USING TOBACCO CHEMISTRY TO HELP EXPLAIN TOXICITY DATA FOR MAINSTREAM SMOKE FROM CIGARILLOS AND FILTERED CIGARS

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Summary

In 2011, Rickert *et al*. [Regul. Toxicol. Pharmacol. 2011 Nov;61(2):199-209] reported mutagenicity, cytotoxicity, and clastogenicity data for mainstream smoke (MSS) from cigarillos and similar products (sometimes called filtered cigars) that have dimensions similar to cigarettes. These products are often wrapped with a reconstituted tobacco wrapper without use of an underlying binder and have cellulose acetate filters similar to filters used on filtered cigarettes. The tobacco blends were suspected to be air-cured as is the case with most cigars, but the toxicity data reported by Rickert did not fully support that conclusion. Initial DS scan GC-MS analyses (SSPT 16, CORESTA Congress Edinburgh, 2010) revealed that products were fabricated from pipe tobacco, blended cigarette tobacco, or what appeared to bepossibly light air-cured tobacco blends that appeared to contain glycerin and sugars. However, the mainstream smoke from many of the filtered cigars had hedonic characteristics unlike larger cigars and unlike experimental cigarettes fabricated only with the grades of burley tobacco used for US-blend cigarettes. Several burley grades likely to be used in filtered cigars were obtained and routine tobacco analytes determined along with the detailed tobacco chemistries previously reported for filler from filtered cigars. Typical blend chemistries for the tobacco from filtered cigars were alkaloids, 1.3 to 1.5%; total sugars, 2.5 to 3.2%; reducing sugars, 2.5 to 3.2%; nitrate, 1.5 to 2.2%; and chloride, 1.5 to 1.9%. DS scan GC-MS data showed evidence for glycerin, fructose, glucose, caffeic acid (trace), sucrose, and chlorogenic acid. GC-MS data on burley grades likely to be used for the blends used for the filtered cigars also showed evidence for the same set of compounds. Such blend chemistries may explain the toxicological findings as well as smoke sensory properties of these products.

Key Words: cigarillo; filtered cigar; routine tobacco chemistry, detailed tobacco chemistry

Introduction

Changes by regulatory agencies in the definitions of cigars and cigar-like products have altered the traditional definitions of what is a small cigar and what is a large cigar. Thus, we will use the term cigarillo to define the traditional small narrow cigars that are similar in size to a 100-mm cigarette, but are doubly wrapped with the traditional binder and wrapper and contain traditional cigar fillers, which are cut as cigar filler in a manner

similar to that used in cigars with larger diameters. In 2011, Rickert *et al*. reported mutagenicity, cytotoxicity, and clastogenicity data for mainstream smoke (MSS) from cigarillos and similar products (sometimes-called filtered cigars) that have dimensions similar to cigarettes. These products are often wrapped with a reconstituted tobacco wrapper without use of an underlying binder and have cellulose acetate filters similar to filters used on filtered cigarettes. The tobacco blends were suspected to be air-cured as is the case with most cigars, but the toxicity data reported by Rickert did not fully support that conclusion. Initial DS scan GC-MS analyses (SSPT 16, CORESTA Congress Edinburgh, 2010) revealed that products were fabricated from pipe tobacco, blended cigarette tobacco, or possibly light air-cured tobacco blends that appeared to contain glycerin and sugars. However, the mainstream smoke from many of the filtered cigars had hedonic characteristics unlike larger cigars and unlike experimental cigarettes fabricated only with the grades of burley tobacco used for US-blend cigarettes. The US FDA ("FDA") has proposed issuance of deeming regulations to regulate cigar products (FDA, 2012, NATO, 2012) so it is essential to have knowledge of the chemical and toxicological properties of these products. Part of the reason for the proposed FDA action may be that the popularity of cigars has been increasing (CDC, 2012; Blank *et al.*, 2011) and according to Richardson *et al.* (2012) much of that increase has come from the 18-29 age group. Very recently, King *et al.*, (2012) reported on the demographics of flavored cigar use.

