Aging of Oral Moist Snuff and the Yields of Tobacco-Specific N-Nitrosamines (TSNA)

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Introduction

In contrast to other tobacco products, the sale of moist snuff has increased significantly over the past two decades (1-3). Epidemiological studies have established that the oral use of snuff is carcinogenic to humans (4-6).
Snuff contains a number of carcinogens, especially the tobacco-specific nitrosamines (TSNA) that are present in relatively high amounts (4,5,7).
The TSNA are formed by nitrosation of nicotine and minor *nicotiana* alkaloids (8).
Previous studies have shown that storage of snuff products at room temperature can cause the TSNA levels to increase (9,10). After eight weeks of storage, the TSNA levels were reported to increase by 40 to 50 % (10).
It was the goal of this study to demonstrate that the aging of snuff applies equally to products sold in the State of Massachusetts. Confirmation of increased TSNA levels upon storage at room temperature may lead to a mandate refrigeration and/or shelf life.

Sampling Background

The following brands, 10 identical cans, were purchased in Massachusetts and shipped in dry ice to Valhalla on Nov. 11, 2000 where they were stored immediately in a refrigerated room (35 °F):

Skoal, Long Cut, Mint (UST)	41/OK, ROFFPBR
Copenhagen (regular) (UST)	Oct. 30, 2000P, ROYFPWR
Timber Wolf, Wintergreen (Swedish Match)	B191Y
Silver Creek, Long Cut, Wintergreen (Swisher)	0220E
Kodiak, Wintergreen (Conwood)	841N121JO

Another brand was shipped via DHL (no dry ice) from Sweden on Nov. 7, 2000 by Britt-Marie Lindblad, arriving in Valhalla Nov. 13, 2000 and stored at 35 °F:

Ettan Snus	5G, 01V04

The samples were analyzed for pH, moisture, nicotine and TSNA.
The TSNA levels were analyzed after aging at room temperature for 0, 2, 4, and 6 months:

Sampling date	months	actual days	cum. days
Nov 14/15	0	0	0

Methods

pH

2 g snuff is suspended in 20 ml water, stirred for 15 minutes and the supernatant is measured with a combination electrode until the pH has stabilized.

Moisture

The moisture content was determined with the Dean-Stark method: 15 g snuff is co-distilled with 100 ml benzene and the resulting water collected is measured.

Nicotine

To 200 mg moist snuff is added 4 ml methanol containing 1 % KOH and 50 ppm quinoline (internal standard). The mixture is then sonicated for one hour, filtered through a membrane filter, diluted ten-fold with methanol containing 50 ppm quinoline and analyzed by gas chromatography-NPD on a 60-m DB-5 capillary column.

Tobacco-Specific Nitrosamines

To 5 g of moist snuff is added 85 ml phosphate-citrate buffer pH 4.5 containing 20 mM ascorbic acid and 10 µg of ethyl-NNK is added as internal standard. The mixture is sonicated for one hour and filtered though Celite. The aqueous solution is extracted with dichloromethane on a Chemelut SPE cartridge and the extract is concentrated to 1 ml. The concentrate is analyzed by GC-TEA on a 60-m DB-5 column.

Results

Table 1 presents the data for pH, moisture and nicotine of the six moist snuff brands tested. In all cases, the pH is high enough to allow nicotine present in its unprotonated form (12 % at pH 7 to 81 % at pH 8.5; 11). Nicotine levels ranged between 2 and 3 %. Considering the relatively high pH of all brands, it seems that these brands are tailored for the seasoned users in contrast to a recently tested brand (Hawken), which was low in nicotine (0.4 %) and pH (6.0), clearly designed for a "beginner" (2). It has been demonstrated that unprotonated (free) nicotine is much quicker absorbed through the mucous membrane than protonated nicotine. This gives the snuff dipper a quicker nicotine effect than the weakly acidic snuff (12). The moisture of all brands varied between 50 and 56 %. The moisture content of all brands was monitored for all six brands over the course of 6 months. Figure 1 illustrates the effect of aging on the moisture content. It can be seen that the two brands sold in cardboard boxes (Copenhagen and Ettan Snus) have the greatest loss of water while those brands sold in plastic cans had a much smaller moisture loss. Figure 1 also shows the uneven loss of moisture (especially Ettan Snus, month 3 and 5 and

