Ethyl carbamate levels in US and Swedish smokeless tobacco products

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1. Introduction:

IARC monograph 89 (1) summarised historical literature on the presence of carcinogens in smokeless tobacco products. 28 Chemical agents are listed, including nitrosamines, carbonyls, benzo(a)pyrene, angelica lactones, coumarin, ethyl carbamate, and a series of metallic and radioactive species.

There are significant data in the literature concerning the levels of a number of nitrosamines in smokeless tobaccos, but relatively little information available for many of the other species. Much of the data is 20-30 years old but smokeless tobacco product styles, ingredients and production practises have undergone significant changes over this time. Research has seen changes in other compounds over this period (2). Moreover most of the existing data have been generated on a small number of brands in each study; with little comprehensive comparative information available on the contents of different product styles.

A study was initiated in 2008 to examine the levels of a number of different chemical species in contemporary smokeless tobacco products (STPs) from the US and Sweden.

2. IARC Classification of compounds (2):

Ethyl Carbamate (also known as urethane) is found in wine and other fermented foods in low concentration as a naturally occurring by-product of fermentation processes. It is also sometimes used in pesticide production. Ethyl carbamate (CAS number 51-79-6) is the ethyl ester of carbamic acid. The two routes of the formation of ethyl carbamate are via hydrogen cyanide and ethanol, or urea and ethanol; the latter probably being the dominant route of formation in STPs (3). In March 2007, IARC reclassified ethyl carbamate from a Group 2B *possible* human carcinogen to a Group 2A *probable* human carcinogen.

3. Smokeless Tobacco Products

The STPs styles analysed in the current work were Swedish pouched and loose snus, US chewing tobacco, pellet (hard and soft), dry snuff, moist snuff and plug. The differences between these product styles are described below:

Dry Snuff: Powdered tobacco, with a significant proportion of fire cured styles and around 10% moisture content, consumed by placing a pinch of powder between gum and cheek.

Moist snuff: ("Dipping tobacco") cut air-cured and fire-cured tobaccos, blended and fermented, with a moisture content of 50-60%. They can be used as small portions or pinches of loose material or in small sachets. They are consumed by positioning in the lower part of the mouth, with occasional expectoration.

Plug: The moist plug tobacco examined in this study has a moisture content around 20%, is composed of mild Burley, Virginia and Philippines tobaccos, is finely ground, soaked in honey and pressed into blocks or "plugs". This product style is consumed by chewing.

Chewing tobacco: Loose leaf chewing tobacco is manufactured from air cured tobaccos which are shredded and cased with sugars and flavourings, they have a moisture content around 20-30%.

Tobacco Pellets: Two forms of tobacco pellets were examined, a hard pellet form containing fine ground tobacco and inorganic materials, with a moisture content of around 5-10%, which is consumed by allowing it to dissolve in the mouth; and a moister (around 20%) small cylindrical soft pellet made from flavoured leaf tobacco which is kept between cheek and gum until the flavour has dissipated.

Snus: Air-cured and sun-cured tobaccos blended and pasteurised, presented as either a loose form (loose snus) or in small sachets (portion snus), with a moisture content of 45-60%. They are consumed by positioning in the upper part of the mouth, without expectoration.

3. Sample details:

70 Smokeless tobacco brands: snus loose and snus portion, chewing tobacco, dry and moist snuff, soft and hard tobacco pellets and plug tobacco, were sourced covering all major manufacturers and representing 80-90% market share in both markets. All samples were sourced in 2008.

3.1 Swedish products

•32 products, 7 different manufacturers

•21 snus portion samples and 11 snus loose samples

•Sourced from Swedish Websites, exported and kept frozen prior to analysis.

3.2 US products

•38 products, 9 different manufacturers

•13 chewing tobaccos, 1 soft pellet, 1 plug, 16 moist snuffs, 2 hard pellets, and 5 dry snuffs

•US samples were sourced from US shops, exported and kept frozen prior to analysis.

4. Methods:

The analysis for ethyl carbamate was undertaken by Eurofins Sweden (TorbjornSynnerdah@eurofins.se) using method 67/1e, as follows:

One gram of sample was taken and dissolved in 10 ml of water. Internal standard was then added and the sample mixed for 30 minutes. 1.5 ml of the sample was taken and centrifuged at 14000 rpm for 5 minutes. The samples were then analysed on a UPLC/MS/MS in APCI mode using a Waters BEH C18 column.