It is important to know the dimensions and other physical properties of the cigars used in a given study. Rickert *et al.* (2011) chose to use the term cigarillo for describing the cigars with dimensions similar to cigarettes. Changes in Canadian (Canadian Parliament, 2009) and US laws and regulations (Tobacco Tax and Trade Bureau, 2009) have reduced the popularity of small cigars (currently defined in Canadian and US regulations as weighing three pounds or less per one thousand cigars). Such products often had cigarette-like filters and resembled king-size cigarettes as pictured in Figure 1 of the article by Rickert *et al.* (2011). Large cigars are those weighing more than three pounds per one thousand cigars (about 1300 mg per cigar). This range of products now classified as large cigars ranges from products with dimensions similar to 100-mm cigarettes all the way up to the largest cigars. Figure 1 of the article, cited as CDC, 2012, gives a good example of the differences in size between the extremes of the large cigar category. However, there can be differences in blend, additives, and other parameters between the filtered cigars that resemble 100-mm filtered cigarettes and the traditional large cigars. One main difference is in how the tobacco filler is wrapped. In traditional large cigars, the tobacco filler is wrapped with a binder and then overwrapped with a wrapper (Weilburg, 1999). This double wrapping is also used in some cigarillos. However, many of the filtered cigars sold in the US are only wrapped with a single paper-type reconstituted tobacco wrapper. This wrapper has sufficient strength that the 100-mm filtered cigars can be made on the same equipment used to make 100-mm filter cigarettes. As noted earlier, in the US, products must now weigh more than 3 pounds 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 filtered cigars to expand.

The main objective for this work stems from the findings of the previously mentioned Rickert study (2011) and the results from the initial GC-MS on tobacco fillers from cigar products we reported at the 2010 CORESTA Congress (Lauterbach and Grimm, 2010). Over the past two years, we have obtained many products that fall into the category of filtered cigars. These products are about 100 mm long. Circumference is about 8 mm, and tipping length is in the range of 32 to 35 mm depending on the brand-style of product. Filter lengths range between 25 and 30 mm with some products having a two-part filter with a 10-mm mouth-end segment and a 20-mm tobacco end segment. The tobaccos on these products are generally cut in the same manner as cigarette tobaccos. In many of the brand-styles, the color and other aspects of the blend resemble the filler used in US-blend cigarettes that do not contain reconstituted tobaccos and expanded tobaccos. However, we will show that the blends in the majority of these products were not typical of cigarette tobaccos. In addition to the GC-MS analyses we used in our earlier study (Lauterbach and Grimm, 2010), we also obtained routine tobacco analytical data and had the levels of endogenous glycerin determined in some of the tobacco samples.

Materials and Method

Samples of cigarillos and filtered cigars were obtained at retail stores and trade shows. Samples of air-cured tobaccos were provided by Dr. William Maksymowicz, Burley Stabilization Corporation, Springfield, TN. Sample identifications are given in Table 1. Routine tobacco analyses were performed by Alliance One International, Wilson, NC. Determinations of endogenous glycerin were provided by Enthalpy Analytical, Durham, NC. GC-MS analyses were performed using the procedures reported by Lauterbach and Grimm (2010). The first technique has been known as the DS scan (Lauterbach and Grimm, 2009; Moldoveanu et al., 1992; Lauterbach, 1988; Alford, 1987). Each sample of tobacco filler, binder, or wrapper (100 mg) was weighed into a GC autosampler vial. BSTFA (800 µL) and DMF (400 µL) were added to the vial. Phenanthrene-d_{10} (10 µg/1200 µL) was used as the internal standard. The vial is sealed and heated for 30 min at 76°C; after heating the supernatant liquid above the tobaccos is ready for analysis. If an autosampler is used, enough replicate samples can be prepared at one time for GC-MS system to run overnight. The second technique has been known as the HFP scan (Lauterbach and Grimm, 2009; Dong et al., 1993). Each sample of tobacco filler (250 mg) was weighed into a GC autosampler vial. However, methanol was used instead of hexafluoroisopropanol (HFP). Phenanthrene-d10 (24 µg/1000 µL) was used as internal standard. The vial was sealed and heated for 30 min at 76°C; after heating the supernatant liquid above the tobaccos was ready for analysis. GC-MS analyses were performed as follows. For both techniques, the following analytical conditions were used. The GC-MS analyses were performed on Agilent 6890 GCs coupled with Agilent 5972 or Agilent 5973 MS. Columns were J&W DB-5ms, 25 m x 0.25 mm ID x 0.25 µm film thickness. MS scan parameters were 40 – 700 amu, EI+, and a solvent delay of 8 min. The injection port temperature was 300°C, transfer line temperature was 280°C, and injection volume was 1 µL with a 10:1 split ratio The DS

