Date: August 21, 2001

To: Public Health Council

From: Gregory N. Connolly, D.M.D., M.P.H.
Director, Tobacco Control Program
Howard Saxner, Deputy General Counsel

Re: Informational Update
Research on Tobacco Specific Nitrosamines (TSNAs) in Oral Snuff and a Request to Tobacco Manufacturers to Voluntarily Set Tolerance Limits For TSNAs in Oral Snuff

In 1985, the Department declared oral snuff a hazardous substance under M.G.L. c. 94B and manufacturers were required to place health warnings on packages. Recently, the Department commissioned a study with the American Health Foundation that measured levels of cancer causing tobacco specific nitrosamines (TSNAs) in a form of smokeless tobacco called oral snuff. The study is appended. The research found that the levels of TSNAs in oral snuff that employed a new manufacturing process to be five to forty times lower than those of products manufactured under standard processes.

Based on the results, we intend to write to the major manufacturers of oral snuff and ask that they voluntarily adopt new manufacturing processes for all brands of snuff sold in Massachusetts that would reduce TSNA content to the lowest possible level but a minimum 10 micrograms per gram. Depending upon the manufacturers' response and a thorough analysis of associated issues, Department staff will consider amending the current hazardous substance regulations to require limits for TSNA levels in oral snuff. The following narrative provides supporting information.

1.) Oral Snuff as a Hazardous Substance

In 1985, the Massachusetts Department of Public Health declared oral snuff to be a hazardous substance under the state Hazardous Substances Act, M.G.L. c. 94B and required health warnings on snuff packages. In response to this action by Massachusetts and other states, a federal law was passed in 1986 restricting advertising of oral snuff and requiring uniform national labels on packages. The Massachusetts labeling requirement was preempted by the federal statute. Oral snuff remains on the Department's list of hazardous substances.

Oral snuff causes oral cancer and the principal cancer causing agents are a class of compounds called tobacco specific nitrosamines (TSNAs). TSNA concentrations are a hundred times greater than that found in regulated products such as meats, beer and baby bottle nipples.

2.) Department of Public Health's Research on TSNA Levels in Oral Snuff

The Department of Public Health contracted with the American Health Foundation to determine how the new and existing technologies affect the levels of TSNAs in six brands of oral snuff. The Department obtained brands of snuff sold in the Commonwealth as well as one brand, Ettan, sold in Sweden. The American Health Foundation research found that the Swedish Match brand and its U.S. subsidiary brand had total TSNA levels between 2.8 ug/g (Ettan) and 7.5 ug/g (TimberWolf). These levels were far lower than that found for the standard brands available in the state. According to Table I, levels for UST, Swisher and Conwood brands ranged from 16.6 ug/g to 127.9 ug/g.

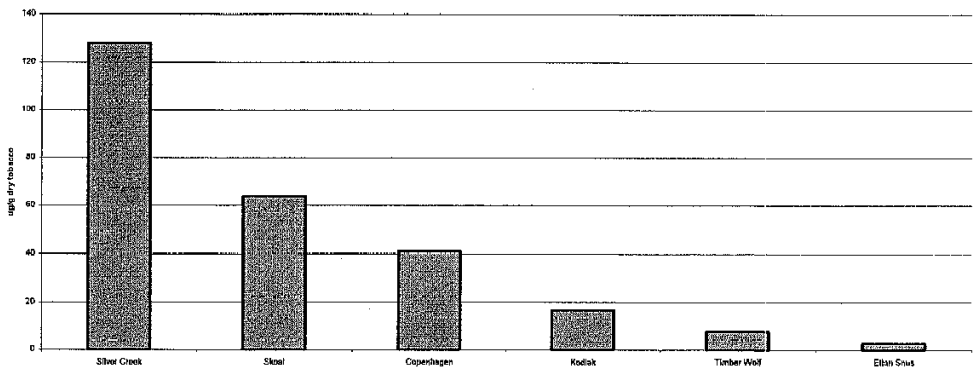
Table I
TSNA Levels in Six Brands of Oral Snuff
(micrograms per gram)

Brand	Company	NNN	NAT	NAB	NNT	Total TSNA
Ettan	Swedish Match	1.12	1.05	.09	0.53	2.8
TimberWolf	Pinkerton (Swedish Sub.)	3.0	3.3	0.25	0.95	7.5
Kentucky Ref.	Uni. KY	8.83	4.2	0.75	2.07	15.85
Kodiak	Conwood	7.4	7.5	0.69	0.97	16.6
Copenhagen	UST	14.3	22.0	1.4	3.4	41.1
Skoal	UST	20.8	36.8	2.1	14.3	64.0
Silver Creek	Swisher	41.4	61.2	7.5	17.8	127.9

