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Tobacco-specific nitrosamines in new tobacco products

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New tobacco products, designed to attract consumers who are concerned about the health effects of tobacco, have been appearing on the market. Objective evaluation of these products requires, as a first step, data on their potentially toxic constituents. Tobacco-specific nitrosamines (TSNAs) are an important class of carcinogens in tobacco products, but virtually no data were available on their levels in these products. In the present study, we analyzed several new products—Ariva, Stonewall, Exalt, Revel, Smokey Mountain, and Quest—for TSNAs and compared their TSNA levels with those in nicotine replacement products and conventional smokeless tobacco and cigarette brands. TSNAs were not detected in Smokey Mountain, which is a tobacco-free snuff product. The lowest levels among the new products containing tobacco were in Ariva and Stonewall (0.26–0.28 μg/g wet weight of product). The highest levels in the new products were found in Exalt (3.3 μg/g tobacco), whereas Revel and Quest had intermediate amounts. Only trace amounts were found in nicotine replacement products, and conventional brands had levels consistent with those reported in the literature. These results demonstrate that TSNA levels in new tobacco products range from relatively low to comparable with those found in some conventional brands.

Introduction

Tobacco-specific nitrosamines (TSNAs) are widely considered to be among the most important carcinogens in smokeless tobacco products and cigarette smoke (Bartsch & Spiegelhalder, 1996; Hecht, 1998; Hecht & Hoffmann, 1988; Magee, 1996; Preston-Martin & Correa, 1989). Two of these tobacco-alkaloid derived compounds, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN), are consistently carcinogenic in laboratory animals, with NNK showing higher activity (Hecht, 1998). The other two commonly measured TSNAs are *N*′-nitrosoanabasine (NAB) and *N*′-nitrosoanatabine (NAT). NAB is a weak carcinogen and NAT apparently lacks activity (Hecht, 1998). NNK and NNN have recently been evaluated by the International Agency for Research on Cancer (2005) as carcinogenic to humans (Group 1).

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In recent years, some new types of tobacco products have been appearing on the market. Ariva is a lozenge that contains compressed powdered tobacco and is advertised as "the alternative for smokers in a smoke-free environment." Stonewall Hard Snuff is another compressed tobacco lozenge. Both products are made with tobacco cured by a process that minimizes nitrosamine formation. General and Exalt are types of Swedish snus in which the GothiaTek process, designed to "continuously reduce or eliminate alleged harmful components in tobacco," is used. Revel is an American smokeless tobacco product designed for "tobacco satisfaction without smoking." Smokey Mountain is a tobacco-free and nicotine-free herbal snuff, designed as a snuff substitute. Quest is a cigarette available in three varieties: 1, low nicotine; 2, extra low nicotine; and 3, nicotine free. Quest cigarettes are "the first that gradually step you to nicotine free smoking."

With the exception of one review that includes data for Ariva, Exalt, and Revel, but without experimental details, we are aware of no published studies on TSNA levels in these products (Rodu & Jansson, 2004). These analyses were carried out here, in parallel with analyses of TSNAs in nicotine replacement products, and in conventional

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smokeless tobacco products and cigarette tobacco, for comparison.

Method

Tobacco samples

The 19 brands collected for analysis represent smokeless spit-free tobacco products, compressed tobacco lozenges, tobacco-free herbal snuff, new cigarettes with reduced nicotine content, nicotine replacement therapy products, and conventional smokeless tobacco products and cigarettes. The products were purchased between June 2003 and January 2005. General was ordered online from Snus Worldwide, Sweden. Exalt was purchased from retail stores in Washington, D.C., and Golden Valley, Minnesota; it was also ordered online from Snus Worldwide, Sweden (for comparison). Revel was ordered online (houseoxford.com) and also purchased in a convenience store in Fort Worth, Texas. Ariva was also obtained from different locations: A pharmacy in Belleville, Illinois; a pharmacy in Richmond, Idaho; and a Virginia cigarette factory outlet. Stonewall was purchased from a tobacco shop in Minneapolis, Minnesota. Quest cigarettes were ordered online through smokes-spirits.com and 001Cigarettes.com; cigarettes purchased in Richmond, Idaho, were analyzed for comparison. Conventional moist snuff, tobacco-free herbal snuff (Smokey Mountain), and commercial premium cigarettes were obtained from retailers in Minneapolis. Nicotine replacement therapy products were purchased from a pharmacy in Minneapolis. For 24 h prior to analysis, the tobacco was conditioned at room temperature in a chamber at a relative humidity of 60%.

