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Stephen B Stanfill, Gregory N Connolly, Liqin Zhang, et al.

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Global surveillance of oral tobacco products: total nicotine, unionised nicotine and tobacco-specific *N*-nitrosamines

Stephen B Stanfill,[1] Gregory N Connolly,[2] Liqin Zhang,[1] Lily T Jia,[3] Jack E Henningfield,[4] Patricia Richter,[5] Tameka S Lawler,[1] Olalekan A Ayo-Yusuf,[6] David L Ashley,[1] Clifford H Watson[1]

[1]Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Emergency Response and Air Toxicants Branch, Atlanta, Georgia, USA
[2]Harvard University School of Public Health, Boston, Massachusetts, USA
[3]Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Organic Analytical Toxicology Branch, Atlanta, Georgia, USA
[4]The Johns Hopkins University School of Medicine and Pinney Associates, Bethesda, Maryland, USA
[5]Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, Georgia, USA
[6]Department of Community Dentistry, Faculty of Health Sciences, University of Pretoria, South Africa

Correspondence to
Stephen B Stanfill, National Center for Environmental Health, 4770 Buford Highway, Atlanta, Georgia 30341, USA; sstanfill@cdc.gov

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ABSTRACT

Objective Oral tobacco products contain nicotine and carcinogenic tobacco-specific *N*-nitrosamines (TSNAs) that can be absorbed through the oral mucosa. The aim of this study was to determine typical pH ranges and concentrations of total nicotine, unionised nicotine (the most readily absorbed form) and five TSNAs in selected oral tobacco products distributed globally.

Methods A total of 53 oral tobacco products from 5 World Health Organisation (WHO) regions were analysed for total nicotine and TSNAs, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), using gas chromatography or liquid chromatography with mass spectrometric detection. Unionised nicotine concentrations were calculated using product pH and total nicotine concentrations. Fourier transform infrared spectroscopy was used to help categorise or characterise some products.

Results Total nicotine content varied from 0.16 to 34.1 mg/g product, whereas, the calculated unionised nicotine ranged from 0.05 to 31.0 mg/g product; a 620-fold range of variation. Products ranged from pH 5.2 to 10.1, which translates to 0.2% to 99.1% of nicotine being in the unionised form. Some products have very high pH and correspondingly high unionised nicotine (eg, gul powder, chimó, toombak) and/or high TSNA (eg, toombak, zarda, khaini) concentrations. The concentrations of TSNAs spanned five orders of magnitude with concentrations of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) ranging from 4.5 to 516 000 ng/g product.

Conclusions These data have important implications for risk assessment because they show that very different exposure risks may be posed through the use of these chemically diverse oral tobacco products. Because of the wide chemical variation, oral tobacco products should not be categorised together when considering the public health implications of their use.

INTRODUCTION

Globally, oral tobacco products represent a diverse assortment of tobacco-containing products that deliver nicotine primarily through the oral mucosa upon placement in the mouth. These products may be chewed, sucked, or held between the gum and teeth for variable time intervals and, in some cases, swallowed in whole or part.[1 2] Oral tobacco product use has varying geographic prevalence. An estimated 8.1 million people use oral tobacco products in the US; however, in Southeast Asia, an estimated 258 million people use oral tobacco products. In addition to its addictiveness, oral tobacco may contribute to diabetes, high blood pressure, cardiovascular disease, oral diseases, and cancers of the oral cavity and pancreas.[1 3] Oral tobacco use is also associated with increased risk of death from myocardial infarction and increased risk of premature birth and pre-eclampsia.[3 4]

Oral tobacco products range from simple cured tobacco to elaborate products containing many non-tobacco ingredients; these products can be handmade or commercially made by using simple or very complex manufacturing processes.[1 5 6] Some oral tobacco products contain significant amounts of plant material (betel leaf, areca nut, catechu, etc.); moreover, additives such as sweeteners, flavour agents and spices (saffron, cardamom, camphor, eucalyptus, etc.) are commonly added. Alkaline modifiers, including certain inorganic salts, slaked lime and ashes produced by burning certain wood (eg, Willow, Mamón) or fungi,[1 5 6] are also added to some oral tobacco products. Unprocessed tobacco is mildly acidic (approx. pH 5–6.5); however, addition of alkaline modifiers boosts product pH converting a greater fraction of nicotine to more rapidly absorbed unionised nicotine,[3 5 6] which contributes to faster spikes in blood nicotine concentrations[7] and could result in such products being more addictive.[7–9]

Regional differences and local preferences contribute to the diversity of oral tobacco products in physical appearance, constituents and chemistry, with some products containing tobacco with little or no alkaline modifier, some augmented with substantial amounts of various alkaline modifiers,[1 5 6] and some mixed with slaked lime (as the alkaline modifier) and areca nut. Some global oral tobacco products have unique characteristics, such as extremely high pH, nicotine-enriched tobacco (eg, *Nicotiana rustica* L.), non-tobacco plant ingredients (eg, catechu, betel leaf, spices, etc.) and alkaline modifiers (plant/fungi ashes; slaked lime, etc.) not associated with Western-style products (ie, snus, snuff, chewing tobacco).[1 5 6] Moreover, some oral tobacco products, particularly those from South Asia (eg, betel quid, gutkha, mawa), also contain appreciable amounts of areca nut, seeds of the Areca palm (*Areca catechu* L.),[5 6] which has been classified as an International Agency for Research on Cancer (IARC) group 1 carcinogen, although the actual carcinogenic constituent(s) has not yet been identified.[10]

Oral tobacco products are known to contain more than 30 carcinogens, including volatile aldehydes, lactones, polycyclic aromatic hydrocarbons (PAHs), heavy metals, radioactive metals and tobacco-specific *N*-nitrosamines (TSNAs).[10] Among these chemical constituents in oral tobacco, TSNAs are considered the most potent classes of carcinogens[11] with *N*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), both IARC group 1 carcinogens,[10] linked to the formation of cancers of the oral cavity, oesophagus, lung and pancreas.[1 3] Another TSNA, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) has recently been reported in US moist snuff products.[12] Clinical studies have shown that NNAL, a metabolite of NNK, is present in the urine of oral tobacco users at levels similar to those found in urine from smokers[13]; the NNAL present in some oral tobacco products[12] may be absorbed directly and add to NNAL formed by metabolism of NNK.

Some products from South Asia, Sudan and South America contain *N rustica*, a tobacco species with higher nicotine and TSNA concentrations than found in cultivated tobacco used in US products (*Nicotiana tabacum* L.).[14] In India, an estimated 35% to 40% of tobacco present in oral tobacco products is *N rustica*.[15 16] Similarly, some Sudanese toombak contains very high concentrations of TSNAs[17] likely from the use of *N rustica*. The type of tobacco and curing of oral tobacco products can influence nicotine content and carcinogenic TSNA content[14 17] and, in turn, could potentially influence addiction and exposure to potent carcinogens.[18]

The primary goal of this study is to provide researchers and policy makers with approximate pH levels and total nicotine, unionised nicotine, and TSNA concentrations for a diverse global set of oral tobacco products. Because all products in this study were tested in a single laboratory using standard methodologies, direct comparisons between product groups are possible.

