of tobacco smoke produced by cigarillos available on the Canadian market ☆

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ABSTRACT

Cigarillos (aka little cigars) have been increasing in popularity unlike cigarettes; but relatively little is known about the toxicology of the mainstream smoke (MSS) from such products. Therefore, the objective of this work was to compare the toxicological properties of the MSS (Health Canada Intensive smoking conditions) from a range of cigarillo products with the toxicological properties of MSS of cigarettes. Three *in vitro* assays were used to evaluate the toxicities of the MSS total particulate matter (TPM): (1) mutagenicity using Ames assay with *Salmonella* strains TA98 and TA100 with S9 metabolic activation (+S9); (2) cytotoxicity using the Neutral Red Uptake (NRU) assay with CHO (Chinese Hamster Ovary) cells; and (3) genotoxicity using the micronucleus assay with CHO cells and short-term exposures (3-h ± S9). The Ames assay (TA100 + S9) and the NRU assay were also applied to the gas/vapour phase of the MSS that passed through the Cambridge pad. On a per-milligram-nicotine basis, the preferred way of comparing toxicities of different types of tobacco products, the MSS from cigarillos was not less toxic, and in some cases more toxic (TPM fraction TA98 + S9, NRU), than the MSS from cigarettes. Thus, our findings support our prior work on smoke mutagenicity that showed MSS from cigarillos was not less toxic than MSS from cigarettes.

1. Introduction

When tobacco was first smoked, it was likely done by rolling up tobacco leaves in the form of a crude cigar, igniting the end, and drawing smoke through the spaces between the layers of rolled leaf. One account of the history of cigars reported that Christopher Columbus observed natives in Cuba smoking tobacco wrapped in plant leaves (Hoffmann and Hoffmann, 1998). More refined methods of cigar production were developed over the centuries including the breeding of tobaccos for cigar products as well as leaf grades for wrapping the cut filler and wrapper grades that give a uniform appearance to the exterior surface of the cigar. Today's all natural handmade cigars are made in much the same traditional manner (Wehlburg, 1999). Advances in the development of machine-made cigarettes have been adopted by the cigar industry over the years. These developments have included the use of reconstituted tobaccos to replace the natural leaf binders and/or wrappers and the use of plastic tips and/or filter cigarette-like cellulose acetate filters.

Consumer preferences have also driven new types of cigars that have a lighter taste and can be enjoyably smoked in less time and in more locations than it would be convenient with conventional cigar products. Furthermore, some manufacturers started adding flavors (e.g., fruit flavors, menthol, and vanilla) to their products to give consumers additional choices when selecting cigar products. Additional consumer choices were provided by manufacturers who added products containing mixtures of flue-cured and burley tobaccos (e.g., pipe-tobacco cigars), blends of light air-cured tobaccos, in addition to products that had only the traditional air-cured and fermented cigar grades. All of these changes have blurred the traditional differences between cigar and cigarette products.

Taxes have been increased on cigarettes and other tobacco products with the objectives of raising revenue, discouraging adult consumption, and raising prices to the extent that they are less affordable to youth. Until recently, most of the increases in tobacco taxes have been on cigarettes. These tax increases have caused some cigarette smokers to use other tobacco products such as cigars, roll-your-own products ("RYO"), and smokeless tobacco products ("STP") such as moist snuff and those products have gained in volume at the expense of cigarettes (Capehart, 2005). In the USA State of New Jersey, cigar sales rose by over twenty percent (20%) from 2003 to 2005 (UMDNJ-School of Public Health, 2006). The same source reported that sales of flavored cigars also increased dramatically during the 2003 to 2005 period. Increases

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in cigar sales appear to be driven by the smaller cigars, cigarillos, and products whose dimensions are very similar to those of cigarettes such as the so-called little cigars (UMDNJ-School of Public Health, 2006). In 2005, the federal excise tax on cigarettes was US $0.39 per pack of 20 while the corresponding tax on little cigars was US $0.04 per pack of 20 (Clarke et al., 2006). In addition to tax rates, the passage in 2009 in the US of the Family Smoking Prevention and Tobacco Control Act (FSPTCA) (USGPO, 2009) outlawed the sales of cigarettes that had characterizing flavors such as strawberry, grape, orange, and clove. However, cigars of all dimensions that had the same characterizing flavors continued to be legal. The Canadian government has outlawed the sales of cigarettes and cigarillos weighing less than 1.4 g (about 3 pound per 1000 cigars) that contain added flavors other than menthol (Canadian Parliament, 2009). In the US, products must now weigh more than 3 pound per thousand and be wrapped in a reconstituted tobacco wrapper that contains more than 67% tobacco to be considered as large cigars and not small cigars that are taxed as cigarettes (Tobacco Tax and Trade Bureau, 2009; Tobacco Tax and Trade Bureau 2006; Herbst, 2007). The difference in taxes has helped the market for so-called filter cigars to expand.