The same study examined the effect of product aging over two, four and six months. Product aging involves placing the tobacco product on a shelf at room temperature and retesting the TSNA levels at the specified time periods. The study found that certain U.S. brands had large increases in TSNA levels. Copenhagen increased 137% over the six-month time period and Skoal increased 20%. Silver Creek increased 9% over a four month time period. No significant changes were observed in the levels of Swedish Match or its subsidiary brands.

The study shows that the levels in the brands manufactured under the new technologies are significantly lower than levels of TSNAs in those brands that are produced under the standard manufacturing processes. Also brands that employ the new processes show no increase in TSNAs when aged. The study demonstrates that it is technologically feasible to produce oral snuff products for adults that are significantly lower in TSNAs than many of those currently on the market.

Inital TSNA Levels in Five Domestic and One Swedish Moist Snuff Brand



3.) The Differences in the TSNA Levels are Due to Different Manufacturing Processes

Swedish Match Company has developed a new method for manufacturing oral snuff that uses selected blends of tobacco as well as a new processing method. Unlike the dark fire cured tobacco common in U. S. snuff, Swedish Match employs tobacco with a low nitrate content, which in itself reduces TSNA levels in the Ettan Brand.

Also, the tobacco is processed in a heated closed system that resembles pasteurization of milk, thus eliminating bacteria that could contribute to TSNA formation. The company also encourages retailers to refrigerate packages to prevent TSNA formation during aging. According to Swedish Match, a somewhat different process is employed in the manufacturing of oral snuff sold in the U.S. (Timberwolf). This brand is priced competitively with other U.S. brands, showing that it is economically feasible to reduce TSNA levels.

Other snuff manufacturers have developed new manufacturing processes that greatly reduce the levels of TSNAs in oral snuff (Pinkerton Tobacco, Star Scientific, and U. S. Tobacco (UST)). The different processes are explained in paragraph 4 below. UST has introduced this technology for a new brand of oral snuff called Revel, but it is unknown if the company will be using this technology in all its brands.

4.) Effects of Setting Limits for TSNAs in Oral Snuff

Research on animal exposure to TSNAs shows a dose response relationship between exposure levels and the incidence of oral tumors. Human data has also found the dose response relationship between the length of oral snuff use and the risk of developing oral cancer. These data strongly suggest that the lowering of TSNA levels in oral snuff could reduce the risk to oral cancer but not eliminate it.

According to the 2000 Surgeon General's Report, if a new technology exists that can significantly reduce levels of known carcinogens in a tobacco product, then that technology should be used.

> "Additionally, as with other consumer products, the manufactured tobacco product should be no more harmful than necessary given available technology." U.S. Surgeon General, Report 2000

Department staff have considered the possibility that setting limits for TSNAs in snuff could have unintended consequences. The history of low yield cigarettes shows how a government mandate for testing cigarette tar and nicotine levels resulted in manufacturers using terms such as "light" and "ultralight" cigarettes to imply that low-yield brands were safe, even though that is not the case. The same could happen with reduced TSNA levels for snuff. However, since the TSNA

reduction would be the same for all brands, there shouldn't be any significant difference in TSNA levels, thus eliminating any marketing claims that one brand is "safer" than another. Regardless, manufacturers are already marketing selected oral snuff brands as having lower TSNA levels (Exalt and Arriva) and implying that they are safe.

Even though TSNA levels can be reduced in oral snuff, the product contains other carcinogens (polonium 210 and benzopyrene) and is highly addictive. Oral snuff is not a safe alternative to cigarette smoking. Pharmacological agents such as nicotine patches and gum are available which have been shown to be safe and at a low risk of causing addiction. The Department will continue to classify oral snuff as a hazardous substance and to maintain that the product is not a safe alternative to cigarettes. The Department will continue to aggressively educate young people and adult users as to the hazards of oral snuff.