METHODS

Sample overview

In total, 65 oral tobacco samples were purchased or obtained by research partners in the following countries of origin: Bangladesh, India, Nigeria, Pakistan, South Africa, Sudan, Sweden, Uzbekistan and Venezuela. In all, 12 samples that lacked detectable nicotine were excluded from the analysis list. This study explores the pH levels and the total nicotine, unionised nicotine and TSNA concentrations in 53 oral tobacco products from 5 WHO regions. The geographic distribution and common constituents of representative oral tobacco products from three broad categories, based on product constituents, is presented in table 1; photographs of selected products from each category are shown in figure 1.

In most cases, a list of locally popular types of oral tobacco products was identified in published reference documents[15] and provided to the research partners. In some cases (eg, Uzbekistan), the research partner identified the type of oral tobacco product for testing. In all cases, the research partner was responsible for locating, purchasing and shipping the items to the Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia, USA). Samples were not stored in the country of origin but were promptly shipped at ambient temperature to CDC; upon receipt, samples were labelled and stored in a freezer at −30°C until analysed to prevent product changes (ie, moisture loss), minimise loss of volatile constituents and inhibit the formation of TSNAs during storage. All pH, nicotine and TSNA measurements were performed in the Tobacco Analysis Laboratory at CDC.

Fourier transform infrared spectroscopy

These products differ widely in tobacco type, additives, non-tobacco constituents and production; in select cases, product composition and identification of tobacco (*N tabacum* and *N rustica*) and non-tobacco components (areca nut, alkaline agents) were aided by using Fourier transform infrared (FT/IR) spectroscopy. Samples were ground and homogenised in a handheld grinder prior to FT/IR analysis. Samples of *N rustica*, provided by the Great Lakes Inter-Tribal Epidemiology Center and the Wisconsin Native American Tobacco Network, and *N tabacum*, cultivated tobacco used in US products, were analysed by FT/IR spectra for comparison with international tobacco products. Chimó, a tar-like product from Venezuela, was applied to the attenuated total reflectance (ATR) crystal, analysed and subsequently removed with methanol. Analyses were performed by using a JASCO 6200 FT/IR spectrometer (JASCO, Inc.; Easton, Maryland, USA) fitted with a diamond crystal ATR (Pike Technologies; Madison, Wisconsin, USA). Absorbance mode spectral detection was accomplished by using a wide-band detector cooled to approximately −70°C with liquid nitrogen. Sample spectra were obtained by averaging 64 scans in the spectral range from 425–4000 cm^{-1} at 4 cm^{-1} resolution.

Total and unionised nicotine quantification

To quantify total nicotine, a 1 g sample of oral tobacco was extracted in 50 ml of methyl *tert*-butyl ether (containing quinoline as an internal standard) and 5 ml of 2 N NaOH. For analysis, 1 µl of the extract was injected into a gas chromatograph/mass spectrometer operated in selected ion monitoring mode[19]; analysis of nicotine was done in triplicate. Measurements of pH (±0.001 pH units) were performed by adding 2 g of oral tobacco product to 10 ml of distilled, deionised water. Many of the products analysed in this study were dry powders. In cases where the product absorbed all the water (resulting in a paste-like consistency), an additional 10 ml of deionised water was added prior to pH measurement.[20] By substituting product pH and the pK_a of nitrogen's pyrollic nitrogen (8.02) into the Henderson–Hässelbalch equation, the proportion of nicotine in the unionised form (α_{fb}) was calculated. The amount of unionised nicotine was determined by multiplying total nicotine by the α_{fb} value.[20]

Tobacco-specific *N*-nitrosamines quantification

The concentrations of five tobacco-specific *N*-nitrosamines ((1) NNN, (2) NNK, (3) *N*′-nitrosoanatabine (NAT), (4) *N*′-nitrosoanabasine (NAB) and (5) NNAL) in oral tobacco samples were measured in triplicate. Samples were ground, and a 0.25 g portion was transferred to an amber extraction vial and spiked with isotopically labelled internal standards. Samples were extracted with aqueous ammonium acetate on a Lab-Line shaker (Melrose Park, Illinois, USA) at 250 rpm for 1 h. Two quality control samples, Copenhagen moist snuff and 2S3 Reference tobacco (University of Kentucky; Lexington, Kentucky, USA), were prepared with each batch of samples. Extracts were filtered with a 0.45 µm nylon syringe filter and a 20 µl aliquot was injected on a Xterra C18 MS liquid chromatography column (Waters Corporation; Milford, Massachusetts, USA). Compound-specific detection was accomplished by using a triple quadrupole mass spectrometer operated under electrospray ionisation and multiple-reaction monitoring mode. All chromatographic data were processed by using Analyst 4.02 software from Applied Biosystems (Forest City, California, USA).

Table 1 Description of representative products from three broad categories of oral tobacco products used globally[1 3 5 6] (some products with the same name can fit in more than one category based on formulation).

Product	Common geographic origins	Common ingredients
Category I: tobacco (with or without flavourants)*		
Tobacco leaf	Bangladesh	Tobacco
Misri	India	Tobacco (powdered)
Qimam (kiman)	India	Tobacco,† additives, spices (aniseed, cardamom, saffron)
Loose leaf	USA	Tobacco (air-cured cigar leaf), sweeteners (sugar, molasses), liquorice
Plug	USA	Tobacco (burley, bright, or cigar tobacco) leaves, sweeteners, liquorice
Twist	USA	Tobacco (dark and air-cured leaf), tar-like tobacco leaf extracts
Dry snuff	USA, UK, India	Tobacco (fermented fire cured, Kentucky and Tennessee), flavourings
Snus	Sweden	Tobacco, sodium carbonate, sodium chloride, moisturisers, flavouring
Moist snuff (lower pH)	USA	Tobacco (fermented air cured or fire cured), flavourings, inorganic salts
Category II: tobacco with various alkaline modifiers‡		
Chimó§	Venezuela	Tobacco, sodium bicarbonate, brown sugar, Mamón tree ashes
Naswar (Niswar, Nass)	Central Asia, Pakistan, Iran	Tobacco,† slaked lime,¶ indigo, cardamom, menthol
Khaini	India	Tobacco,† slaked lime paste (sometimes areca nut)
Toombak	Sudan	Tobacco (fermented),** sodium bicarbonate
Iq'mik	USA (Alaska)	Tobacco (air cured or fire cured), willow or punk fungus ashes
Gul	Central/eastern India	Tobacco powder,** molasses, alkaline modifiers
Snuff (higher pH)	USA, South Africa	Tobacco (fermented air cured or fire cured), flavourings, various alkaline modifiers
Category III: tobacco with slaked lime (as an alkaline modifier) and areca nut††		
Gutkha	India, Southeast Asia, UK	Tobacco, slaked lime, areca nut, catechu, saffron, saccharine, flavourings
Mawa	India	Tobacco, slaked lime, areca nut
Manipuri	Pakistan	Tobacco, slaked lime, areca nut, spices
Zarda	India, Arab countries	Tobacco, slaked lime, usually areca nut, spices, vegetable dyes
Betel quid (with tobacco)	South Asia, Southeast Asia, China‡‡	Tobacco, slaked lime, areca nut, flavourings§§ wrapped in betel leaf