Apparatus

Tobacco-specific nitrosamines were analyzed on a model 5890 gas chromatograph (GC; Hewlett Packard, Palo Alto, California) interfaced with a model 610 thermal energy analyzer (TEA; Orion Research, Beverly, Massachusetts). The gas chromatography conditions were as follows: DB-1301 capillary column (30 m × 0.32 mm × 0.25 µm; 6% [cyanopropylphenyl]methylpolysiloxane; J&W Scientific, Folsom, California) and a 2 m × 0.53 mm deactivated fused silica pre-column; flow rate 2.6 mL/min He; splitless injection port temperature 225°C. The following oven temperature program was used: 80°C for 2 min, then 12°C/min to 150°C, then 7 min at 150°C, then 12°C/min to 200°C, then 10 min at 200°C.

Reagents

Reference NNN, NNK, NAB, 5-methyl-*N*′-nitrosonornicotine (5-McNNN), and 5-(methylnitrosamino)-1-(3-pyridyl)-l-pentanone (C5-NNK) were synthesized as described previously (Amin, Desai, Hecht, & Hoffmann, 1996; Carmella, McIntee, Chen, & Hecht, 2000; Stepanov, Carmella, Hecht, & Duca, 2002). NAT was purchased from Toronto Research Chemicals Inc., Toronto, Ontario, Canada.

Tobacco-specific nitrosamine analyses

Moist snuff, cigarette tobacco, and lozenges. We used a slight modification of a method described previously (Adams, Brunnemann, & Hoffmann, 1983; Stepanov, Hecht, Ramakrishnan, & Gupta, 2005). Humidity-conditioned tobacco (200–500 mg) and 10 mL of citrate-phosphate buffer (pH = 4.5) containing ascorbic acid were added to a 30 mL Nalgene centrifuge tube (Nalge Nunc International, Rochester, New York) to which 200 ng each of 5-MeNNN and C5-NNK internal standards were added. The samples were homogenized for 30 s with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, New York) and sonicated for 1 hr. The buffer extracts were separated from the particles of tobacco by high-speed centrifugation (15,000 rpm, 10 min). The extracts were filtered into 50-mL glass screw-top centrifuge tubes (Kimble, Vineland, New Jersey), and the pH was adjusted to 7 by adding 100 µL of 10 N NaOH. Each sample was applied to a 20-mL ChemElut cartridge (Varian, Harbor City, California), eluted with 3 × 20 mL CH_2Cl_2, and the eluants were combined and concentrated to dryness with a model SVT200H Speedvac concentrator (Thermo Savant, Farmingdale, New York). Residues were dissolved in 0.5 mL of CH_2Cl_2 and further purified by solid-phase extraction using Sep-Pak Plus silica cartridges (Waters Corp., Milford, Massachusetts), pre-equilibrated with CH_2Cl_2. The cartridges were washed with 5 mL of CH_2Cl_2/ethyl acetate: 50/50, and the TSNAs were eluted with 10 mL of ethyl acetate. The ethyl acetate eluants were concentrated to dryness (Speedvac). The dry residues were transferred into gas chromatography microvials with 3 × 50 µL methanol, concentrated to dryness, and redissolved in 100 µL of acetonitrile. Then 3 µL of the prepared sample was injected into the GC-TEA.

Patch and gum. The patches were cut in three pieces and, after removal of the backing, rolled into 30 mL Nalgene centrifuge tubes containing 10 mL of citrate-phosphate buffer (pH = 4.5). The gum was cut into small pieces, added to Nalgene tubes containing 10 mL of citrate phosphate buffer, and homogenized for 30 s with a Polytron tissue homogenizer. Then 100 ng each of 5-MeNNN and C5-NNK internal standards were added to the samples. The samples were incubated overnight at 37°C and the next day

were sonicated for 1 hr. The buffer extracts were separated from the particles of patch or gum and transferred into 50-mL glass screw-top centrifuge tubes. The pH was adjusted to 7 by adding 100 µL of 10 N NaOH, and each sample was extracted three times with equal volumes of ethyl acetate. The extracts were combined, dried with approximately 10 g of Na_2SO_4 and concentrated to dryness with a Speedvac concentrator. Residues were dissolved in 0.5 mL of CH_2Cl_2 and further purified by solid-phase extraction and analyzed as described for tobacco products.