Silver Creek, month 3). This demonstrates that in spite of the same lot number, there is uneven aging among the same brands.
Table 2 lists the initial TSNA values of the six brands. As can be seen, the levels vary dramatically between brands. While those levels for Skoal and Copenhagen are consistent with the values observed in the past, we were surprised at the high levels of Silver Creek (a total amount of 128 µg per g of dry tobacco). We had not analyzed Silver Creek for its TSNA content previously. In 1995 we reported for Silver Creek 51.9 % moisture (here 50.3), 1.71 % nicotine (1.90) and a pH of 6.28 (7.01; 13). Kodiak showed medium levels of TSNA (16.6 µg/g), while the Swedish brand Ettan Snus had by far the lowest levels of TSNA (2.8 µg/g). Interestingly, Timber Wolf, made by Swedish Match, had the lowest TSNA values of the domestic brands tested. This illustrates that the technology exists to manufacture moist snuff with low levels of carcinogenic nitrosamines.
Interestingly, recently purchased brands of Silver Creek and Copenhagen had significantly lower TSNA levels than the lots tested in this study.
Table 3 lists the numeric values of the TSNA in the six brands for month 0, 2, 4, and 6 while Figures 2-7 graphically show the effects of storage conditions on the levels of TSNA.
While in some cases there is a linear increase of TSNA over time (Fig. 3, Copenhagen, in six months to a TSNA total of 94.3 or 130 %), some other brands have either a marginal increase (Skoal, Fig. 2, total TSNA after four months of aging 77 µg/g or 20 % and Silver Creek, Fig. 5, after four months of aging 152 µg/g or 19 %) or some small changes (Timber Wolf, Fig. 4, total TSNA after 4 months 8.2 µg/g or 9 %) or no significant changes for total TSNA in Kodiak (Fig. 6) or Ettan Snus (Fig. 7) during 6 months of aging at room temperature.
On the basis of uneven water loss, we did expect a somewhat uneven effect of storage.

Precision of the TSNA Assay

In order to assure that our assay procedure is consistent during the test period, we analyzed the TSNA in the University of Kentucky Reference snuff 1S3 (made in 1986) over the course of the study. During that time, 17 samples of KY 1S3 were analyzed. Table 4 lists the data obtained; the standard deviation varied from 5.6 % (NNN) to 8.6 %(NNK). Since the levels of TSNA are in the mid-level of those for the six brands tested, they are considered as representative.
When the same sample (Copenhagen) was analyzed on the same day (n=5), the standard deviation varied between 2.4 and 3.2 %. This demonstrates that our assay procedure is validated.

Discussion

The concentration of the total TSNA in the leading U.S. moist oral snuff brands varies greatly, from 7.5 to 128 µg per g dry tobacco (Table 2). For comparison with the TSNA concentrations in the leading five U.S. snuff brands, a popular Swedish snuff brand, which was produced under anaerobic conditions, had even lower TSNA values (2.8 µg/g) than the lowest U.S. snuff brand. This demonstrates that snuff can be produced with low concentration of TSNA and

with it, with a significantly reduced potential for carcinogenic activity. The technology clearly exists to manufacture snuff with low levels of TSNA, as shown by the Swedish brand and the brand made by Swedish Match.
The TSNA levels in the two leading U.S. snuff brands, accounting for 69 % of the 1999 U.S. market (14), were found to increase during 6 months storage at room temperature between 30 and 130 %, while the TSNA concentration in the Swedish brand increased inconsequentially. These observations suggest the possibility that Government agencies may mandate that commercial snuff brands significantly reduce their levels of TSNA with the goal of ≤10 µg/g, and that snuff be kept stored refrigerated by wholesale and retail stores. In future studies it must be documented at which temperature snuff can be stored without an increase of the concentrations of TSNA.

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Table 1. Chemical Parameters of Five Domestic and One Swedish Moist Snuff Brand

Brand	pH	Moisture (%)	Nicotine (%)
Skoal	8.00	52.6	2.98
Copenhagen	7.86	53.7	3.01
Timber Wolf	7.72	51.3	2.71
Silver Creek	7.01	50.3	1.90
Kodiak	8.40	51.3	1.89
Ettan Snus	8.52	55.9	2.01

Table 2. Initial TSNA Levels in Five Domestic and One Swedish Moist Snuff Brand (µg per g dry tobacco)

Brand	NNN	NAT	NAB	NNK	Total TSNA
Skoal	20.8	36.8	2.1	4.3	64.0
Copenhagen	14.3	22.0	1.4	3.4	41.1
Timber Wolf	3.0	3.3	0.25	0.95	7.5
Silver Creek	41.4	61.2	7.5	17.8	127.9
Kodiak	7.4	7.5	0.69	0.97	16.6
Ettan Snus	1.12	1.05	0.09	0.53	2.8

Table 3. TSNA Levels as a Function of Storage Conditions

Skoal

Months	NNN	NAT	NAB	NNK
0	20.8	36.8	2.1	4.3
2	26.0	38.7	2.6	6.0
4	30.4	37.1	2.9	6.6
6	29.3	36.1	3.0	5.9

Copenhagen

Months	NNN	NAT	NAB	NNK
0	14.3	22.0	1.4	3.4
2	20.7	30.9	2.2	6.8
4	26.0	38.7	3.0	8.2
6	31.6	45.2	4.1	13.4

Timber Wolf

Months	NNN	NAT	NAB	NNK
0	3.0	3.3	0.25	0.95
2	3.4	3.2	0.28	0.90
4	3.8	3.1	0.29	1.0
6	3.6	3.0	0.32	0.90

Silver Creek

Months	NNN	NAT	NAB	NNK
0	41.4	61.2	7.5	17.8
2	53.8	67.2	10.7	22.4
4	55.9	66.5	9.3	20.3
6	51.5	63.7	8.5	17.6

Table 3 cont.