*These products may contain small amount of compounds that boost alkalinity.
†These products are made with *Nicotiana tabacum* L. (cultivated tobacco) and/or *Nicotiana rustica* L. (Aztec or shamanic tobacco) that has a higher nicotine content.
‡Alkaline modifiers, which that can boost product pH, may include inorganic salts (sodium bicarbonate, sodium carbonate, potassium carbonate, etc.), slaked lime (calcium hydroxide) and ashes from various plants, Mamón (*Melicocca bijuga* L.) and willow (*Salix* spp.) trees and from punk fungi (*Phellinus igniarius* (L.) Quél.).
§Chimó may also contain banana peel, avocado seed and yoco (*Paullinia yoco* L.) as flavourings.
¶Slaked lime (ie, calcium hydroxide) can be obtained from coral, shellfish, or quarried limestone.
**This product may be made of *N tabacum*, *N rustica*, or *Nicotiana glauca* Graham (Brazilian tree tobacco).
††These products made with areca nut (*Areca catechu* L.) can be made with or without piper betel leaf (*Piper betle* L.) and catechu (*Acacia catechu* L.).
‡‡Betel quid with tobacco is used in countries including India, Sri Lanka, Pakistan, Bangladesh, Myanmar, Thailand, Cambodia, Malaysia, Singapore, Philippines, New Guinea, Taiwan, China and Guam.
§§The flavourings used can include menthol, camphor, sugar, rosewater, aniseed, mint and other spices; this handmade product may also contain catechu.

RESULTS

Product characterisation by FT/IR

Of the samples analysed, 25 had definitive product labelling. For example, several samples believed to be gutkha were analysed by FT/IR and the resulting interferograms were matched to known examples. Nut-like plant material from products presumed to contain areca nut were collected, washed and dried to remove other product constituents. The material was then examined by light microscopy. These samples were further analysed by FT/IR analysis and compared with spectra of fresh areca nut for confirmation. In this instance, the samples in question contained unique IR peaks corresponding to areca nut and were confirmed to be handmade gutkha.

Analyses of mawa and mainpuri by FT/IR confirmed the presence of areca nut. One zarda sample from Bangladesh contained areca nut while another zarda sample did not. Neither of the Indian khaini samples contained an FT/IR signature indicative of the presence of areca nut, a popular but non-essential additive in these products. Of the five Sudanese toombak samples received, one product was a coarse, tan-coloured powder that lacked detectable nicotine. Furthermore, FT/IR analysis revealed that this sample did not contain characteristic spectral peaks indicative of tobacco (*N tabacum* or *N rustica*); this non-tobacco product was excluded from further analysis. Another toombak sample, a dry, light brown product, contained nicotine and total TSNA levels three times higher than the other three toombak products, which were black and appeared similar to moist snuff. Determination of the type of tobacco present in the areca nut-containing zarda (Hakim Pury) was inconclusive due to interferences due to the areca nut present in that sample.

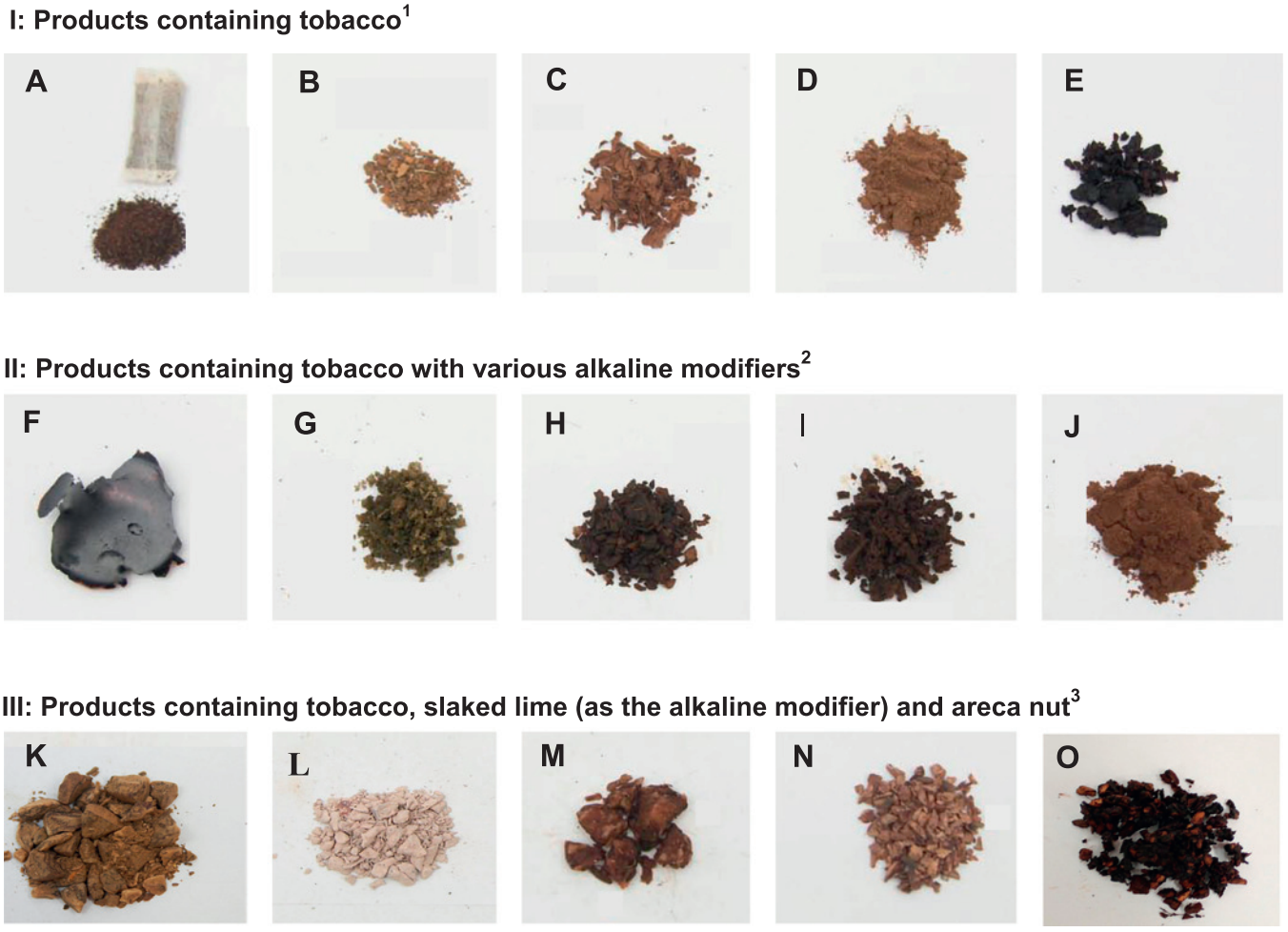
Because some oral tobacco products from Sudan contain *N rustica*, a nicotine-enriched tobacco variety,[5 14 17] we compared the IR spectra of pure *N rustica* tobacco with each of the four toombak samples. A light brown toombak product (sample 5) exhibited spectral patterns corresponding to known samples of *N rustica* tobacco. The three other toombak samples had spectra similar to that found for products made with *N tabacum*, the species commonly used in US products. Some samples of gul and zarda with very high nicotine concentrations had spectral patterns most similar to *N rustica*.; however, these samples did not contain extremely high TSNA concentrations. Identity of tobacco species (*N rustica* or *N tabacum*) in tobacco samples was further confirmed by the ratio of IR-specific absorbances.