scan oven temperature program was initial temperature: 50°C, initial time was 2 min, ramp rate was 2°C/min, final temperature: 300°C, hold time was 23 min, and total run time was 150 min. The HFP scan GC oven temperature program was initial temperature was 40°C, initial time was 0 min, ramp rate was 2°C/min, final temperature was 300°C, hold time was 20 min, and total run time was 150 min. Data reduction was done with WSearchPro software (www.wsearchpro.com.au).

Results

Table 1 shows the cigarillo and filtered cigar samples used in this study. The results of routine tobacco chemical analyses on the tobacco filler are also shown. Samples A – G are filtered cigars wrapped with a single paper reconstituted tobacco wrapper. Sample H was also a filtered product, but was double-wrapped in the manner of a traditional European cigarillo. Sample I was a traditional unfiltered European cigarillo. Table 1 shows data for three grades of air-cured tobaccos believed to be similar to some of the grades of air-cured tobaccos used in filtered cigars. The final two rows show data for reconstituted tobacco wrapper that was removed from products we purchased. Thus, the data we are reporting may not be reflective of wrapper that has not been in contact with tobacco filler. Dr. Gene Gillman of Enthalpy Analytical provided data on endogenous glycerin levels in the three grades of air-cured leaf. The values ranged between 0.13% and 0.15% for B3VF, B4K, and C4M grades of air-cured tobacco. Numerous GC-MS analyses [DS Scan, HFP (MeOH)] scan were obtained on the air-cured leaf, filler taken from product, and the wrappers removed from the product. However, the space limitations of this extended abstract allow for only two chromatograms. Figure 1 shows the DS Scan total ion chromatogram (“TIC”) for the filler from Brand D. Figure 2 shows the DS Scan TIC for air-cured tobacco Grade B3VF. Peak-by-peak comparison of the chromatography peaks and their mass spectra show a high degree of correlation between the two chromatograms with the expected much increased nicotine peak in the TIC of the B3VF.

Discussion

When we first started our investigations on filtered cigars, we found that our GC-MS analyses indicated that most products had air-cured tobacco blends. However, the smoke was easily inhaled and the strands of tobacco had a light color that was more typical of cigarette blends than it was cigar blends. The GC-MS work showed a hint of additives except in cases where product was flavored, and the main components of the additives were present. When we spoke with experts on air-cured tobaccos, we were told about drought-stressed burley tobaccos. These tobaccos are apparently unsuitable for use in cigarette blends, but apparently can be used in filtered cigars. However, based on the analytical data we have obtained to date, there is nothing in the routine analytical data and in the GC-MS scan data that allows us to clearly distinguish good cigarette burley from drought-stressed burley. Yes, the alkaloid levels in the blends used for most filtered cigars (particularly low-cost, US-made product is low in alkaloids relative to cigarette blends). This may account for the low sensory smoke impact relative to the impact from US blended cigarettes and the impact from straight-grade burley cigarettes.