Kodiak

Months	NNN	NAT	NAB	NNK
0	7.4	7.5	0.69	0.97
2	9.1	7.3	0.71	1.04
4	8.8	6.3	0.80	0.90
6	8.1	5.4	0.74	0.73

Ettan Snus

Months	NNN	NAT	NAB	NNK
0	1.12	1.05	0.09	0.53
2	1.20	0.86	0.08	0.48
4	1.22	0.74	0.16	0.43
6	1.34	0.87	0.12	0.39

Table 4. TSNA Assay Precision (KY 1S3)

	NNN	NAT	NAB	NNK
n=17				
Mean	8.83	4.20	0.75	2.07
Std. Dev.	0.49	0.30	0.05	0.18
% Std. Dev.	5.57	7.05	6.33	8.59

Figure 1: Snuff Moisture Change

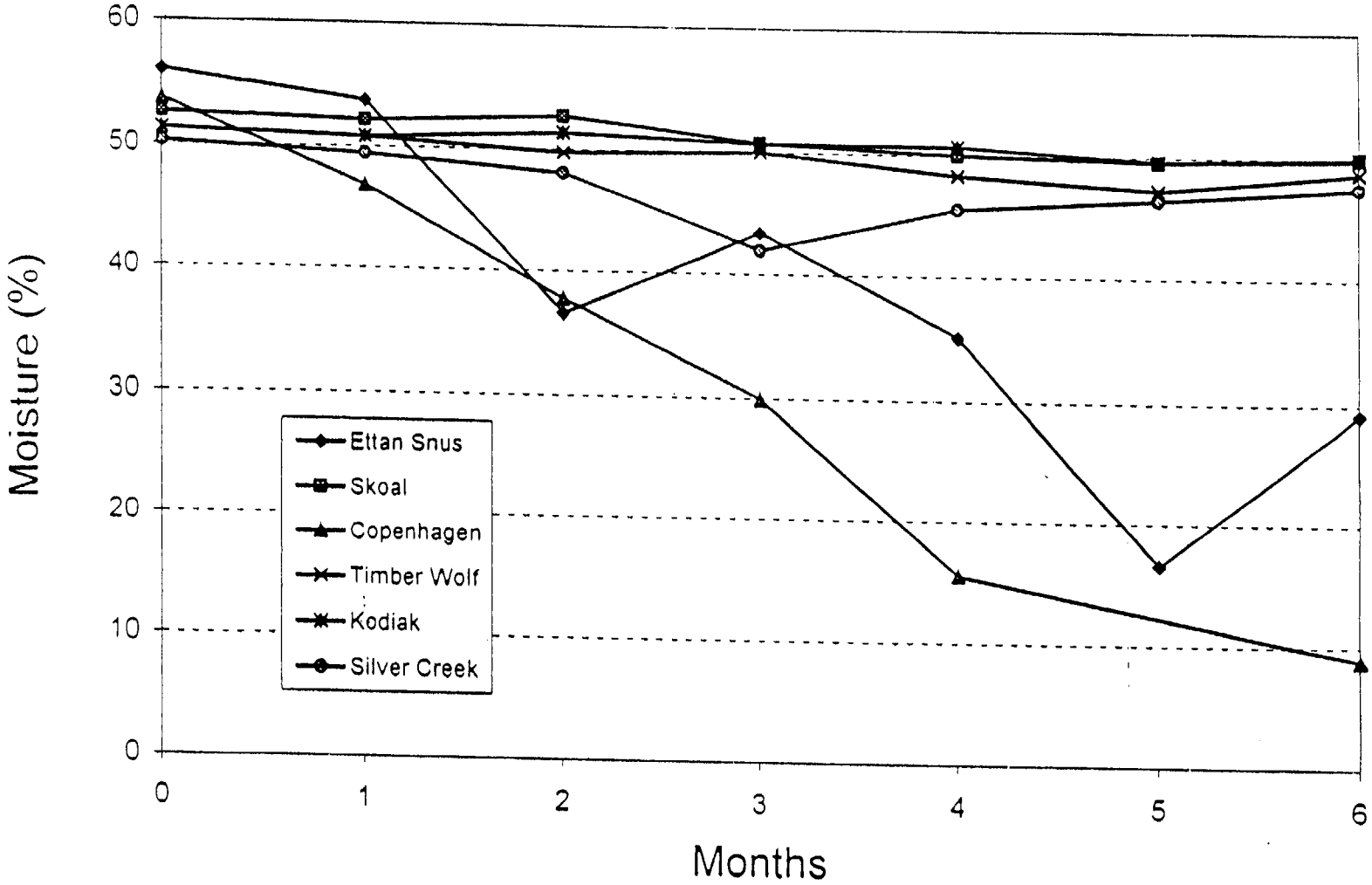

Figure 3. Effect of Storage on TSNA Levels
Copenhagen

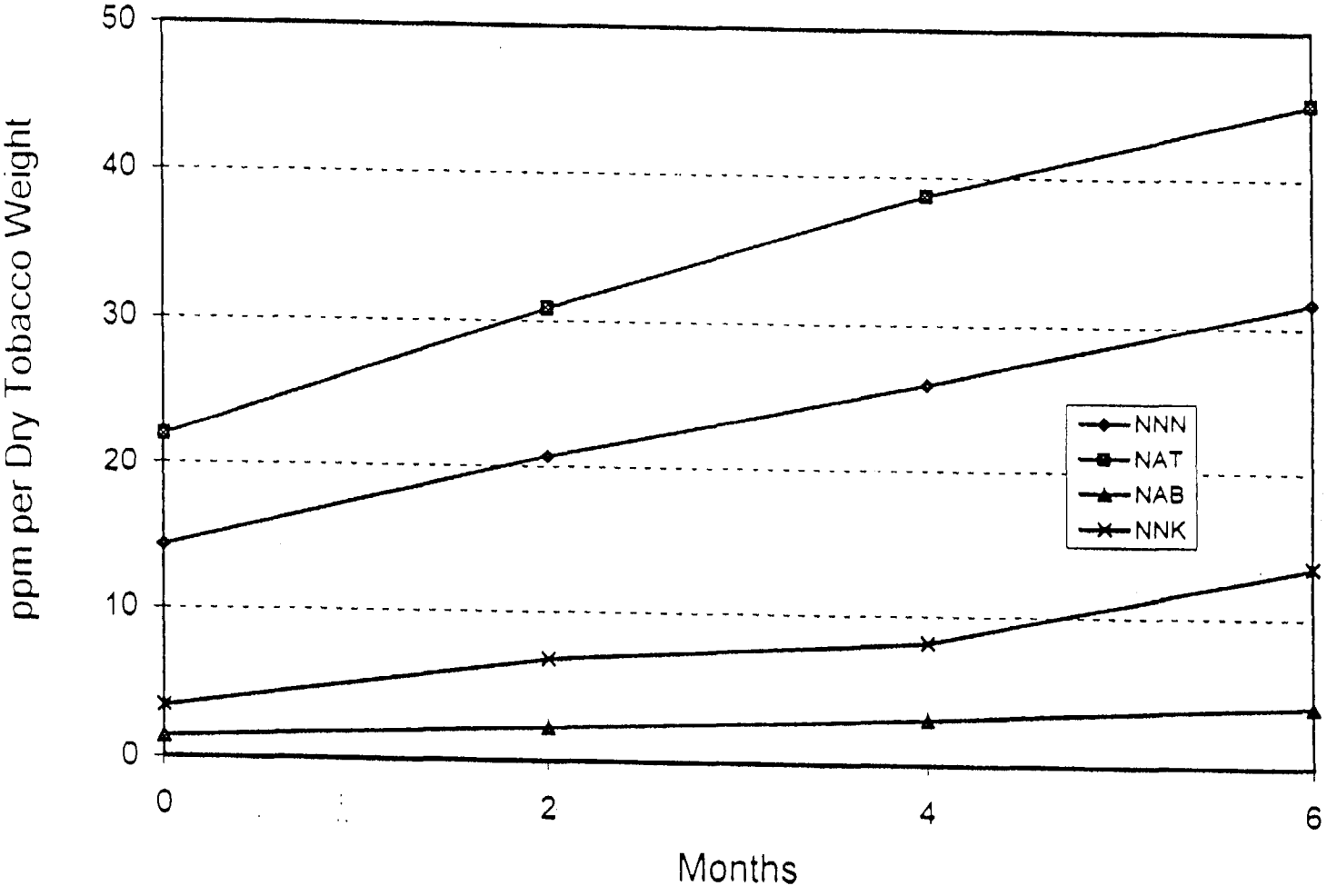

Figure 7. Effect of Storage on TSNA Levels
Ettan Snus