Product designations

Product designation categories used in table 2 are based on product constituents listed on the package labelling (if available), product pH and, in the case of some products, confirmation of the presence or absence of areca nut by FT/IR analysis and light microscopy.

pH

The pH in international oral tobacco products tested in the study ranged from pH 5.2 to 10.1, which translates to 0.2% to 99.1% of nicotine being in the unionised form (figure 2). The



1 These products may also contain spices, sweeteners, flavor chemicals, and low levels of alkaline modifiers.

2 These products may also contain spices, sweeteners, flavor chemicals, and substantial amounts of alkaline modifiers that may include sodium bicarbonate, slaked lime, ashes from fungi or plants, or inorganic salts that increase product pH.

3 These products may also contain piper betel leaf, catechu, and various spices.

Figure 1 Photographs of representative products from three broad categories of oral tobacco products used globally. Examples of category I are (A) pouch snus (Sweden), (B) tobacco leaf (Bangladesh), (C) natural leaf chewing tobacco (USA), (D) dry snuff (USA) and (E) low pH moist snuff (USA). Examples of category II are (F) chimó (Venezuela), (G) naswar (Uzbekistan), (H) khaini (India), (I) toombak (Sudan) and (J) medicated dry snuff (South Africa). Examples of category III are (K) handmade gutkha (India), (L) manufactured gutkha (India), (M) mawa (Pakistan), (N) mainpuri (Pakistan) and (O) zarda, areca nut-containing variety (Bangladesh).

highest pH values were found in khaini (India), toombak (Sudan) and snuff (South Africa) (table 2). In terms of pH, handmade gutkha (pH 7.4–8.6) had a wider range of pH than the commercially manufactured gutkha (pH 8.5–8.9) analysed. The pH level in tobacco-only products (pH 5.2–7.2) was generally lower than oral products known to contain alkaline modifiers (pH 7.0–10.1) (figure 1), whereas areca nut-containing products ranged from pH 6.5–8.9.

Total nicotine

Nicotine concentrations ranged from 0.2–34.1 mg nicotine/g product (mg/g) (table 2). Total nicotine in most products ranged from 0.2–21.3 mg/g; however, a few products, such as gul powder (Bangladesh), zarda (India), chimó (Venezuela) and toombak (Sudan), had higher nicotine concentrations ranging from 27.5–34.1 mg/g. One toombak sample (sample 5), with a FT-IR spectral pattern most similar to *N rustica*, had a nicotine concentration (28.2 mg/g) that was almost three times higher than the other three toombak samples (9.56–10.7 mg/g). Several other products, including Eagle Gul, Baba Zarda and tobacco leaf (Bangladesh), with higher nicotine values (19.7–33.4 mg/g) had FT/IR spectral features consistent with *N rustica*. Some chimó samples had high nicotine values (27.5–30.1 mg/g); however, FT/IR was inconclusive as to the tobacco type it contains.

Unionised nicotine

Unionised nicotine content, calculated by using product pH and measured total nicotine, spanned over four orders of magnitude (table 2). Calculated unionised nicotine concentrations for most products ranged from about <0.1–13.8 mg/g, except for two chimó products (27.4 and 30.1 mg/g) and two gul powder products (29.1 and 31.0 mg/g). Unionised nicotine was lowest in handmade gutkha from Pakistan (0.1 mg/g), Sada Pata tobacco leaf (0.2 mg/g) and wet zarda (0.2 mg/g). In terms of unionised nicotine concentrations, handmade gutkha (0.2–3.3 mg/g) was

Table 2 Levels of pH, nicotine and unionised nicotine found in international oral tobacco products

