Levels of non-tobacco specific nitrosamines in US and Swedish smokeless tobacco products

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1. Introduction:

There has been considerable interest in recent years in the chemical composition of smokeless tobacco products (STPs), primarily based around health concerns associated with their use. IARC Monograph 89 (1) summarised the presence of 28 chemical agents in STPs including Benzo(a)pyrene (B(a)P), metals, aflatoxins, and number of nitroso species (TSNAs, nitrosoacids, and a range of non-tobacco specific nitrosamines - NTSNA). IARC has classified STPs as carcinogenic to humans (Group 1).

Over the last thirty to forty years there has been a considerable amount of research conducted to establish the levels of tobacco-specific nitrosamines (TSNAs) in tobacco products. However, in contrast, research on the NTSNA species has been less frequent, with correspondingly less quantitative data on the levels of NTSNAs in STPs. Research has shown changes in levels of TSNAs in STPs over the last 30 years(2), therefore an up-to-date survey on the levels of NTSNAs was considered necessary in order to more fully characterise currently available STPs.

A subcommittee of the FDA Tobacco Products Scientific Advisory Committee (TPSAC) identified (3) the following NTSNA compounds on their draft initial list of harmful or potentially harmful constituents: N-Nitrosodiethanolamine (NDELA), N-Nitrosodiethylamine (NDEA), N-nitrosodimethylamine (NDMA), N-Nitrosoethylmethylamine (NEMA), N-nitrosomorpholine (NMOR), N-Nitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPYR).

A Tobacco Industry trade association, the European Smokeless Tobacco Council (ESTOC) has recently (4) proposed a regulatory limit of 10ng/g on the NDMA contents of STPs, in addition to limits on the combined concentrations of four TSNAs, the content of B(a)P, Lead, Cadmium and the sum of four aflatoxins.

Given the lack of recent quantitative information on non-tobacco specific nitrosamines (NTSNAs) in smokeless tobacco, and the emerging regulatory interest in these species there is clearly a need for greater insight into the levels in these constituents in STPs. The current study was conducted in order to establish the NTSNAs profiles of contemporary STPs, and to understand the impact of proposed regulatory NDMA limits on the range of product styles available in the USA and Sweden.

2. Smokeless Tobacco Products (5,6):

The STPs 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, processed into fine particles (fine-cut) or strips (long cut), with a high 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, sucking, and 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 flavours; they have a moisture content around 20-30%.

Tobacco Pellets: Two forms of tobacco pellets were examined, a hard pastille 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 (hard pellet); and a moister (around 20%) small cylindrical pellet made from single leaf flavoured tobacco (soft pellet) which is kept between cheek and gum until the taste has gone.

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 normally in the range of 45-60%, although some lower moisture content products have been observed. They are consumed by positioning in the upper part of the mouth, without expectoration.

3. IARC Classification of Nitrosamines (7):

IARC has examined evidence for the carcinogenicity of a number of nitrosamines and assigned the following groupings:

Nitrosamine	IARC Group
N'-Nitrosonornicotine (NNN)	1
4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK)	1
N-Nitrosodimethylamine (NDMA)	2A
N-Nitrosodiethylamine (NDEA)	2A
N-Nitrosomethylethylamine (NEMA)	2B
N-Nitrosodi-*n*-propylamine (NDPA)	2B
N-Nitrosodibutylamine (NDBA)	2B
N-Nitrosopiperidine (NPIP)	2B

Nitrosamine	IARC Group
N-Nitrosopyrrolidine (NPYR)	2B
N-Nitrosomorpholine (NMOR)	2B
N-Nitrosodiethanolamine (NDELA)	2B
N'-Nitrosoanabasine (NAB)	3
N'-Nitrosoanatabine (NAT)	3
N-Nitrosodiisopropanolamine (NDIPLA)	3
N-nitrosodiisopropylamine (NDIPA)	not classified
N-Nitrosodibenzylamine (NDBzA)	not classified

Key:
Group 1: Carcinogenic to humans, Group 2A: Probably carcinogenic to humans,
Group 2B: Possibly carcinogenic to humans, Group 3: Not classifiable as to carcinogenicity to humans

TSNA NTSNA

4. Methods:

In order to quantify the levels of the 28 chemical agents in contemporary smokeless tobacco products we commenced a study in October 2008 measuring the toxicant contents of 70 US and Swedish products. Eight different types of smokeless tobacco, all major manufacturers and approximately 80-90% market share for each category were represented by 32 Swedish loose and pouched snus products and 38 US products covering chewing tobacco, dry snuff, hard and soft pellets, moist snuff and plug.