The "as received" wet weight basis (WWB) limit of detection was 20 ng/g. Moisture content was determined using weight loss on drying by Eurofins Sweden to provide a dry weight basis value (DWB).

In addition to ethyl carbamate the samples were also analysed for moisture content, total and reducing sugars, total nicotine alkaloids, nitrate, reducing and total sugars. Some other compounds were chosen for analysis as ethyl carbamate levels were thought to be possibly related to fermentation type processes and as such there may be relationships between moisture, sugars and nitrate levels. Total nicotine alkaloids were obtained as part of the sugar analysis method.

Total nicotine alkaloids, reducing and total sugars were analysed at BAT Southampton using continuous flow analysis. An aqueous extract of the sample was prepared and the reducing sugars determined by the reduction of the cupric chelate of neocuproine in alkaline solution to the highly coloured cuprous form which was measured at 460 nm. Non-reducing sugars were hydrolysed by the action of the enzyme invertase within the flow system, and the total non-reducing sugars then present determined. The total sugars were calculated as the sum of reducing and non-reducing sugars. The total nicotine alkaloids were determined by reaction with sulphanilic acid and cyanogen chloride. The developed colour was measured at 460 – 480 nm.

Nitrate was determined by continuous flow analysis at GR&D Southampton. The nitrate content was determined by reduction of the nitrate to nitrite with hydrazinium sulphate, followed by reaction with sulphanilamide to form the diazo compound. This was coupled with N-1-naphthyl-ethylenediamine dihydrochloride to form a coloured complex. The developed colour was measured at 550 nm.

5.0 Comparison of ethyl carbamate levels across smokeless tobacco categories

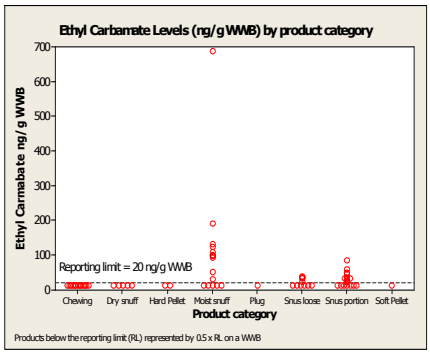


Figure 1: As received (WWB) ethyl carbamate levels in contemporary STPs

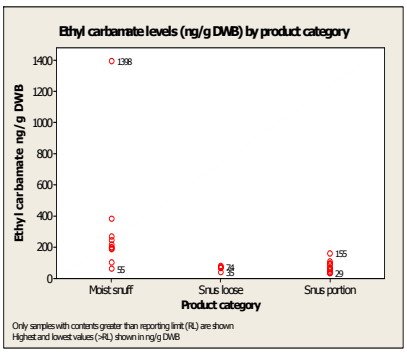


Figure 2: Dry Weight Basis ethyl carbamate levels in contemporary STPs

WWB = Wet weight basis DWB = Dry weight basis

Figure 3

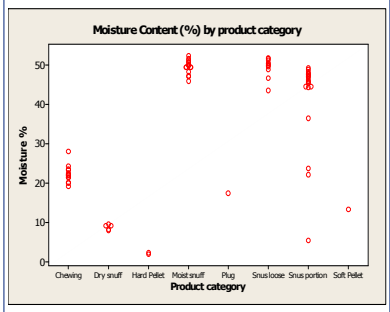


Figure 4

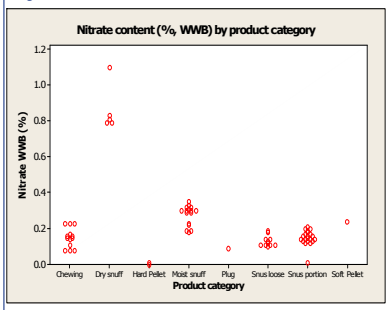
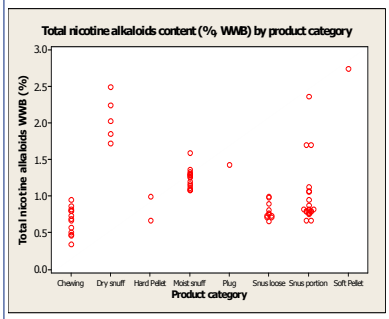


Figure 5



5. Results:

5.1 Ethyl carbamate

The levels of ethyl carbamate found in contemporary STPs are shown graphically in Figure 1 on an as received or wet weight basis (WWB) and in Figure 2 on a dry weight basis (DWB).