Product description/name	Product category*	Country of origin	WHO region†	pH		Total Nicotine		Percentage of nicotine unionised	Unionised nicotine, mg/g
				Mean	SD	mg/g	SD		
Special Gul Powder	II	Bangladesh	SEARO	8.79	0.25	34.1	0.1	85.2	29.1
Eagle Gul Powder‡§	II	Bangladesh	SEARO	9.22	0.13	33.4	0.1	92.8	31.0
Sada Pata Tobacco Leaf§	I	Bangladesh	SEARO	5.92	0.14	19.7	0.2	0.77	0.15
Hakim Pury Wet Zarda (with areca nut)¶**	III	Bangladesh	SEARO	6.51	0.04	21.3	0.2	2.95	0.63
F. Rahman & Co Zarda††	I	Bangladesh	SEARO	6.28	0.03	9.55	0.15	1.76	0.17
Baba Zarda 120§	I	India	SEARO	5.22	0.02	30.4	0.8	0.16	0.05
Super Raja Khaini††	II	India	SEARO	9.65	0.02	4.79	0.22	97.7	4.68
Spitt Raja Chap Khaini**††	II	India	SEARO	9.79	0.09	2.53	0.04	98.3	2.48
Gutkha product 1 (handmade)¶	III	India	SEARO	7.45	0.19	0.91	0.21	20.8	0.19
Gutkha product 2 (handmade)¶	III	India	SEARO	7.99	0.08	0.92	0.19	47.4	0.44
Gutkha product 3 (handmade)¶	III	India	SEARO	8.60	0.05	1.41	0.19	78.6	1.11
Gutkha product 4 (handmade)¶	III	India	SEARO	8.48	0.07	2.24	0.52	50.2	1.13
Gutkha product 5 (handmade)¶	III	India	SEARO	8.61	0.25	4.20	0.61	79.4	3.33
Gutkha product 7 (handmade)¶	III	India	SEARO	7.43	0.01	1.76	0.59	20.1	0.35
Rajdarbar Gutkha	III	India	SEARO	8.46	0.02	1.57	0.17	72.8	1.14
Shikhar Gutkha	III	India	SEARO	8.88	0.07	1.67	0.22	87.7	1.47
Sitar Gutkha	III	India	SEARO	8.59	0.02	1.09	0.16	78.3	0.86
Bahar Gutkha‡‡	III	India	SEARO	8.64	—	1.29	0.15	80.3	1.03
Dhamaal Gutkha (Saffron)‡‡	III	India	SEARO	8.54	—	2.33	0.08	76.4	1.78
RMD Gutkha‡‡	III	India	SEARO	8.49	—	1.73	0.46	74.3	1.28
Gutkha (handmade; Karachi)¶	III	Pakistan	EMRO	8.48	0.03	0.16	0.01	73.6	0.12
City Gutkha (Saffron)	III	Pakistan	EMRO	8.20	0.03	2.08	0.05	43.1	0.90
JM Extra Strong Gutkha	III	Pakistan	EMRO	8.54	0.12	1.41	0.25	76.5	1.08
Mawa¶	III	Pakistan	EMRO	8.31	0.02	0.16	0.02	65.4	0.11
Mainpuri¶	III	Pakistan	EMRO	7.65	0.22	1.28	0.14	29.3	0.38
Naswar, sample 1§§	II	Pakistan	EMRO	9.14	0.02	14.2	0.1	92.8	13.2
Naswar, sample 2§§	II	Pakistan	EMRO	8.76	0.04	10.5	0.0	84.4	8.84
Toombak, sample 1 (black)§§	II	Sudan	EMRO	9.84	0.07	10.3	0.1	98.5	10.2
Toombak, sample 2 (black)§§	II	Sudan	EMRO	10.1	0.0	9.56	0.23	99.1	9.47
Toombak, sample 5 (brown)§	II	Sudan	EMRO	7.38	0.05	28.2	0.5	18.3	5.16
Toombak, sample 7 (black)‡‡	II	Sudan	EMRO	9.88	0.20	10.7	0.4	98.6	10.6
Nigerian Snuff (traditional)**	II	Nigeria	AFRO	9.42	0.16	2.49	0.33	96.1	2.39
Joseph & H. Wilson Medicated 99 Snuff$$	II	Nigeria	AFRO	9.02	0.17	7.41	0.07	90.7	6.72
NTSU Ugway Snuff$$	II	South Africa	AFRO	9.15	0.14	14.9	0.1	92.9	13.8
South African Snuff (traditional)**	II	South Africa	AFRO	9.29	0.03	5.29	0.16	94.8	5.01
Singleton's Super Menthol Snuff**	II	South Africa	AFRO	9.35	0.10	2.95	0.02	95.4	2.82
Super Taxi Snuff§§	II	South Africa	AFRO	10.1	0.2	1.17	0.04	99.1	1.16
Peter Stuyvesant Menthol Snus	I	South Africa	AFRO	6.79	0.08	14.1	0.1	5.44	0.77
Peter Stuyvesant Blue Snus	I	South Africa	AFRO	6.48	0.02	17.2	0.7	2.74	0.47
Svenskt Tobacco-rette Snus	I	South Africa	AFRO	6.56	0.05	15.0	0.1	3.28	0.49
Lucky Strike Original Red Snus	I	South Africa	AFRO	7.02	0.17	13.4	0.2	8.90	1.19
Lucky Strike Menthol Snus	I	South Africa	AFRO	6.66	0.00	15.2	1.3	4.09	0.62
General Original Snus	I	Sweden	EURO	7.01	0.02	8.34	0.08	8.98	0.75
General Loose Snus	I	Sweden	EURO	6.61	0.00	7.79	0.07	3.77	0.29
General White Portion Wintergreen Snus	I	Sweden	EURO	7.07	0.01	7.76	0.24	10.0	0.78
General White Portion Snus	I	Sweden	EURO	6.86	0.04	8.09	0.03	6.48	0.52
Catch Peppermint Snus	I	Sweden	EURO	7.21	0.02	15.2	0.3	13.3	2.03
Nasway¶¶**	II	Uzbekistan	EURO	8.43	0.09	8.89	0.64	71.5	6.36
Vencedor Chimó**	II	Venezuela	AMRO	6.98	0.09	16.1	0.2	8.18	1.32
Fabrica De Chimó**	II	Venezuela	AMRO	9.40	0.03	5.29	0.14	95.9	4.99
El Tigirito Chimó**	II	Venezuela	AMRO	8.56	0.03	10.4	0.3	77.2	8.02
El Tabacote Chimó**	II	Venezuela	AMRO	9.12	0.08	27.5	1.2	92.5	25.4
Chimó La Chinata C.A.**	II	Venezuela	AMRO	9.04	0.01	30.1	2.2	91.1	27.4

Total nicotine values represent measurements made in triplicate unless noted otherwise; total nicotine and calculated unionised nicotine are presented as mg/g wet weight. pH and total nicotine values were produced form measurements of three separate samples of tobacco (n=3) unless otherwise noted.

*Product categories are: I) tobacco only (with or without flavorants), II) tobacco with alkaline modifier, and III) tobacco with areca nut and slaked lime (with or without piper betel leaf and catechu). These product designations were made based on ingredients listed on packaging, product pH and the presence or absence of areca nut based on analysis by Fourier transform infrared spectroscopy (FT/IR).

†WHO Regions: SEARO=Southeast Asia; EMRO=Eastern Mediterranean; AFRO=Africa; EURO=Europe; AMRO=Americas.

‡Due to limited sample size, pH measurements were made in duplicate (n=2).

§The tobacco in this product is most similar to *Nicotiana rustica* L., a high nicotine-containing species, as determined by FT/IR.

¶The presence of areca nut (*Areca catechu* L.) in this product was confirmed by FT/IR.

**FT/IR determination of tobacco type was inconclusive because the sample did not match the spectra for *N tabacum* or *N rustica*; these products may contain another tobacco species. Identification of chimó by FT/IR may be affected by product preparation.

††FT/IR analysis revealed that these products contain little or no areca nut (*A catechu* L.).

‡‡Due to limited sample size, only one pH measurement was performed.

§§The tobacco in this product is most similar to *N tabacum* the species most commonly used in U.S. products when analysed by FT/IR.

¶¶Total nicotine values for this product were measured eight times (n=8).

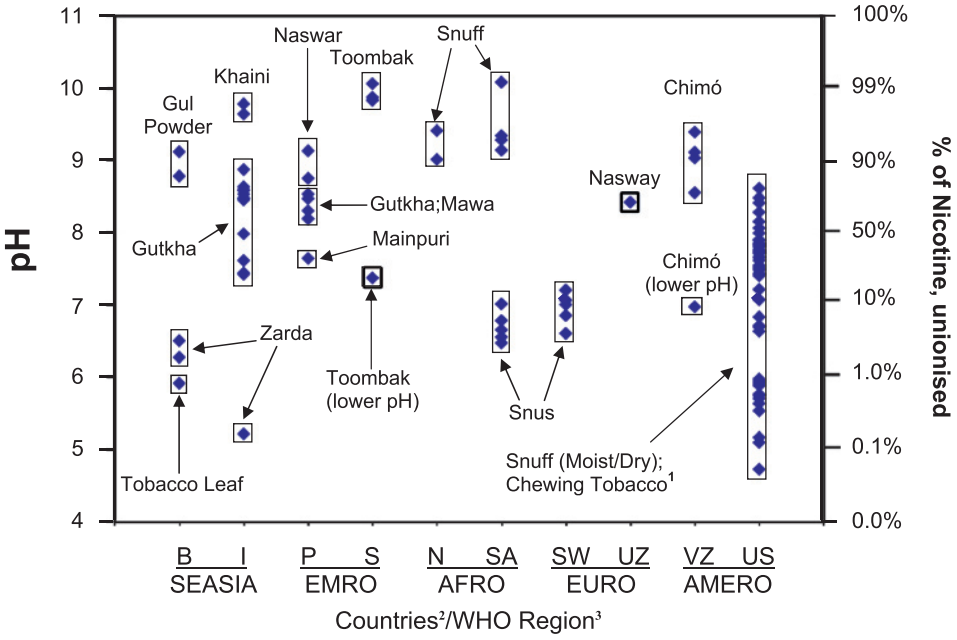


Figure 2 The range of pH and percentage of unionused nicotine in various oral tobacco products from 10 countries from 5 WHO regions.

