Determination Of The Levels Of Polycyclic Aromatic Hydrocarbons (PAH) in US and Swedish Smokeless Tobacco Products

Kevin G. McAdam[1], Arif Faizi[1], Harriet Kimpton[1], Christopher G. Wright[1] and Brad Rodu[2]

[1]British American Tobacco, Group Research and Development, Regents Park Road, Southampton, SO15 8TL, United Kingdom

[2] University of Louisville, Clinical Translational Research Building, 505 South Hancock Street, Louisville, KY 40202, USA

Contact: Kevin_McAdam@bat.com

1. Introduction:

In recent years interest has grown considerably 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 a number of tobacco specific nitrosamines, benzo(a)pyrene (B(a)P), trace metals, volatile nitrosamines and aflatoxins. IARC has classified smokeless tobaccos as carcinogenic to humans (Group 1).

B(a)P, formed by the incomplete combustion of organic material, is thought to be an environmental contaminant present into tobaccos as a result of exposure to combustion emissions during plant growth, curing and processing (2). B(a)P is a Group 1 IARC carcinogen (3), and its presence in STPs has been the focus of concern in the public health community. This concern has recently led to two organisations recommending regulatory limits on the levels of B(a)P and other toxicants in STPs.

The WHO Study Group on Tobacco Product Regulation (TobReg) proposed regulatory limits (on a dry weight basis) of 5ng/g for B(a)P contents of STPs, in addition to limits for the combined concentrations of NNN and NNK (2). The European Smokeless Tobacco Council (ESTOC), a tobacco industry organisation, has proposed a limit of 20ng/g for the B(a)P content of STPs; in addition to limits on the combined concentrations of four TSNAs, NDMA, Lead, Cadmium, and the summed contents of four aflatoxins in STPs, also on a dry weight basis (4).

However, the presence of many additional PAHs in tobacco products has long been known, with 575 PAHs having been reported in mainstream cigarette smoke, and 86 PAHs reported to be present in tobacco (5). Despite this knowledge, very little quantitative information is available on the levels of PAHs in STPs. The most comprehensive study published to date is that of Stepanov et al. (6) who quantified the levels of 23 PAHs in US moist snuff and pouched snus products, concluding that their findings "rendered PAHs one of the most prevalent groups of carcinogens in smokeless tobacco."

Since there is strong reulatory focus on B(a)P and a lack of quantitative information on PAHs in STPs other than moist snuff and US snus, there is clearly a need for greater insight into PAH levels in contemporary STPs. The present study was therefore conducted in order to establish data on the PAH profiles and content of a greater range of STPs than is currently available in the literature, and to understand the impact of proposed regulatory limits for B(a)P on the range of product styles available in the USA and Sweden.

2. IARC Classification of PAHs (8):

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

PAH	IARC Group
Benzo[a]pyrene	1
Dibenzo(a,l)pyrene	2A
Cyclopenta[cd]pyrene	2A
Dibenz[a,h]anthracene	2A
5-Methylchrysene	2B
Benz[a]anthracene	2B
Benz[e]acephenanthrylene (benzo(b)fluoranthene)	2B
Benz[j]aceanthrylene	2B
Benzo[c]phenanthrene	2B

PAH	IARC Group
Benzo[k]fluoranthene	2B
Chrysene	2B
Dibenzo(a,i)pyrene	2B
Indeno[1,2,3-cd]pyrene	2B
Naphthalene	2B
Dibenzo(a,h)pyrene	2B
Dibenzo[b,def]chrysene	2B
Benzo[j]fluoranthene	2B
Dibenzo(a,e)pyrene	3

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

3. Smokeless Tobacco Products (7, 8):

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, 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 flavourings, 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 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.

4. Methods:

In order to quantify the levels of the 28 constituents in contemporary STPs a study was commenced in October 2008 to measure the toxicant contents of 70 US and Swedish products. Eight different types of smokeless tobacco, comprising products from all major manufacturers with approximately 80-90% market share for each category, were represented by 32 Swedish loose and portion snus products and 38 US products that included chewing tobacco, dry snuff, hard and soft pellets, moist snuff and plug.

Measurement of 21 PAHs (naphthalene, 1-methylnaphthalene, 2-methylnapthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo (b) fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(a)pyrene, benzo(e)pyrene, perylene, indeno (1,2,3-cd) pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene) in each of the 70 products was conducted by Labstat International ULC (www. labstat.com), a laboratory with significant experience in the measurement of PAHs in smokeless tobaccos; B(a)P was also measured by BAT Group R&D analytical laboratories.

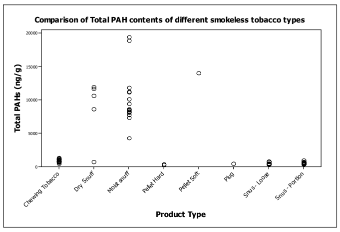
The Labstat method for PAH analysis involved extraction with alkaline ethanol, solvent partitioning, SPE clean-up, hexane elution, evaporation and quantification using HRGC-LRMS in SIM mode. The wet weight basis (WWB) data were also adjusted to provide concentrations (ng/g) on a dry weight basis (DWB) following water determination of the STPs.

The measurement of B(a)P by BAT was conducted using GR&D method T115U (V3 issued on 15 Jan 2008). A 2g aliquot of tobacco product was extracted using cyclohexane and acetonitrile. The extracts were analysed by High Performance Liquid Chromatography (HPLC) with fluorescence detection. The reporting limit was 0.25ng/g (WWB).