None of the data we obtained sheds light on the toxicity findings reported by Rickert *et al.* (2011). Rickert and colleagues reported that the differences in mainstream smoke ("MSS") cytotoxicity between all burley and all flue-cured cigarettes reported by Bombick *et al.* (1998) were not seen when the MSS from cigarillos and filtered cigars were assayed along with the MSS of US blend cigarettes. There was one important difference between the Rickert study and the Bombick study. The Rickert study was done with Health Canada Intensive smoking conditions (Health Canada, 1999) as those are specified for analyses of cigarillos and filtered cigars. However, the Bombick study was done with the Federal Trade Commission smoking conditions (FTC, 1967). Research reported by Rickert *et al.* (2007) showed that the relative rankings for particulate-phase (total particulate matter, "TPM") cytotoxicity were unchanged.

If it is not the blend that is causing the MSS cytotoxicity findings reported by Rickert *et al.* (2011), then is it the tobacco-containing wrapper or wrapper/binder combinations that are responsible for the observed responses seen in the cytotoxicity assays? The materials contain many compounds not found in cigarette paper and in cigarette paper that has been in contact with the tobacco column in a cigarette. More research will be needed to ascertain the contribution of cigar wrappers and binders to the chemical and toxicological properties of the mainstream smoke from filtered cigars and cigarillos.

Conclusions

This research did not yield conclusive evidence as to the relative rankings of the particulate-phase cytotoxicity results for cigarillos, filtered cigars, and cigarettes that have been reported in the literature. Further research will be needed and should focus on the differences in smoke composition between products fabricated with tobacco containing wrappers (or wrapper/binders) and those fabricated with cigarette paper.

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Figure 1. DS Scan of tobacco filler from Brand D.