1 Data for U.S. moist snuff brands was previously reported[12]. The data range of pH and percentage of unionised nicotine for chewing tobacco (e.g., loose leaf, plug, and twist) and dry snuff were determined by Lawler *et al.* (unpublished results from the CDC Tobacco Analysis Laboratory).

2 Countries: B=Bangladesh; I=India; P=Pakistan; S=Sudan; N=Nigeria; SA=South Africa; SW=Sweden; UZ=Uzbekistan; VZ=Venezuela; US=United States.

3 WHO Regions: SEASIA=Southeast Asia Region; EMRO=Eastern Mediterranean Region; AFRO=African Region; EURO=European Region; AMERO=Region of the Americas.

similar to the manufactured gutkha (1.0–1.8 mg/g) samples analysed in this study.

Tobacco-specific *N*-nitrosamines

The TSNA concentrations varied widely among the international samples (table 3). The highest concentrations of NNK were found in toombak from Sudan (516 000 ng/g). Dry zarda from Bangladesh had 3840 ng/g of NNK, much higher than most of the products tested. The highest concentrations of NNN were found in products from Sudan (368 000 ng/g), Bangladesh (28 600 ng/g) and India (18 600 ng/g) (table 3). Handmade gutkha and mawa from Pakistan contained the lowest NNK concentrations. Oral tobacco products contained a wide range of NNAL concentrations (3.58–6770 ng/g), unlike cigarette smoke, which does not usually contain detectable concentrations of this compound. The highest NNAL concentrations were found in four samples of toombak, and also in dry zarda and khaini.

All four nicotine-containing toombak samples from Sudan had high TSNA concentrations. This toombak product, with the highest NNK concentrations (516 000 ng/g) and extremely high nicotine (28.2 mg/g), was identified by FT/IR as containing *N rustica*. Zarda (Pakistan) and khaini (India) analysed in this study had very high TSNA concentrations. The NNN content in Zarda exceeded 28 000 ng/g and concentrations in khaini exceeded 17 000 ng/g. Among the gutkha products analysed, a handmade gutkha (product 1; Secunderabad, India) had the highest concentration of all five TSNAs, whereas a handmade gutkha from Pakistan had the lowest concentrations of the five TSNAs. The concentration of total TSNAs in the international products analysed in this study ranged from 83.9–992 000 ng/g (table 3).

DISCUSSION

Confirmation of product identity or composition using FT/IR was performed on 34 samples, including gul powder, tobacco leaf, zarda, khaini, gutkha, mawa, mainpuri, naswar, toombak, snuff, nasway, and chimó. Furthermore, FT/IR was used to determine whether a product contained tobacco similar to that used in U.S. products (*N tabacum*) or a higher nicotine-containing tobacco species (*N rustica*) or neither and whether or not it contained areca nut (*A catechu*). In a few cases, products contained a spectral pattern unlike either *N tabacum* or *N rustica* and may indicate the use of a different tobacco species (such as *Nicotiana glauca* Graham) in these products. Chimó is made by cooking tobacco, sodium bicarbonate, flavouring, brown sugar and Mamón tree ashes until the mixture becomes a concentrated black tar.[1 5] Some products made in South America contain *N rustica*[14]; however, due to the tar-like consistence of chimó, FT/IR was inconclusive in determining the tobacco species present in these products. The high concentration of nicotine in chimó products is undoubtedly influenced by the nicotine content of the tobacco used and the preparation of the product.

The pH values in the international products (pH 5.2–10.1) (see figure 2) exceed the pH values found recently among top selling US moist snuff products (pH 5.5–8.6). Approximately 40% of the international products had pH values exceeding the highest value found for US moist snuff products (pH 8.6).[12] Total nicotine among the international brands ranged from 0.16 to 34.1 mg/g product. For comparison, US moist snuff products range from 4.4–14.2 mg/g product with a single product as high as 25.0 mg/g product.[12] Due to higher alkalinity and, in some cases, higher nicotine values, unionised nicotine had a much wider range (0.05–31.0 mg/g product) in many international products than found among US moist snuff products (<0.1–7.8 mg/g product).[12]

In this study, one toombak product had the highest concentrations of all five TSNA compounds, with NNN and NNK concentrations of 368 000 and 516 000 ng/g product, respectively. For comparison, the highest levels of NNN and NNK in

Table 3 The concentrations of five tobacco-specific *N*-nitrosamines (TSNAs) found among various international oral tobacco products