5.) Summary

The existing evidence indicates that US manufacturers of oral snuff are selling a product that contain between 2 and 42 times the level of nitrosamines as products sold by Swedish companies. Moreover, aging increases the levels of nitrosamines even higher in the US product, whereas the Swedish product does not change with age. US manufacturers have the manufacturing technology to reduce the level of nitrosamines to the same level as those found in Swedish products, yet they have not done so. There can be no excuse for this failure to remove a known toxin from an already hazardous product, when the technology exists and US manufacturers should respond immediately. Adequate post market surveillance is needed to know ultimate benefits of removing this toxin. No other manufacturer of a consumer product would be allowed to leave in a known toxin once it could be removed. The smokeless industry should do this minimum.

Based on the findings of this study, the Department intends to request that manufacturers who sell oral snuff in Massachusetts adopt new technologies to reduce TSNA content to the lowest possible level but at a minimum below 10 ug/g.

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

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Progress Report

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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
Jan. 16/17	2	63	63
Mar. 14/15	4	57	120
May 15/16	6	62	182

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

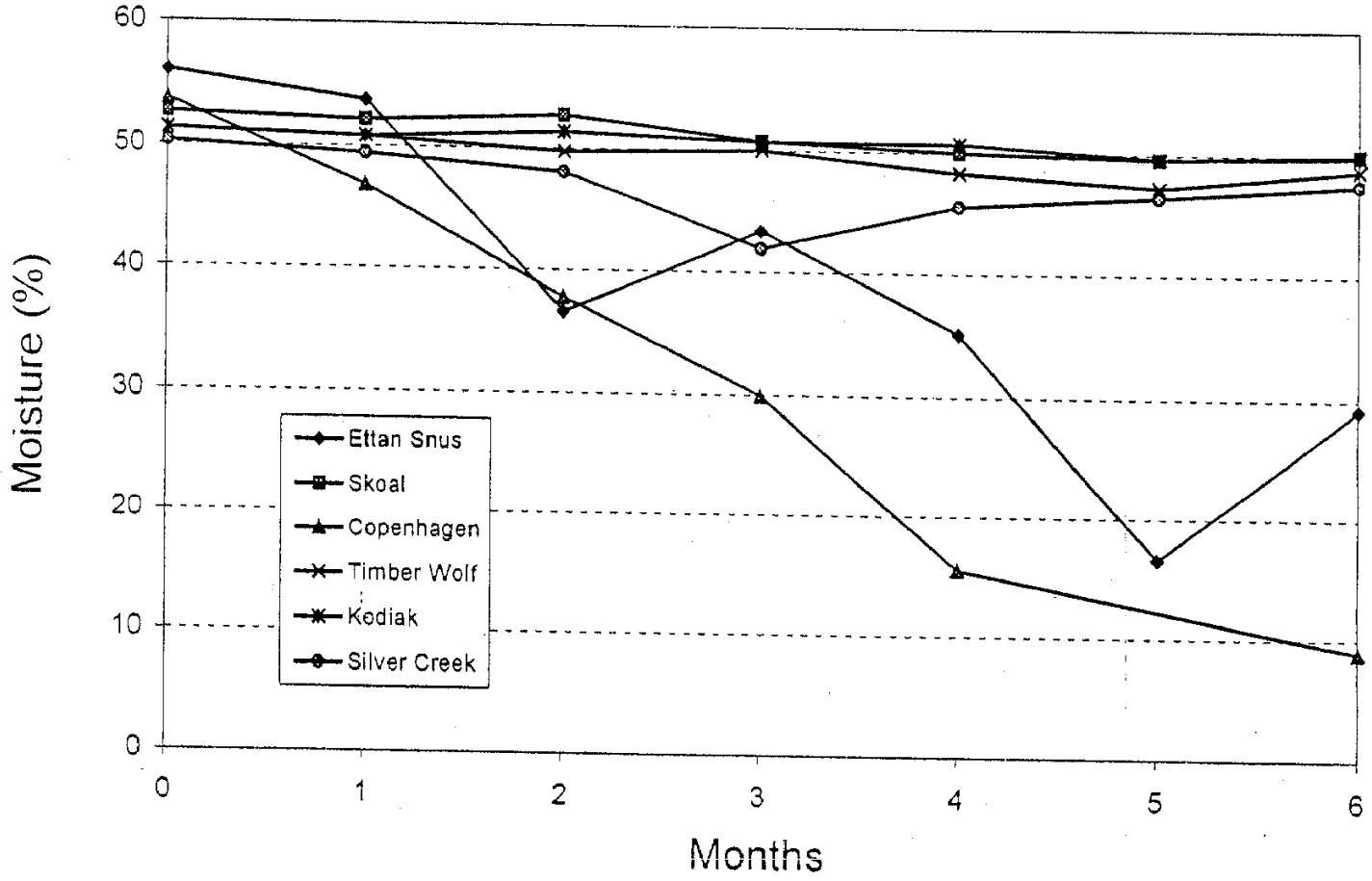

Figure 3. Effect of Storage on TSNA Levels
Copenhagen

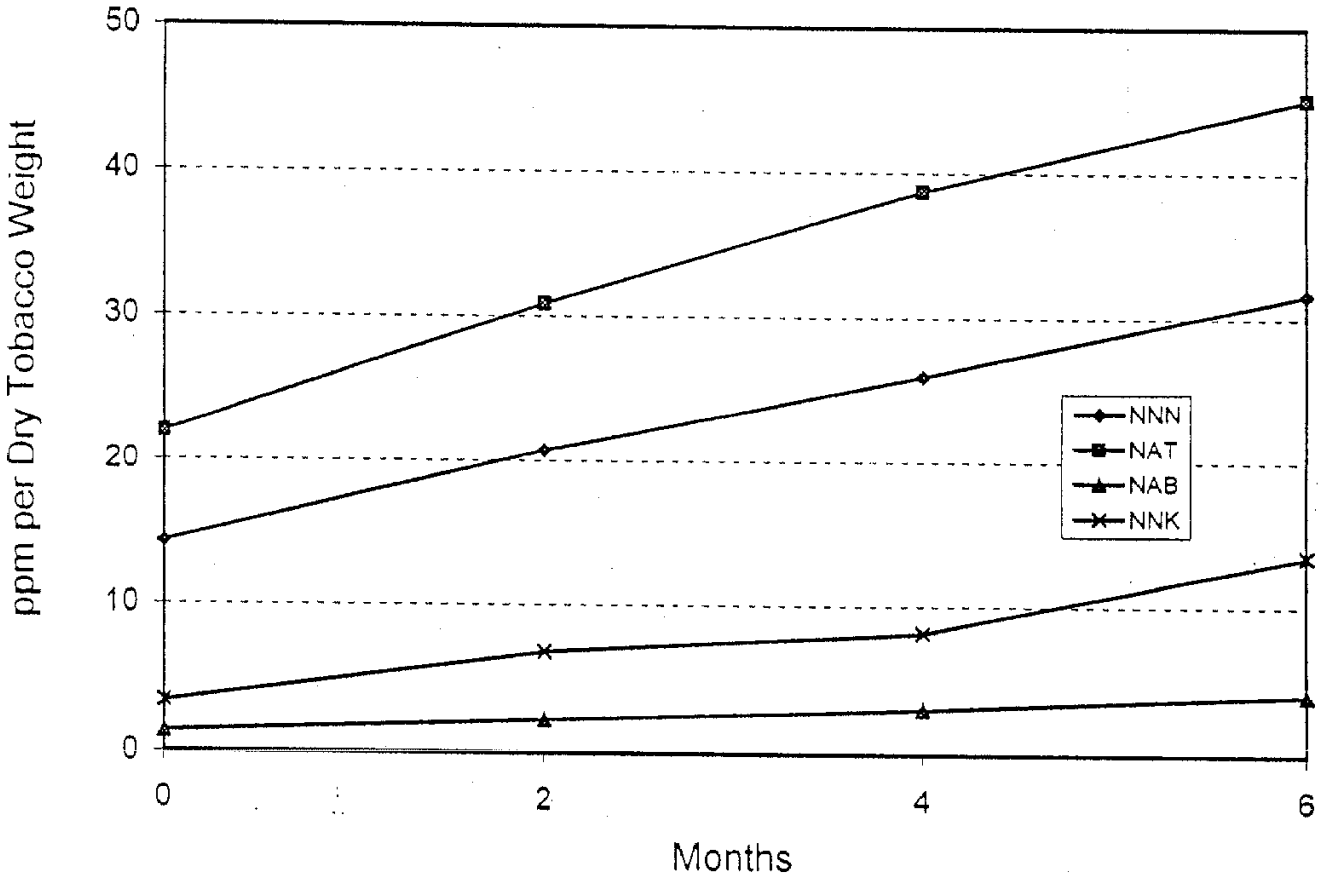

Figure 7. Effect of Storage on TSNA Levels
Ettan Snus