In total 40 of the 70 products (57%) were below the reporting limit. All of the samples measured for chewing tobacco, dry snuff, hard pellet, soft pellet and plug product styles had ethyl carbamate levels below the limit of quantification. Within the three categories that had measurable levels of ethyl carbamate, 5 moist snuff products (31% of category), 6 loose snus products (55%) and 7 portion products (33%) were below detection limits.

Values of ethyl carbamate contents for moist snuff ranged from below reporting limit (BRL) – 688 ng/g WWB (BRL – 1398 ng/g DWB), values for loose snus ranged from BRL – 37 ng/g WWB (BRL -74 ng/g DWB), and for pouched snus from BRL – 84 ng/g WWB (BRL -155 ng/g DWB).

One of the moist snuff products, Marlboro moist snuff contained a level of ethyl carbamate, 688 ng/g WWB (1398 ng/g DWB), that was over 3 times higher than the next highest product in this study.

5.2 Moisture

The moisture contents of the STPs are given in Figure 3. The STPs have a wide range of moisture from 3.8 – 57.7%. The two hard pellet samples (Ariva and Stonewall Wintergreen) had the lowest moisture and the loose snus, pouched snus and moist snuff had the highest. The pouched snus had the widest range of moisture as a product group from 9.6 – 56% which may indicate important differences in product compositions.

5.3 Nitrate

Values for nitrate ranged from the reporting limit (0.04% on a WWB) to 1.1% (Figure 4). The dry snuff samples showed significantly greater levels of nitrate (at 95% confidence, from one way ANOVA) than all the other products. Values for dry snuff ranged from 0.79 – 1.1% on a WWB.

5.4 Total nicotine alkaloids (TNA), total and reducing sugars

TNA contents of the samples are shown in Figure 5. The values ranged from 0.3% to 2.7%. Chewing tobacco was found to contain significantly less % TNA than dry and moist snuffs and the soft pellet (95% confidence, one way ANOVA). The soft pellet sample contained the highest % TNA content of all the samples analysed.

For the two types of sugars measured, only chewing tobacco, both pellet types and plug contained levels of reducing sugars greater than the reporting limit of 2% and only chewing tobacco, both pellet types and plug had levels of total sugars above the reporting limit of 2%.

6. Discussion

One way ANOVA was undertaken on the data from the samples with measurable ethyl carbamate levels (with Marlboro moist snuff excluded). Moist snuff was found to be statistically higher in ethyl carbamate than loose and pouched snus. It is interesting to note that all the products that have measurable values of ethyl carbamate are from categories that tend to be high in moisture. However, within the categories there is no strong correlation between moisture and ethyl carbamate levels (Pearson correlation = 0.33). There were also no strong correlations (greater than Pearson correlation = 0.7) between ethyl carbamate and any of the other analytes measured.

6.1 Comparison with historical data:

There is very limited historical data on levels of ethyl carbamate in smokeless tobacco. Ethyl carbamate was measured in 1992 by Hoffmann et al in chewing tobacco and found to contain 310-375 ng/g (4). The absence of measurable levels of ethyl carbamate in contemporary chewing tobacco contrasts with this historic data. The analytical method, which was originally developed for cigarette tobacco and smoke, involved multiple liquid extractions with chlorinated solvent followed by GC/MS and as such differs from the method detailed in this work (5).

7. Disclosure: The study was funded by British American Tobacco.

8. Conclusions:

This work has significantly extended the amount of information available on ethyl carbamate levels in contemporary US and Swedish smokeless tobaccos. Fresh insight has been provided on the relative levels in different smokeless tobacco types, with only three product categories having measurable levels of ethyl carbamate. The results were compared to levels of other smokeless tobacco constituents but no strong correlations were identified.

9. References:

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Poster Presented at 2010 CORESTA Conference, Edinburgh September 2010: SSPTPOST14