Sample description	Country of origin*	TSNAs,† ng/g										Total TSNAs (ng/g)
		NAB		NAT		NNK		NNN		NNAL		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Special Gul Powder	B	2370	20	4770	90	1370	50	8020	370	590	40	17100
Eagle Gul Powder	B	1980	30	4240	80	1330	20	5190	410	630	80	13400
Sada Pata Tobacco Leaf	B	68.9	10.4	294	25	21.7	4.1	165	15	24.5	7.6	574
Hakim Pury Zarda	B	6030	190	11800	500	3840	250	28600	1600	3460	310	53700
F. Rahman & Co Zarda	B	1020	5	3110	60	457	4	4280	120	248	13	9120
Baba Zarda 120	I	210	11	1150	50	829	29	2910	120	390	30	5490
Super Raja Khaini	I	2580	70	2220	30	502	23	16800	400	1440	30	23500
Spitt Raja Chap Khaini	I	2190	120	303	10	288	30	17500	700	1350	50	21600
Gutkha product 1 (handmade)‡	I	1600	450	2310	600	375	84	18600	4800	1030	290	23900
Gutkha product 2 (handmade)	I	10.0	1.8	51.8	7.8	20.2	5.3	154	28	27.7	4.8	264
Gutkha product 3 (handmade)	I	13.9	0.6	41.5	3.2	7.1	1.2	192	3	23.4	1.4	278
Gutkha product 4 (handmade)	I	9.64	0.94	125	2	20.4	0.8	208	17	10.8	0.9	374
Gutkha product 5 (handmade)	I	184	12	284	10	47.3	2.4	1610	30	57.9	2.2	2180
Gutkha product 7 (handmade)	I	28.6	1.1	85.4	7.8	46.2	5.3	292	23	13.5	0.9	466
Rajdarbar Gutkha	I	11.3	3.3	111	19	57.1	9.3	167	24	23.2	2.7	370
Shikhar Gutkha	I	6.2	0.69	110	14	58	6.5	177	28	36.2	4.4	387
Sitar Gutkha	I	85.3	8.9	282	15	241	29	1080	80	77.2	6.6	1770
Bahar Gutkha	I	16.2	4.6	100	25	68.7	20.4	206	50	29.6	6.2	420
Dhamaal Gutkha-Saffron	I	133	30	126	31	456	121	1280	280	258	77	2250
RMD Gutkha	I	52.9	7.5	118	26	236	42	587	96	103	15	1100
Gutkha (handmade; Karachi)§	P	5.44	0.64	14.4	1.1	11.6	1.1	45.4	4.9	7.02	1.52	83.9
City Gutkha-Saffron	P	12.8	0.6	76.9	6.7	64.5	2.3	174	10	37.3	3.2	366
JM Gutkha Extra Strong	P	91.4	13.2	290	29	208	12	913	39	53.5	10.6	1560
Mawa	P	5.49	0.23	16.2	2	4.47	1.4	65.5	4.2	3.98	0.24	95.6
Mainpuri	P	17.3	1.8	63.5	3	6.05	1.26	106	3	25.9	2	219
Naswar, sample 1	P	19.8	0.6	56.9	1	29.4	3.4	363	16	8.56	1.48	478
Naswar, sample 2	P	85.3	8.9	342	13	309	12	545	14	104	1	1380
Toombak, sample 1 (black)	S	119000	400	17100	200	149000	3000	119000	300	5790	4950	302000
Toombak, sample 2 (black)	S	302000	200	17200	1300	152000	8000	119000	7000	4550	230	305000
Toombak, sample 5 (brown)	S	41500	800	59600	4900	516000	53000	368000	3000	6770	360	992000
Toombak sample 7 (black)	S	11100	200	16600	300	147000	6000	115000	2000	5470	3590	295000
Nigerian Snuff (traditional)	N	50.2	4.2	444	20	285	3	711	19	29.5	6.3	1520
Joseph & H. Wilson 99 Snuff	N	51.9	2.0	418	50	365	54	1460	180	125	16	2420
NTSU Ugway Snuff	SA	29.4	12.9	653	21	130	6	892	94	3.58	2.35	1710
South African Snuff (traditional)	SA	629	7	12600	30	1610	78	5570	150	71.8	6.8	20500
Singleton's Super Menthol Snuff	SA	58.0	12.4	696	80	347	69	1590	250	40.1	19.2	2730
Super Taxi Snuff	SA	175	14	565	49	242	77	3400	60	287	27	4670
Peter Stuyvesant Menthol Snus	SA	65.4	8.8	827	33	275	37	1290	40	30.4	13.0	2490
Peter Stuyvesant Blue Snus	SA	41.9	0.8	521	26	202	48	925	61	30.1	7.5	1720
Svenskt Tobacco-rette Snus	SA	114	4.0	1360	10	1340	20	2950	110	84.2	0.4	5850
Lucky Strike Original Red Snus	SA	73.0	11.2	632	135	171	35	1190	260	18.6	8.7	2080
Lucky Strike Menthol Snus	SA	86.4	7.9	881	76	267	31	1440	40	29.4	8.7	2700
General Original Snus	SW	20.8	0.4	248	14	96.4	4.2	345	32	12.5	0.7	723
General Loose Snus	SW	17.7	1.1	224	13	105	4	293	12	12.8	3.0	652
General White Wintergreen Snus	SW	17.1	1.8	214	24	89.8	9.5	267	23	12.8	1.3	601
General White Portion Snus	SW	17.5	1.5	225	10	96.8	4.6	296	22	13.1	2.8	648
Catch Peppermint Snus	SW	13.4	1.4	229	18	84.5	8.2	295	23	8.57	0.77	630
Nasway	UZ	71.4	7.2	297	39	88.3	8.6	628	43	10.5	2.1	1100
Vencedor Chimó	V	57.3	2.8	602	16	902	65	3310	210	290	66	5160
Fabrica De Chimó	V	173	11	668	56	2600	100	4620	240	1330	110	5880
El Tigirito Chimó	V	103	2	965	96	1760	160	2620	92	431	37	954
El Tabacote Chimó	V	21.7	2.4	224	30	532	46	329	30	53.3	12.4	1160
Chimó La Chinata CA	V	19.1	5.9	292	49	310	131	318	87	14.9	5.6	9390

All TSNA measurements were made in triplicate and presented as ng/g wet weight. TSNA values were produced from measurements of three separate samples of tobacco (n=3) unless otherwise noted.

*Tobacco-specific N-nitrosamines measured in the study include N′-nitrosoanabasine (NAB), N′-nitrosoanatabine (NAT), N′-nitrosonornicotine (NNN), 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and 4-(methylnitrsoamino)-4-(3-pyridyl)-1-butanol (NNAL).

†Countries are identified as B=Bangladesh, I=India, P=Pakistan, S=Sudan, N=Nigeria, SA=South Africa, SW=Sweden, UZ=Uzbekistan, and V=Venezuela.

‡Handmade gutkha products bought from street vendors in Secunderabad, India.

§Handmade gutkha products bought from street vendors in Karachi, Pakistan.

US moist snuff products were 42 600 ng/g and 9950 ng/g product, respectively.[12] The highest NNK levels in one toombak product was 50 times greater than the maximum concentration found among US moist snuff products.[12] The total TSNAs (sum of all five TSNAs) in the international products analysed in this study ranged from 83.9–992 000 ng/g (table 3), whereas total TSNAs in US moist snuff ranged from approximately 4900–90 000 ng/g.[12] A combination of factors, such as pH, tobacco type, nitrate fertilisation/uptake, curing, fermentation and storage conditions, could contribute to these extremely high TSNA concentrations. Moreover, salivary TSNA concentrations in the oral cavity of toombak users reach the low ppm (μg/ml) range,[17 18] thus, it is not surprising that 68% of oral cancers in Sudanese men are attributed to the use of toombak or other oral products.[3]

In addition to toombak, several other products, including zarda (Pakistan) and khaini (India), also had very high TSNA concentrations compared with US moist snuff.[12] Even though oral tobacco products, such as zarda, gutkha, or snuff, share the same name, in different countries the chemical composition can be different. Swedish snus had relatively low concentrations of most of the TSNAs, particularly NAB, NNK and NNAL. Although snus products purchased in South Africa had relatively low TSNA concentrations, Swedish snus products were at least four times lower. The concentrations of TSNAs in South African snus were also higher than a local South African snuff product (NTSU) not manufactured by using GothiaTek,[21] the strict Swedish tobacco industry standards governing allowable toxicant content in snus. This observation, together with lower TSNA concentrations in other traditional products, suggests that such products could be produced with lower TSNA concentrations. Effective product regulation and testing and the dissemination of best manufacturing practices across nations, particularly with respect to manufacture of traditional products, could have a positive net effect in reducing carcinogen levels. The Surgeon General concluded that tobacco products should be no more harmful than necessary given available technology.[22]

Our findings confirm that TSNA levels vary widely in oral tobacco products. Factors, such as pH, nitrate content, tobacco type, curing, fermentation and storage conditions, which can be altered, could influence the TSNA content of a product. The data in this paper suggest that oral tobacco products can be produced with lower TSNA content. Efforts to reduce TSNAs in tobacco would likely reduce exposure to these known human carcinogens. Moreover, individual use factors, such as the type and amount of product used and duration of use, may affect the delivery, exposure and health risk. Oral tobacco products are highly diverse and present a complex array of potential health hazards. The use of cigarettes and oral tobacco makes estimating the cumulative exposure risks more difficult. Even among dedicated oral tobacco users, the availability and opportunity for using a wide variety of different oral tobacco product types makes risk assessment more challenging.