The NTSNA species examined in the current study were NDMA, NEMA, NDEA, NDPA, NDBA, NPIP, NPYR, NDIPA, NMOR, NDELA, NDIPLA, and NDBzA. Analysis for NTSNAs was undertaken by Labstat International using established methods (NDMA, NEMA, NDEA, NDPA, NDBA, NPIP, NPYR) and methods developed for this study (NDIPA, NMOR, NDELA, NDIPLA, and NDBzA).

Analysis of NDMA, NPYR, NEMA, NDEA, NDPA, NDBA, NPIP, NDIPA, and NMOR was conducted by extracting 3g of tobacco with a citrate-phosphate buffer containing ascorbic acid, clean-up using SPE, elution with dichloromethane, concentration and quantification using GC-TEA. Analysis of NDELA and NDIPLA was conducted by extracting 1g of tobacco with water at 40ºC, centrifuged, filtered and acidified, cleaned up by SPE, concentrated, derivatised and analysed using GC-NCI MS in SIM mode. Analysis of NDBzA was conducted by extracting 2g of tobacco with methanol, clean-up using reverse phase SPE, and quantification using LC-APCI and tandem mass spectrometry in MRM mode.

The following limits of quantification and detection were achieved

Nitrosamine	Units	LOD	LOQ
NDMA	ng/g (as rec'd)	1.18	3.9
NDEA	ng/g (as rec'd)	1.4	4.67
NEMA	ng/g (as rec'd)	1.35	4.51
NDPA	ng/g (as rec'd)	1.51	5.05
NDBA	ng/g (as rec'd)	2.11	7.04
NPIP	ng/g (as rec'd)	2.29	7.63

Nitrosamine	Units	LOD	LOQ
NDIPA	ng/g (as rec'd)	0.579	1.93
NPYR	ng/g (as rec'd)	1.66	5.53
NMOR	ng/g (as rec'd)	0.53	1.77
NDBzA	ng/g (as rec'd)	0.177	0.591
NDELA	ng/g (as rec'd)	0.784	2.61
NDIPLA	ng/g (as rec'd)	0.643	2.14

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

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6. Results:

The analysis exercise showed very low levels of NTSNAs in most samples.

With the majority of the NTSNAs the 70 smokeless samples examined had NTSNA contents either below the quantification limit, or were not detected at all.

Only with NDMA, NDPA, NPYR and NMOR were quantifiable levels found with the US and Swedish smokeless samples.

None of the 70 samples examined in this study showed the presence of NDELA, NDIPLA or NDBzA.

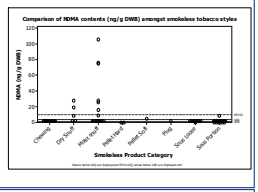
IARC Group	NTSNA	Samples with Quantifiable NTSNA Values	Samples with NTSNA contents below the Quantification limit (Below LOQ)	Samples where the NTSNA was Not Detetcted (Below LOD)
2A	NDMA	12	49	9
	NDEA	-	15	55
2B	NEMA	-	16	54
	NDPA	3	19	48
	NDBA	-	22	48
	NPIP	-	17	53
	NPYR	16	40	14
	NMOR	3	45	22
	NDELA	-	-	70
3	NDIPLA	-	-	70
NC	NDIPA	-	40	30
	NDBzA	-	-	70

6.1 Contents of IARC Group 2A (Probably carcinogenic to humans) NTSNAs in STPs

No quantifiable values were obtained for the NDEA contents of the 70 smokeless tobaccos, and 79% of the samples had no detectable NDEA content.

With NDMA the highest contents were found with moist and dry snuff, the levels of which were considerably higher than found with the other tobacco categories. Quantifiable levels were also found with the soft pellet and one portion snus product.

Comparison of the NDMA levels to the ESTOC proposed limit shows that 6 of the 16 moist snuff products and 2 of the 5 dry snuff products had contents above 10 ng/g.

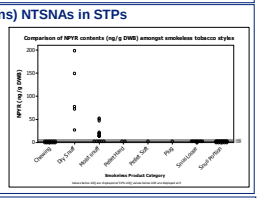


6.2 Contents of IARC Group 2B (Possibly carcinogenic to humans) NTSNAs in STPs

No quantifiable values were obtained for the NEMA, NDBA, NPIP and NDELA contents of the 70 smokeless tobaccos, and 70-100% of the samples measured showed no detectable content of these NTSNAs.

