Analysis of Tobacco Constituent Extraction by Snus Users

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Introduction:

• A better understanding of exposure and intake of tobacco constituents during snus use can provide valuable insights into it's potential effects.

• A variety of techniques have been employed to assess exposure, from biomarkers for NNK[1] and nicotine[2], to the use of *in vitro* extraction systems to examine toxic metals[3].

• These approaches are restricted by either the limited range of available biomarkers, or difficulties in ensuring that *in vitro* systems mimic real-world use.

• A versatile alternative approach is chemical analysis of the snus portion before and after use[2] to determine the level of constituent extraction.

Analytical approach:

• The chemical content of used snus was measured and compared with levels of constituents in unused "control" samples; the amount extracted from the snus portion by the user was calculated as follows:

Amount extracted = Quantity in unused snus – Quantity in used snus

% Extracted = 100(Amount extracted/Quantity in unused pouch)*

• A number of "multi-analyte" methods were developed to examine multiple constituents from the same snus portion, as shown in Figure 1.

• Snus samples were solvent extracted; the choice of solvent was dictated by the target analytes, as shown in Figure 1.

Basic techniques:

• Water extraction: Snus was extracted in 40ml of water in a 50ml plastic centrifuge tube, shaken for 30 minutes, centrifuged, and analysed

• Methanol extraction: Snus was extracted in 20ml of methanol in a 20ml plastic centrifuge tube, shaken for 30 minutes, centrifuged, and analysed

• Ethanol extraction: Snus was extracted in 20ml of ethanol in a 20ml glass tube, shaken for 30 minutes, centrifuged, and analysed.

Five Stage Method Validation Process:

• Equivalence of results from new method against existing pre-validated individual analyte methodology

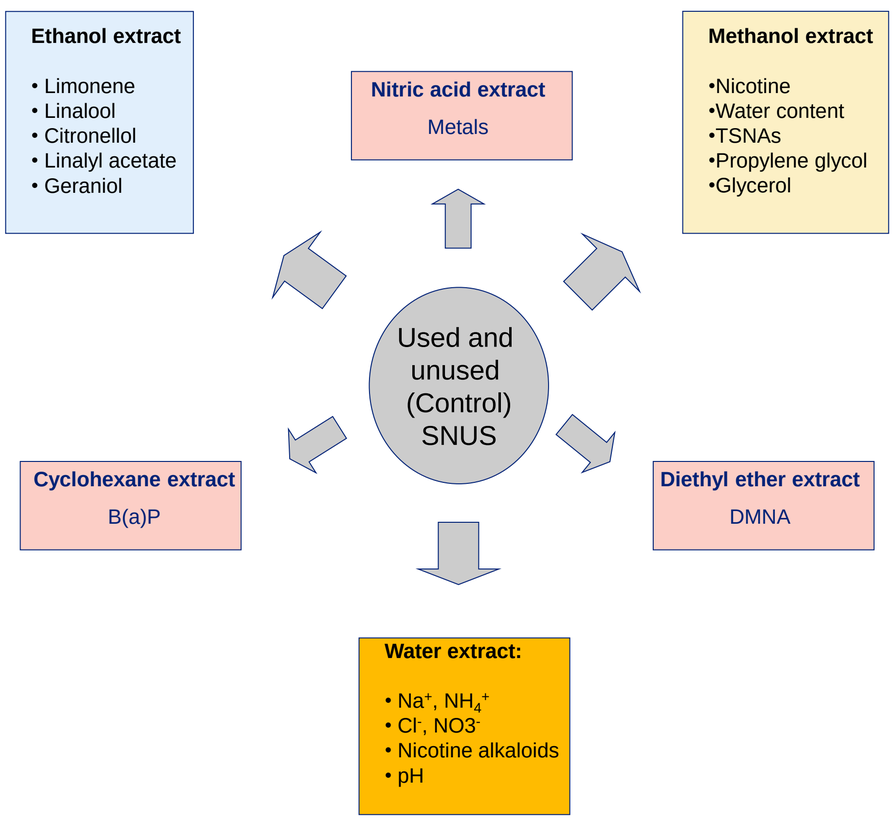
• No perturbation of analysis through presence of saliva

