DIFFERENTIATING THE REAL AND POLITICAL TOXICOLOGY OF SMOKELESS TOBACCO PRODUCTS

John H. Lauterbach, PhD, DABT
Lauterbach & Associates, LLC
Macon, GA 31210 USA

Outline for presentation

- Background for the presentation
- Objectives for the presentation
  - Chemistry of smokeless tobacco products
  - Toxicology of smokeless tobacco products
- Conclusions

Background – 1

- Smokeless tobacco products have been around for eons, but much interest in them recently
  - No smoke-related health risks of cigarettes
  - No ETS issues
  - New product introductions implying reduced risk
  - Some health experts suggesting that smokers who cannot quit switch to low-TSNA smokeless tobacco
- However, there is considerable debate on the safety and desirability of such products
  - Nitrosamine issues
  - Safety issues other than nitrosamines
  - Safety versus nicotine replacement therapy

Background – 2

- Part of this debate appears to be associated with confusion over product composition
  - Several types of commercial products sold in USA
  - Changes in commercial products over time that have resulted in reduced TSNA levels
  - Products with higher levels of TSNAs and other contaminants available in some parts of the world
- Another part of the confusion appears political
  - By presenting image all smokeless products unsafe, some researchers might get more funding
  - Likewise, if all smokeless products deemed unsafe, the sales of NRT might grow faster than otherwise

Objectives for presentation

- Review literature on chemistry of contemporary products made in USA
  - KY Reference smokeless tobacco products
  - Commercial products
  - Compare real chemistry with “political” chemistry
- Review literature on toxicology of contemporary products made in USA and compare findings with the “political” toxicology
- Focus on chemistry and toxicology other than those related to tobacco specific nitrosamines and nicotine

Review of smokeless product chemistry – 1

- KY Reference smokeless tobacco products – formulas

	1S1	2S1	1S2	1S3	2S3
Ingredient	Loose-leaf	Loose-leaf	Dry snuff	Moist snuff	Moist snuff
Dark fired tobacco			22.75	25.73	25.63
Fire-cured Virginia tobacco			19.66		
Air cured tobacco				7.83	7.80
Burley stems				3.73	3.72
Air cured stem			33.03		
Flue-cured stem			15.20		
Wisconsin air cured	17.40	17.40			
Pennsylvania air cured	15.47	15.47			
Crushed burley stems	5.80	5.80			
Sodium chloride	1.60	1.60	0.36	7.40	7.32
Sodium carbonate				0.51	0.72
Sodium proprionate	0.28	0.28			
Moisture	23.48	23.48	9.00	54.80	54.81
Glycerin	3.75	3.75			
Sucrose	23.01	23.01			
Dextrose	1.70	1.70			
Maltose	1.30	1.30			
Other corn syrup solids	6.21	6.21			

Review of smokeless product chemistry – 2

- KY Reference smokeless tobacco products – analysis

Analyte (all analyses wet basis)	1S1 Loose-leaf	2S1 Loose-leaf	1S2 Dry snuff	1S3 Moist snuff	2S3 Moist snuff
Nicotine	0.76 ± .05	0.84 ± 0.06	1.32 ± .04	1.25 ± .08	1.34 ± .11
Total nitrogen	1.20 ± .13	1.62	3.12	1.33 ± .19	1.70
Nitrate nitrogen	0.20 ± .01	0.29 ± .01	0.75	0.28 ± .04	0.29 ± .03
Total sugars	26.50 ± 1.42	27.81 ± 1.92	0.36	0.20	0.23
Reducing sugars	4.18 ± .65	4.14 ± 1.59	0.24	0.04	0.23
Moisture	23.20 ± .61	21.99 ± 1.01	11.75 ± .41	55.00 ± .29	54.46 ± .22
pH	6.42 ± .13	5.81 ± .05	6.29 ± .07	8.01 ± .15	7.32 ± .20
Ash	11.00 ± .12	10.41 ± .17	20.62 ± .27	17.30 ± .51	16.32 ± .30
Potassium	2.09 ± .01	2.27 ± 0.22	5.17 ± .26	1.78 ± .15	1.65 ± .13
Sodium	0.78 ± .05	0.59 ± .04	0.26 ± .02	2.80 ± .02	3.25 ± .17
Calcium	1.37 ± .07	1.31 ± .03	2.85 ± .01	1.44 ± .07	1.39 ± .03

Source: Smokeless Tobacco Research Products - North Carolina Agricultural Research Service