Poster 15710: ACS Fall Meeting, Boston, August 2010

5. Results

5.1 Comparison of PAH levels in different smokeless tobacco types



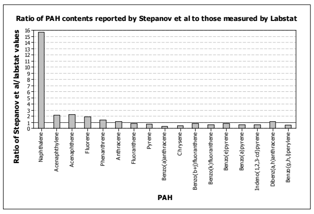
Highest total PAH contents were found with moist and dry snuff, and with soft pellet products. Other smokeless products had considerably lower contents.

The same general relative contents were found with most individual PAHs, other than the naphthalenes, where generally comparable contents were observed across different product categories.

Phenanthrene, naphthalene, fluoranthene and pyrene were the PAHs present at highest levels in all STPs.

5.2 Interlaboratory consistency of data

5.2.1 Comparison of PAH contents reported by Labstat and Stepanov et al (6) for moist snuff samples

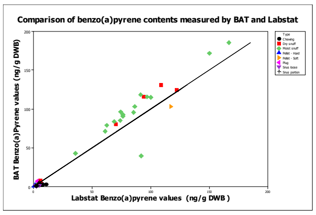


The naphthalene contents reported by Stepanov *et al.* were fifteen times greater than the values found in this study. The ratio of PAH contents observed in the two studies decreases with increasing PAH size, and from fluoranthene upwards the present study reports slightly higher values

At present we do not have an explanation for the differences found between the two studies, but it is highly unlikely that the differences are due to different sampling periods or slightly different products in the two studies

5.2.2 Comparison of B(a)P contents reported by Labstat and BAT

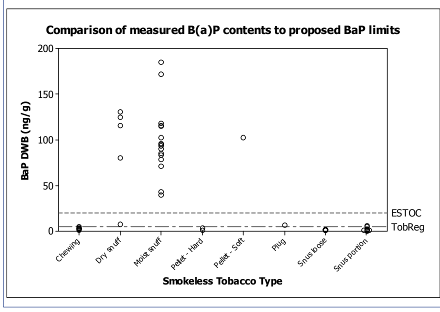
BaP BAT (DWB) = - 1.41 + 1.12 BaP Labstat (DWB) r2 = 0.96



Taken as a whole the B(a)P values measured by BAT are higher than reported by Labstat; in general by around 12%.

However, examination of the agreement between laboratories on the basis of product style showed that for all of the chewing tobaccos, loose snus and most of the pouched snus, the BAT reported values were lower than the Labstat reported values. These results indicate that there may be matrix-related differences in the results for different product styles.

5.3 Impact assessment of proposed limits on B(a)P contents of STPs



The B(a)P contents of all of the dry and moist snuff products, and the plug and soft pellet products, were greater the proposed TobReg limit (5ng/g dwb); all of the loose snus, hard pellet and chewing tobacco products were below the limit; all but one of the pouched snus products were also below the proposed limit.

A similar picture emerged with the proposed ESTOC limit (20ng/g dwb), but with slight differences. All of the moist snuff, all but one of the dry snuff and the soft pellet products exceeded the proposed limit. All of the loose and pouched snuff, hard pellet and chewing tobacco products were below the proposed limit.

6. Conclusions:

There are significant differences in the total and individual PAH contents of different smokeless tobacco product styles, with substantially higher levels found in moist and dry snuff and the soft pellet product than in the other smokeless tobacco styles.

A comparison of PAH concentrations measured by two different laboratories suggests that there are systematic inconsistencies between the two laboratories; for naphthalene these differences were extremely high.

The differences found between two laboratories in measurements of B(a)P were reasonably small, but showed evidence of matrix effects in the different methods used by the two laboratories. Interlaboratory consistency in benzo(a)pyrene measurement could therefore be a major issue in assessing compliance with the proposed limits if they were enacted.

Measurement of the B(a)P contents in this study allowed an impact assessment of two sets of proposed B(a)P limits. A fairly consistent picture emerged between the two limits, which indicated that in general dry and moist snuff products and the soft pellet product were above the limits, and the snus products and chewing tobaccos were below the limits.

The presence of PAHs in STPs, as measured in this study, does not in itself establish any toxicological relevance with respect to their use. It is also necessary to determine how much of the compounds transfer from the product during use in order to conduct a risk assessment. Transfer is probably low due to the low solubility of PAHs in water, and exposure of smokeless tobacco users may therefore be much lower than suggested by the contents measured in this study.

7. References:

1. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, IARC Press, Lyon, France, Volume 89, 2007, 55-60, Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php
2. WHO Study Group on Tobacco Product Regulation, WHO Technical Report Series 955, ISBN 978 92 4 120955 7, http://www.who.int/tobacco/global_interaction/tobreg/publications/tsr_955/en/index.html
3. IARC Monograph 92, 100F, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php
4. ESTOC Regulatory Proposal, http://www.estoc.org/uploads/Documents/documents/ESTOC_Regulation_Proposal_Part1.pdf
5. Rodgman A, and Perfetti T, The Chemical Components of Tobacco and Tobacco Smoke, CRC Press, 2009, ISBN 978-1-4200-7883-1
6. Stepanov I.,Villalta P., Knezevich A., Jensen J., Hatsukami D., Hecht S., Chem. Res. Toxicol, *23* (1), pp 66–73, 2010
7. Klus *et al*, Beitrage zur Tabakforschung International, (2009), Volume 23, No.5, 248-276
8. Smokeless tobacco facts, CDC, http://www.cdc.gov/tobacco/data_statistics/fact_sheets/smokeless/smokeless_facts/index.htm
9. http://monographs.iarc.fr/ENG/Classification/index.php

Disclosure: The study was funded by British American Tobacco.