With NPYR the highest contents were found with dry and moist snuff samples, the levels in some dry snuff samples were considerably higher than found with the other tobacco categories. Quantifiable levels were not found with any of the other samples measured.

Two moist snuff and one portion snus sample gave measurable NMOR values (13-20ng/g) and three portion snus samples gave measurable NDPA values (24-57ng/g).



6.3 IARC Group 3 (Not classifiable as to carcinogenicity to humans) and unclassified NTSNAs in STPs

NDiPLA and NDBzA were not found in any of the 70 smokeless tobacco products examined in this study. There was no quantifiable level of NDiPA in any of the samples, although NDiPA was detected in 57% of the samples.

6.4 Comparison of NTSNA contents across STP categories

Loose snus: None of the loose snus samples showed any quantifiable levels of the NTSNAs examined in this study. However, all of the NTSNAs other than NDELA, NDiPLA and NDBzA were detected in some proportion of the loose snus samples.

Pouched snus: Quantifiable contents of NDMA, NDPA and NMOR (1-3 samples each) were found with a small proportion of the pouched snus samples. None of the other NTSNAs were found at quantifiable levels. However, a significant proportion of pouched snus samples contained detectable levels of the other NTSNAs apart from NDELA, NDiPLA and NDBzA.

Chewing tobacco: None of the NTSNAs were found in the chewing tobacco samples at quantifiable levels. NDEA, NDPA, NDELA, NDiPLA and NDBzA were not detected in any of the chewing tobacco samples. The other 7 NTSNAs were detected but could not be quantified.

Dry snuff: Quantifiable levels of NDMA and NPYR were found in the majority of dry snuff samples; a significant proportion of dry snuff samples contained detectable levels of the other NTSNAs apart from NDELA, NDiPLA and NDBzA.

Moist snuff: A significant proportion of the moist snuff products showed quantifiable levels of NDMA, NPYR and NMOR. NDELA, NDiPLA and NDBzA were not detected in any of the moist snuff products. A substantial proportion of the moist snuff products showed detectable levels of the other NTSNAs.

Hard Pellet: Most of the NTSNAs were not detected in the two hard pellet products. Non-quantifiable levels of NMOR and NDiPA were found in one product.

Soft Pellet: Quantifiable levels of NDMA were found with the soft pellet product. NDiPA was detected in the soft pellet product, but non of the other NTSNAs were detected.

Plug: None of the NTSNAs were quantifiable in the plug product; NDMA, NPYR and NMOR were detected but not quantified. None of the other NTSNAs were detected in the plug sample.

6.5 Implications of measured NTSNA contents for the recommended FDA H/PH TPSAC subcommittee Draft Initial List of Harmful/Potentially Harmful constituents of smokeless tobacco products

Of the seven NTSNA compounds identified by the sub-committee of the FDA's TPSAC as being Harmful or Potentially Harmful compounds in smokeless tobacco products only three (NDMA, NPYR and NMOR) were found in quantifiable levels in the 70 smokeless tobacco products measured in this study. Three of the identified NTSNAs: NDEA, NEMA and NPIP were detected but were present at levels too low to be quantified, and one NTSNA (NDELA) was not detected in any of the products.

The levels of N-nitrososarcosine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol have not been measured in the NTSNA study but are currently being examined in other parallel studies.

7. Conclusions:

The levels of twelve NTSNAs were examined in 70 contemporary US and Swedish smokeless tobacco products, covering all major product styles, manufacturers and significant market share for each category. Only four NTSNAs were found to be present in quantifiable levels, and three NTSNAs were not detected in any of the products sampled.

None of the NTSNAs were found at quantifiable levels in any of the Loose Snus, Chewing Tobaccos, Hard Pellet and Plug Tobacco products examined in this study. Small numbers of pouched snus products contained quantifiable levels of NDMA, NDPA and NMOR. Relatively high levels of two NTSNAs, NDMA and NPYR, were found with the dry and moist snuff products.

The impact of the ESTOC proposal of a limit for the NDMA contents was assessed and a number of dry and moist snuff products were found to have contents higher than 10 ng/g.

Only three of seven NTSNA compounds identified by the FDA TPSAC sub-committee were found to be present at quantifiable levels in these contemporary smokeless tobacco products.

8. References:

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