Review of smokeless product chemistry – 3

- pH, moisture, and sugars in commercial brands – 2005

Product type	Snuff	Chewing tobacco
Total number samples	n = 11	n = 9
pH range	7.13 - 8.93	5.12 - 6.23
Average pH	7.64	5.65
σ, pH	0.51	0.39
Moisture range	36.79 - 54.32	15.65 - 26.34
Average moisture	50.38	23.04
σ, moisture	4.78	3.30
Glucose range	ND - 0.16	0 - 10.43
Average glucose	0.02	5.88
σ, glucose	0.05	3.25
Fructose range	ND - 0.02	1.89 - 20.96
Average fructose	0.01	8.96
σ, fructose	0.01	7.08
Sucrose range	ND	0.026 - 22.46
Average sucrose	ND	12.83
σ, fructose	0.01	7.08

Source: Adapted from Clarke *et al.*, *J. Agric. Food Chem*. 2006;54:1975-81

Review of smokeless product chemistry – 4

- Examples of TSNA levels in moist snuff products

Source	Year	Number of Samples	NNK (μg/g)	NNN (μg/g)	Total TSNA (μg/g)
Sweden	2002	24	0.2	0.5	1.0 (0.2 - 3.1)
Norway	1983	2	6.6 (5.4-7.78)	42 (26-58)	77 (52-108)
Sudan (Toombak)	1991	20	2310 (620-7870)	1130 (500-3080)	3740 (1160-13580)
USA	1987	5	1.3 (0.1-3.1)	16.6 (5.8-64.1)	85.6 (9.6-288)
USA	2003	7	0.9 (0.4-1.6)	4.5 (2.4-6.4)	8.4 (4.5-12.3)

Sources: Adapted from Nilsson, "De minimus non curat lex -- virtual thresholds for cancer initiation by tobacco specific nitrosamines --prospects for harm reduction by smokeless tobacco," *Int. J. Occup. Med. Environ. Health* 2006;19(1):6-35 and Rodu & Jansson, "Smokeless tobacco and oral cancer: a review of the risks and determinants," *Crit. Rev. Oral Biol. Med.* 2004;15(5):252-63

Review of smokeless product chemistry – 5

- Analysis of plug-style chewing tobaccos – 1994

	Brand A	Brand B	Brand C	Brand D
Alkaloids	0.84	0.97	0.76	1.55
Nitrates	1.08	0.99	0.77	1.05
Chlorides	1.99	1.96	1.73	4.00
Phosphates	0.39	0.35	0.44	0.39
Total sugars	27.70	27.10	33.00	20.70
Reducing sugars	6.10	6.00	9.10	6.40
Tobacco pH	6.17	5.81	6.30	5.12
Moisture	20.70	18.70	22.50	24.40
Saccharin (ppm)	2200	2200	1800	2200

Source: Adapted from Cantrell, B&W Memo, July 6, 1994, Bates numbers 415000155/0156

Review of smokeless product chemistry – 6

- Analysis of chewing tobaccos & dark air-cured leaf – 1985

	Units	Brand A	Brand B	Brand C	Brand D	DAC KY 160	DAC KY 171
NNN	μg/g	0.75	6.50	1.50	0.65	2.16	0.48
NNK	μg/g	0.30	0.50	0.55	1.05	0.17	0.12
Nicotine	mg/g	7.99	4.73	13.19	5.49	29.0	44.5
Nornicotine	mg/g	0.07	0.06	0.18	0.03		
Ananbasine	mg/g			0.10			
Anatabine	mg/g	0.13	0.05	0.31	0.03		
Total alkaloids	mg/g	8.19	4.84	13.78	5.54		
Malic acid	mg/g	7.24	1.85	8.73	3.37	50.1	88.4
Citric acid	mg/g	4.79	3.68	9.09	5.22		
α-glucose	mg/g	4.02	17.03	37.15	21.29		
β-glucose	mg/g	31.19	21.28	47.42	26.67		
Fructose	mg/g	13.27	11.82	52.18	19.09		
Sucrose	mg/g	175.6	187.0	168.1	224.6		
Chlorogenic acid	mg/g	0.43	0.31	0.45	0.26	1.4	0.5
Solanesol	mg/g	4.43	1.86	6.13	1.37	28.2	21.3