Results

Table 1 summarizes levels of TSNAs in the products. TSNAs were not detected in Smokey Mountain, a tobacco-free snuff product. The lowest TSNA levels among the new products that contain tobacco were found in the compressed tobacco lozenges Ariva and Stonewall, which had totals of 0.19 and 0.28 µg/g wet weight of product, most of which was due to NAT. The highest nitrosamine levels among the new tobacco products were found in Exalt, and these were independent of the place of purchase. TSNA levels were relatively low in Quest cigarette tobacco, as compared with Marlboro, Camel, Winston, and Newport. The amounts of NNN were similar in Quest 1, 2, and 3; the levels of the other TSNAs were similar in Quest 1 and 2 and lower in the nicotine-free Quest 3.

Nicotine replacement therapy products did not contain detectable levels of NAT or NAB. Traces of NNN were found in three out of six gum pieces taken for analysis (0.002 µg/piece), and traces of NNK were found in six patches analyzed (0.008 µg/patch). The levels of TSNAs in the conventional products represent means for two to six analyses, carried out at different times.

We observed substantial variation in nitrosamine content measured in the same brand at different times (see Table 2 for some examples). The Swedish snus General, produced in 2003, was found to contain lower levels of TSNAs compared with that produced in 2002. The tobacco of Marlboro and Camel had variable levels of individual TSNAs, with the total amount in Marlboro cigarettes generally lower in 2004 than in 2001 (Table 2).

Discussion

Tobacco companies are trying to create alternative smokeless tobacco products and safer cigarettes, which would potentially reduce the risk of tobacco-attributable cancers. A number of new brands are being test marketed in the United States. These products are targeted to smokers and smokeless tobacco users who wish to reduce or quit tobacco use or who want to use "safer" products. Manufacturers' claims include statements of reduced toxin content and implied reduced risk, but it may take years before the real health effects of these new tobacco products are known. TSNAs are among the most important carcinogens in tobacco, and it is imperative that objective data on levels of these compounds be available.

The lowest TSNA levels in the tobacco-containing products we analyzed were found in the compressed tobacco lozenges Ariva and Stonewall. Levels of the strongly carcinogenic NNN and NNK were only 56–99 ng/g, with most of the TSNA content comprised of NAT, which is apparently noncarcinogenic. These products use Star Scientific specially cured tobacco known to be low in TSNAs. The emergence of these new products with relatively low levels of carcinogenic TSNAs is an encouraging sign.

The Swedish snus General, which is manufactured using the GothiaTek process and quality standard designed to minimize nitrosamine contamination, contained relatively low levels of TSNAs, compared with conventional smokeless tobacco products. The variation in TSNA content observed in General in 2002 and 2003 (Table 2) is consistent with a study by the Swedish National Food Administration (Österdahl, Jansson, & Paccou, 2004) that demonstrated a noticeable decrease in TSNA content in moist snuff on the Swedish market. However, TSNA levels in Exalt, which is supposedly produced by the same technology, were comparable with those in some conventional commercial brands of smokeless tobacco such as Copenhagen and Kodiak, which have had relatively high amounts of these compounds for many years (Hecht & Hoffman, 1988; Hoffmann et al., 1995; Rodu et al., 2004). Lower levels were found in Revel; however, these levels were still considerably higher than nitrosamine levels in other products such as food and beer (Bartsch & Spiegelhalder, 1996). Based on comprehensive dietary surveys, exposure to carcinogenic volatile nitrosamines such as *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine is estimated to be about 1 µg/day (Bartsch & Spiegelhalder, 1996). The same exposure to carcinogenic TSNAs would be reached by using just 1–2 g of Revel. One packet of Revel contains approximately 27 g tobacco.

Quest cigarette tobacco contained substantial amounts of NNN, which did not decrease with reduced nicotine content. Levels of NNN and other TSNAs in tobacco are a major determinant of smoke levels (Fischer, Spiegelhalder, Eisenbarth, & Preussmann, 1990). Therefore, cigarettes lacking nicotine will still expose smokers to significant amounts of known carcinogens. However, NNN

Table 1. Tobacco-specific nitrosamine levels.