• No influence of combined extraction and sub-sampling

• Stability under transport and storage conditions

• Execution of a small-scale pilot study

Figure 1: Multi-analyte analysis approach



References:
1. S.S. Hecht *et al*. (2007), *Cancer Epidemiology, Biomarkers & Prevention* **16**, 1567-1572
2. E. Lunell, and M. Lunell, (2005), *Nicotine and Tobacco Research*, **7**, 397-403
3. R.S. Pappas *et al*., (2008), *Journal of Analytical Toxicology*, **32**, 281- 291
4. C. Proctor *et al*., (2008), *Poster presentation POS1-41 SRNT 14th Annual Meeting, Portland, Oregon,*

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Trial Protocol:

• Trials conducted in Stockholm and Lund, Sweden by an established consumer research agency, GfK

• Recruited existing snus users, aged 18-72, who use at least 8 samples per day for 1 hour+ per sample

• Each volunteer provided informed consent

• Paid for involvement

Recruitment of Panellists:

• Volunteer snus users

• Contacted and invited to participate by telephone

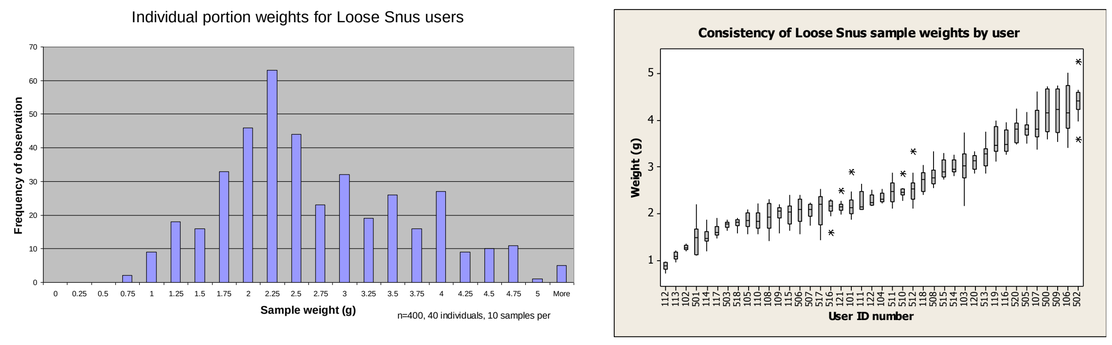
Screening steps

• Loose users invited pre-trial to try product to evaluate acceptability

• Loose snus sample sizes: Users prepared 10 samples in their normal way; samples weighed and consistency checked (results are shown in Figure 2); panellists selected from near the mean of the sample set

• Pouched users were interviewed about product preferences to assess compatibility with trial products

Figure 2: Analysis of loose snus sample sizes from screening trial



Trial Procedure:

• Trials conducted as monitored "Central-Location" trials,

• No snus use 1 hour prior to trial,

• Loose snus: Two loose portions prepared by each panellist; samples weighed and lowest used; highest cut down to same weight as other sample to act as control,

• Pouched snus: "Control" pouches were sampled at the same time from the same container as the test sample,

• Snus was held in-mouth by panellists for 60 minutes, consistent with consumption data reported previously[4] for pouched snus,

• Panellists used four samples per session; each panellist attended multiple sessions, depending on the number of replicates required,

• No food or drink during use other than water between snus uses if needed,

• Trial observations recorded and reported back at end of trial.

Sample transport

• Post-use, snus samples were stored individually in glass or plastic vials, depending upon the method for the target analyte,

• Samples were frozen at -18°C,

• Couriered in a cool-box with freezer blocks to British American Tobacco's Southampton laboratories for analysis,

• Samples frozen until analysis.

Results from pilot studies on pouched snus (n=20):

Pouch weight gain:	27%	Nicotine extracted:	31%
Pouch moisture pre-use:	48%	Pouch pH pre-use:	8.18
Moisture post-use	78%	Pouch pH post-use:	7.98

Extraction data submitted for presentation at SRNT Dublin 2009

Conclusions:

The methodology provides a flexible and robust approach for measurement of constituent extraction from snus by users.