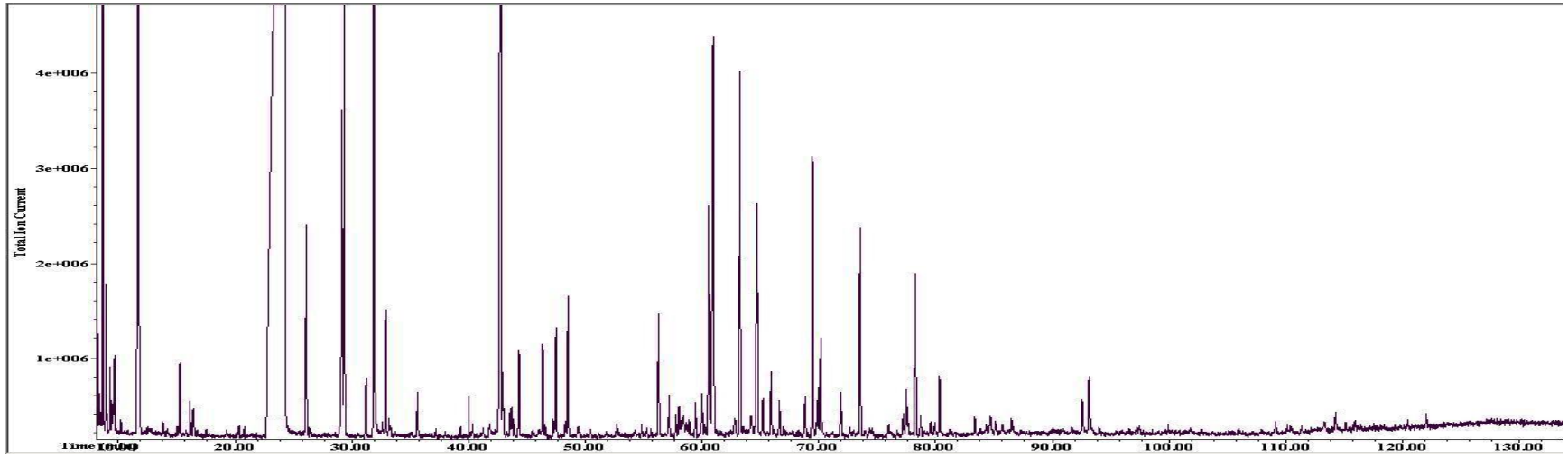

Figure 2. DS Scan of air-cured tobacco Grade B3VF

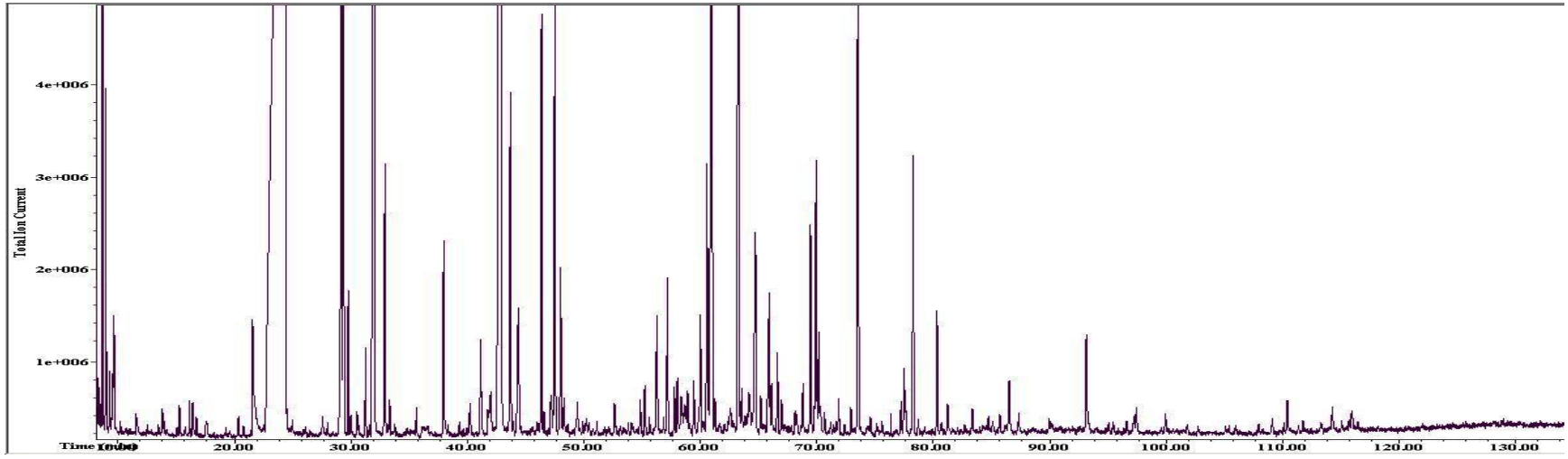

Table 1. Data from routine tobacco chemical analyses.

Brand	Flavor	Wrapper	Filter	Nicotine	Total Sugar	Reducing Sugar	Nitrate	Chloride	Moisture
A	Cherry	Single	Y	1.46	2.48	2.43	1.51	1.63	10.40
B	Sweet	Single	Y	2.02	2.29	2.20	1.17	0.95	10.07
C	Peach	Single	Y	1.35	2.99	2.78	2.17	1.91	9.74
D	Lights	Single	Y	1.29	3.19	2.78	1.59	1.69	10.90
E	Vanilla	Single	Y	1.47	3.79	3.54	0.64	1.22	10.12
F	Regular	Single	Y	1.35	3.06	3.01	1.95	1.52	9.44
F	Cherry	Single	Y	1.57	2.21	2.31	1.68	2.07	10.69
G	Coconut	Single	Y	1.23	2.79	2.53	1.82	1.59	10.37
H	Sweet	Double	Y	1.80	6.85	6.42	0.87	0.79	10.38
I	N/A	Double	N	1.38	1.63	1.58	1.61	1.48	NM
B3VF	N/A	N/A	N/A	4.35	3.27	2.93	1.56	0.70	10.12
B4K	N/A	N/A	N/A	5.16	2.82	2.71	1.55	0.61	10.38
C4M	N/A	N/A	N/A	4.75	2.55	2.44	2.03	1.10	9.71
B-wrapper	Sweet	Wrapper	N/A	0.37	0.85	1.10	1.60	1.10	NM
F-wrapper	Cherry	Wrapper	N/A	0.17	0.91	1.10	2.35	1.33	10.69

Notes:

N/A Not applicable
NM Not measured
Brand E has black-colored wrapper
Brand I is traditional European cigarillo