Filtered cigars are similar in appearance to cork-tipped KS or 100-mm cigarettes except for the brown color of the reconstituted tobacco wrapper around the tobacco rod in place of cigarette paper. Generally, both the filter weight and the tobacco section weight are heavier than they are for cigarettes to exceed the 3 lb per thousand minimum weight that is used for regulatory classification in Canada and tax classification in the US. Manufacturers of filtered cigars are often not large vertically integrated tobacco manufacturers, but small business tobacco product manufacturers. They purchase blends of ready-to-use cut tobacco, filter rods, and other fabrication materials, and only assemble and package the filtered cigars. An essential fabrication material for these filtered cigars is a high-strength paper-type reconstituted tobacco that is used as the wrapper on these products (Greiwe, 2007; Herbst, 2007). Most filtered cigars are made on equipment similar to cigarette makers, the reconstituted tobacco wrapper is used in the same manner as cigarette paper is used in cigarettes, and the tobacco blends in many instances do not contain flue-cured and oriental tobaccos, but are composed of cigar-type air-cured tobaccos and burley tobaccos (Greiwe, 2007). However, recent analytical work on filtered cigars showed that some products (often the flavored ones) contain cigar tobaccos fillers that have been cased with humectants and sugars (Lauterbach and Grimm, 2010). Thus, mainstream smoke from such filtered cigars may have some flue-cured character that would not be expected in the smoke from more traditional cigars. The MSS from flavored products also contains the components of flavors that transfer intact and flavor pyrolysis products.

It should be clear to the reader that similar products may be in different tax classes depending on the jurisdiction and have different legal names. On the other hand, very different products may have the same legal name for tax purposes. While the sample set for the research reported here was obtained in Canada, our findings will likely be of interest to regulators in the US and other countries. Therefore, for the remainder of this report, we will use the term cigarillo to refer to the products studied in our research even though the marking on the packaging may include such terms as filtered cigar, little cigar or small cigar.

One concern of tobacco regulators is that smokers, especially the younger ones, will perceive that smoking cigarillos presents less health risks than smoking cigarettes (Health Canada, 2009). There is little in the toxicological literature on contemporary smoking products to come to a clear conclusion. In 2007, Rickert and colleagues reported that TPM (ISO smoking conditions) from two types of cigarillos was more mutagenic (TA98 + S9, TA100 + S9) than the TPM from the KY2R4F and CIM-7 reference cigarettes when the mutagenicity was expressed on a revertants-per-milligram-nicotine basis (Rickert et al., 2007a). There are apparently no other reports in the literature dealing with mutagenicity of smoke from little cigars or reports on other measures of tobacco smoke toxicity such as cytotoxicity and clastogenicity (CORESTA *in Vitro* Task Force, 2007). Therefore, the objective of this research was to compare the toxicological properties of the MSS [Health Canada Intensive (HCI) smoking conditions] of a range of cigarillo products with the toxicological properties of MSS of other smoking products such as cigarettes. Three *in vitro* assays were used to evaluate the toxicities of the MSS total particulate matter (TPM): (1) mutagenicity using Ames assay with strains TA98 and TA100 with S9 metabolic activation (+S9); (2) cytotoxicity using the Neutral Red Uptake assay (NRU) with CHO cells; and (3) genotoxicity using the micronucleus assay with CHO cells and short-term exposures (3-h ± S9). In addition, the mutagenicity and cytotoxicity of the MSS gas vapor phase (GVP) were estimated using the Ames assay (TA100 + S9) and the NRU assay.

2. Materials and methods

Except for the KY3R4F reference cigarettes, the cigarillo and cigarette samples were selected by Health Canada and were received from Health Canada between July and September 2008. As such, they represent products made before passage of Bill C-32, which prohibited flavors (with the exception of menthol) on cigarettes and cigarillos weighing less than 1.4 g (Canadian Parliament, 2009). Sample descriptions and other relevant data are shown in Table 1.

Based on trademark data, Cigarillos 1, 3, 4, and 5 were likely fabricated by the same manufacturer. Cigarillos 2 and 6 likely came from other manufacturers. Cigarillo samples 1–6 are commonly known as "filtered cigars." Samples 7 and 8 were manufactured in Europe, and they came from two different tobacco companies. Both did not have filters.

Also as noted in Table 1, the sample set selected by Health Canada contained two cigarette brand-styles. One brand-style (Blended 1) was a no-additive blended cigarette that has been on the Canadian market for many years. The second brand-style (Blended 2) contained additives that would be typical for many nonmenthol brands sold in the USA.

At the request of Health Canada, the Health Canada Intensive (HCI) smoking protocol was used for routine smoke determinations and to collect the TPM and GVP fractions for toxicological studies. The smoke collection assembly was the same as that used for Health Canada Method T-502, Determination of Cytotoxicity with the Neutral Red Uptake Assay (CHO cells). However, in addition to the cytotoxicity assays on TPM and GVP, the TPM fraction was also used for the Ames assays (TA98 + S9, TA100 + S9) and the *in vitro* micronucleus assays; and the GVP fraction was used for Ames assays (TA100 + S9) to determine mutagenicity of the GVP. The modified microsuspension procedure for the Ames assay as described by Kado et al. (1983) was used for the evaluation of the mutagenicity of the GVP fraction. The Ames assays on the mainstream TPM were done with Health Canada Method T-501. The *in vitro* micronucleus assays on mainstream TPM were conducted with Health Canada Method T-503 with CHO cells and short-term exposures (3-h ± S9). Health Canada tobacco and tobacco smoke analytical methods are at http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/index-eng.php. Summaries of Health Canada tobacco smoke toxicity methods (T-501, T-502, and T-503) and instructions on how to obtain copies of them can be found at http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/tox-eng.php. Further descriptions