Product	Tobacco-specific nitrosamine level (μg/g product wet weight)				
	NNN	NNK	NAT	NAB	Total
New tobacco products					
Hard snuff					
Ariva	0.019	0.037	0.12	0.008	0.19[a]
Stonewall	0.056	0.043	0.17	0.007	0.28[b]
Swedish snus					
General	0.98	0.18	0.79	0.06	2.0[c]
Spit-free tobacco packets					
Exalt					
Purchased in Sweden	2.3	0.27	0.98	0.13	3.7[d]
Purchased in the United States	2.1	0.24	0.68	0.05	3.1[b]
Revel					
Mint flavored	0.62	0.033	0.32	0.018	0.99[b]
Wintergreen flavored	0.64	0.032	0.31	0.017	1.0[b]
Tobacco-free snuff					
Smokey Mountain	nd	nd	nd	nd	nd[b]
Nicotine-reduced cigarettes					
Quest 1 (low nicotine)	0.93	0.17	0.31	0.013	1.4[d]
Quest 2 (extra-low nicotine)	0.82	0.19	0.19	0.01	1.2[d]
Quest 3 (nicotine free)	0.83	0.054	0.045	0.003	0.93[d]
Nicotine replacement therapy products					
NicoDerm CQ (patch, 4-mg nicotine)[f]	nd	0.008	nd	nd	0.008[b]
Nicorette (gum, 4-mg nicotine)[f]	0.002	nd	nd	nd	0.002[b]
Commit (lozenge, 2-mg nicotine)[f]	nd	nd	nd	nd	nd[b]
Conventional tobacco products					
Smokeless tobacco					
Copenhagen					
Snuff	2.2	0.75	1.8	0.12	4.8[b]
Long cut	3.9	1.6	1.9	0.13	7.5[b]
Skoal					
Long cut straight	4.5	0.47	4.1	0.22	9.2[b]
Bandits	0.9	0.17	0.24	0.014	1.3[b]
Kodiak					
Ice	2.0	0.29	0.72	0.063	3.1[b]
Wintergreen	2.2	0.41	1.8	0.15	4.5[b]
Cigarette tobacco					
Marlboro					
Full flavor	2.9	0.96	2.3	0.1	6.3[e]
Light	2.8	0.68	1.1	0.051	4.6[b]
Ultra-light	2.9	0.75	1.1	0.058	4.8[b]
Camel					
Full flavor	2.5	0.90	1.7	0.091	5.2[e]
Light	2.7	0.55	1.3	0.061	4.6[b]
Ultra-light	2.8	0.77	1.2	0.055	4.8[b]
Winston (full flavor)	2.2	0.58	0.56	0.025	3.4[b]
Newport (full flavor)	1.1	0.83	1.9	0.055	3.9[b]

Note. NAB, *N*′-nitrosoanabasine; NAT, *N*′-nitrosoanatabine; nd, not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-nitrosonornicotine. [a]Mean of five analyses, each performed in duplicate. [b]Single analysis performed in duplicate. [c]Mean of two analyses, each performed in duplicate. [d]Mean of three analyses, each performed in duplicate. [e]Mean of four analyses, each performed in duplicate. [f]Values are expressed per piece.

Table 2. Variation of tobacco-specific nitrosamine levels in some products.

Product	Date of purchase	Number of samples	Tobacco-specific nitrosamine level (μg/g tobacco)				
			NNN	NNK	NAT	NAB	Total
General	2002	2	1.2	0.28	0.93	0.076	2.5
	2003	2	0.78	0.075	0.65	0.049	1.6
Marlboro (full flavor)	July 2001	2	4.3	1.8	2.7	0.14	8.9
	Oct. 2003	2	3.0	1.2	4.9	0.19	9.3
	Oct. 2004	2	2.0	0.37	0.71	0.03	3.1
	Jan. 2005	2	2.5	0.49	1.0	0.046	4.0
Camel (full flavor)	July 2001	2	3.1	1.4	1.6	0.11	6.2
	Oct. 2003	2	1.9	1.2	2.8	0.15	6.1
	Oct. 2004	2	2.1	0.40	0.75	0.032	3.3
	Jan. 2005	2	3.0	0.56	1.5	0.074	5.2

Note. NAB, *N*′-nitrosoanabasine; NAT, *N*′-nitrosoanatabine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-nitrosonornicotine.

and NNK levels in Quest were substantially lower than in currently available conventional brands.

Overall, the results of the present study demonstrate that TSNA levels in new tobacco products range from relatively low to comparable with those found in some conventional brands.

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