Source: Adapted from Chamberlain *et al.*, *J. Agric. Food Chem*. 1988; 36:48-50

Review of smokeless product chemistry – 7

- Analysis of moist snuff – 1985

	Units	Brand A	Brand B	Brand C - Mint	Brand D - WG	Brand E	Brand F - WG
NNN	μg/g	2.20	17.75	9.25	Not detemin.	1.55	4.00
NNK	μg/g	7.30	3.20	3.75	Not detemin.	2.10	1.10
Nicotine	mg/g	3.87	14.61	23.97	29.47	3.25	12.05
Nornicotine	mg/g	0.01	0.02	0.29	0.2	0.05	0.06
Ananbasine	mg/g		0.04	0.10	0.12		0.06
Anatabine	mg/g	0.02		0.19	0.22	0.03	
Total alkaloids	mg/g	3.90	14.87	24.54	30.29	3.46	12.16
Malic acid	mg/g	2.08	0.45		0.56	5.36	11.45
Citric acid	mg/g	8.82	0.70	1.54	2.79	7.70	3.09
α-glucose	mg/g	23.47	0.30			36.67	
β-glucose	mg/g	31.14	0.12			354.0	
Fructose	mg/g	32.63	0.30			53.5	
Sucrose	mg/g	139.9	0.75			136.8	
Chlorogenic acid	mg/g	0.22	0.27	3.43	0.22	0.75	0.33
Solanesol	mg/g	4.19	6.02	8.50	8.29	2.25	11.30

Source: Adapted from Chamberlain *et al.*, *J. Agric. Food Chem*. 1988;36:48-50

Other analyses reported for smokeless products

- Flavoring agents
  - LaVoie *et al.*, *J. Agric. Food Chem.* 1989;37:154-7
  - LaVoie *et al.*, *J. Agric. Food Chem.* 1985;33:876-879
- Microbiology
  - Rubinstein & Pedersen, *Clin. Diagn. Lab. Immunol.* 2002;9:1057-60
  - Rubinstein, *Clin. Diagn. Lab. Immunol.* 2000;7:794-802
  - Warke *et al.*, *J Food Prot.* 1999;62:678-81
- Silylation of acids & hydroxylated compounds
  - Alford *et al.*, B&W report, November 3, 1989, Bates numbers 569203168-569203185

Chemistry – real versus "political"

- The chemistry of conventional smokeless tobacco products made in the USA does not appear to have changed much in recent times although TSNA levels have decreased
- TSNA levels have decreased to near those found in Swedish snus, thus those who depend on the "political" chemistry for arguments against US made smokeless tobacco products are having a harder time keeping to the science

Why not discuss TSNAs in more detail?

- Subject has been well reviewed by others
  - Nilsson, "De minimus non curat lex -- virtual thresholds for cancer initiation by tobacco specific nitrosamines --prospects for harm reduction by smokeless tobacco," *Int. J. Occup. Med. Environ. Health* 2006;19(1):6-35
  - Accortt *et al*., "Cancer incidence among a cohort of smokeless tobacco users (United States)," *Cancer Causes Control* 2005; 16:1107-15
  - Rodu & Jansson, "Smokeless tobacco and oral cancer: a review of the risks and determinants," *Crit. Rev. Oral Biol. Med.* 2004;15(5):252-63
- Evidence suggests current TSNA levels (or less) in US and Swedish commercial products, are likely not an issue

What about other contaminants?

- Several contaminants (e.g., B[a]P, metals, volatile carbonyls) reported in dry and moist snuff
  - Hoffmann *et al.*, *JNCI* 1987; 79:1281-86
  - Presence of these contaminants cited as reasons against smokeless tobacco use
  - However, intake from other dietary sources could be comparable (Rodu & Jansson, *op. cit.*)
- Some snuff products show higher levels of D-amino acids than found in other tobaccos
  - Kullman *et al.*, *Chirality* 1999; 11:669-73
  - Ali *et al.*, *Food Chemistry* 2006; 99:803-12
  - Thought formed from bacterial action and/or generated from Amadori rearrangement products

Ames assay & smokeless tobacco extract (STE)

- Conflicting results obtained in different studies
  - Guttenplan assayed STE from US snuffs and chewing tobaccos (*Carcinogenesis* 1987;8:741-3)
    - Aqueous STE were mutagenic with TA100+S9
    - Some activity found in DCM extraction of aqueous STE
  - Jansson *et al.* assayed aqueous and DCM STE of Swedish snus (*Mutat. Res*. 1991;261:101-115)
    - Aqueous STE inactive
    - DCM extracts active in both TA98+S9, TA100+S9
  - Stamm *et al.*, assayed DCM/MeOH/Me_2CO STE of two types of products (*Mutat. Res.* 1994; 321:253-64)
    - Five strains ±S9: YG1024, YG1021,TA98NR, TA98DNP, TA98
    - Wet snuff STE (+S9): YG1024>>TA98~YG1021~TA98NR>TA98DNP
    - Chewing tobacco STE (+S9): YG1024 only

Ames assay & smokeless tobacco

- Conflicting results obtained in different studies
  - Rickert *et al.*, assayed DMSO STE of regular and low TSNA products on US market (2004 TSRC, Paper #37)
    - DMSO extraction likely to extract both polar & nonpolar mutagens & STE + with TA100+S9, – with TA98+S9
    - STE from low TSNA products as active as regular products so no apparent TSNA effect as predicted
    - Activity may be from Maillard reaction products formed during processes that heat the tobaccos
- Urinary mutagen levels – snuff use vs. smoking
  - Curvall *et al.*, *Mutat. Res.* 1987;188:105-10
  - Urine mutagenicity (10^3 revertants/24-hour):
    - Smokers (8.6) >> snuff users (1.3) ~ abstinent snuff users (1.3) ~ non-tobacco users (0.9)

Toxicology – real versus "political"

- The reviews of the toxicological properties of US-made smokeless tobacco products by Nilsson (*op. cit.*) and Rodu and Jansson (*op. cit.*) have done much to put the health risks associated with such products into the proper prospective
- Rodu and Jansson (*op. cit.*) have also put Hoffmann's 1987 data on minor toxicants into prospective by comparing estimated intakes from other dietary sources versus those from smokeless tobacco products

Smokeless tobacco & oxidative stress (OS)

- Some health-related effects that have been ascribed to smokeless tobacco use cannot be explained in terms of TSNA levels and/or levels of minor contaminants in tobacco
  - Leukoplakia
  - Possible cardiovascular effects not attributable to nicotine alone as in NRT (Gupta *et al.*, Arch. Intern. Med. 2004;164:1845-9)
- Such effects may be caused by OS caused by substances extracted from the product during use (essentially aqueous extraction by saliva)

Aqueous STE (AqSTE) & OS – 1

- Several researchers have linked AqSTE with OS
  - Barley *et al.*, *J. Dent. Res.* 2004;83:903-8
  - Lam *et al.*, *Tobacco Induced Diseases* 2003;1:207-11
  - Mangipudy & Vishwanatha, *Mol. Cell. Biochem.* 1999;200:51-7
  - Yildiz *et al.*, *Arch. Environ. Contam. Toxicol.* 1999;37:434-9
  - Hassoun *et al*., *Free Radic. Biol. Med*. 1995;18:577-83
  - Bagchi *et al.*, *Int. J. Exp. Path.* 1994;75:197-202
- It has been hypothesized that leukoplakia may be caused by oxidative stress from AqSTE
  - Rohatgi *et al.*, *Toxicology* 2006;224:1-13
  - Rohatgi *et al.*, *Oral Oncol*. 2005;41:806-20
  - Barley *et al.*, *op. cit.*

AqSTE & OS – 2

- Also, supplementing diet with β-carotene produces remissions in patients with leukoplakia
  - Garewal *et al.*, Arch. Otolaryngol. Head Neck Surg. 1999;125;1305-10
- There apparently are no published data on the chemical composition of AqSTE
  - It is likely to be variable depending on product and extraction procedures
  - Compounds readily water-soluble may predominate
    - Phenolics – antioxidants (Rodu and Jansson, Op. Cit.)
    - Inorganic salts such as nitrate and nitrite, which can lead to reactive oxygen species
- If the hypothesis on OS is correct, are reductions in nitrate and nitrite a solution?

Conclusions – 1

- The chemistry of conventional smokeless tobacco products in the USA does not appear to have changed much over the last decade or so although TSNA levels have decreased
- Chemistry of USA products is unlike that of products from other parts of the world that use different ingredients and have high TSNA levels
- Thus, attempts to equate the chemistry of commercial products made in USA with those made in certain foreign countries use “political” chemistry to justify the “political” toxicology

Conclusions – 2

- The levels of trace contaminants found in USA smokeless tobacco products are not unlike those found in food products
- Several studies have shown that smokeless tobacco extracts are mutagenic but mutagenicity is not from TSNAs
- These factors along with decreased TSNA level put much of the published critique of smokeless tobacco products made in the USA into the realm of “political” toxicology

Conclusions – 3

- Some studies have shown that aqueous extracts of smokeless tobacco cause oxidative stress in different bioassays
- It has been hypothesized by some researchers that oxidative stress may be the cause of the oral leukoplakia that occurs in users of smokeless tobacco products
- This may be where real toxicology is needed