The concentration of unionised nicotine in an oral product is likely the primary characteristic that determines the extent of tobacco-dependent addiction that, in turn, results in repeated exposure to many harmful tobacco-related constituents during long-term use. The concentration of total nicotine alone may not adequately explain nicotine delivery and response. Alkaline agents can substantially increase nicotine absorption rates by converting nicotine to its most rapidly absorbed form. Moreover, use of nicotine-enriched tobacco (ie, *N rustica*) in some products (eg, gul powder, toombak) or processes that concentrate nicotine in a tar-like product (ie, chimó), could contribute to high nicotine concentrations, which in the presence of elevated pH, yield high unionised nicotine concentrations (figure 3). Potential modification of pH levels through addition of varying levels of alkaline modifiers could produce products with lower unionised nicotine levels suitable for initiation and products with progressively higher unionised nicotine and greater addiction potential that might facilitate product graduation.[3 7 8] The wide concentration ranges seen among certain product types (eg, toombak, chimó, snuff) could help provide data useful on an empirical basis for specifying different or multiple maximum allowable concentrations for nicotine, pH and various toxicants as a function of product type by organisations such as WHO[23] and the US Food and Drug Administration.[24]

Limitations

Oral tobacco products sent to CDC were a convenience sample available to our research partners at the time of the request and with their available financial resources; research partners were not reimbursed for the purchase or shipping of these products. These products do not represent an exhaustive sampling of individual countries geographically or a particular product group (eg, snuff, chimó, etc.). Due to wide variety of values found

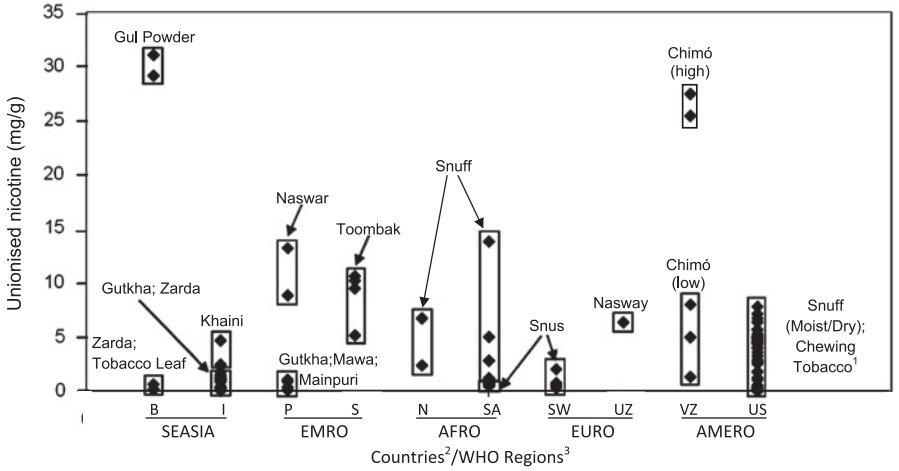


Figure 3 Unionised nicotine concentrations (mg/g) in oral tobacco products from 10 countries from 5 WHO regions.

1 The data plotted or the U.S. includes a total of 51 brands of snuff (moist/dry) and chewing tobacco (twist, plug and leaf). Data for 40 U.S. moist snuff brands were reported in reference 16; range s of un-ionized nicotine for chewing tobacco (loose leaf, plug, and twist) and dry snuff are results determined in the CDC Tobacco Laboratory by Lawler *et al*.

2 Countries: B=Bangladesh, I=India, P=Pakistan, S=Sudan, N=Nigeria, SA=South Africa, SW=Sweden, UZ=Uzbekistan, VZ=Venezuela, US=United States.

3 WHO Regions: SEASIA=Southeast Asia Region, EMRO=Eastern Mediterranean Region, AFRO=African Region, EURO=European Region, AMERO=Region of the Americas.

among the entire set of global products, analysis of one or a few samples of a particular product type gives a limited but informative view of product constituents. Further research with a larger set of products from each product group and more extensive sampling (ie, greater number of analyses) will be required to fully characterise these products so that they can be compared within a product group. In this study, sample measurements were made in triplicate. A greater number of measurements would be required to provide the level of statistical power necessary to make meaningful comparisons between individual products with very similar values. The results for these international products represent the analyte concentration in the products at the time of testing. The amount measured also does not translate directly into absorbed amount. The amount actually absorbed by users depends on numerous product characteristics, use parameters (e.g. amount used) and physiological differences in individual users. Even with these limitations, a clearer picture of oral tobacco as an inhomogeneous and diverse group of products has emerged from this global study.

Conclusions

Oral tobacco products are a chemically diverse group of products that can contribute to numerous health problems, including cancer and cardiovascular disease. Differences in the tobacco used, the various methods of curing and preparation, and the nature of other substances added to these products prior to use yield a varied group of products. When referring to such a diverse group of products, the term 'oral tobacco' is preferable to 'smokeless tobacco', as some of these products (eg, snuff, fire-cured dry snuff, iq'mik)[1 5 6] are made using fire-cured tobacco that contains smoke-derived chemicals including phenols, PAHs and TSNAs also found in cigarette smoke.[10 25]

The global sample of oral tobacco products analysed here contained a wide range of pH levels and total nicotine, unionised nicotine, and TSNAs concentrations with some products containing NNN and NNK levels exceeding daily levels delivered in cigarette smoke.[26] Tobacco products with higher unionised nicotine and TSNA levels generally leads to greater deliveries[7 13 26] and, in some cases, may translate to higher risks for adverse health outcomes.[3] Our data does not support oral tobacco products, as a class, being viewed as 'safer' or as providing a 'reduced harm' alternative to smoking. The possibility of dual use of tobacco products expands the potential for addiction and exposure to harmful constituents and may reduce the likelihood of complete abstinence from all tobacco products. At present, the only known means to reduce risk from tobacco is through cessation.

Oral tobacco products should not be routinely lumped together as a homogenous product category and considered as a single, equivalent product nor should their use be considered in isolation from other concurrent tobacco use. The drawing of broad conclusions about oral tobacco products based on limited data obtained on select samples from specific localities could be very misleading. Further studies to better characterise individual oral tobacco products, their diverse contents, the exposure of users to these products and the role of oral tobacco taken alone or in combination with other forms of tobacco are needed to provide crucial science to help inform consumers and also those involved in policy decisions and recommendations for tobacco control.

What this paper adds

- Oral tobacco is often viewed as a homogenous set of products; however, on a global basis, oral tobacco products vary from simple tobacco-only products to those containing substantial amounts non-tobacco ingredients including various flavour additives or pH modifiers. Past studies have focused primarily on groups of related products or products from a particular geographical region or country. This study treats smokeless tobacco on a global basis to show the ranges of nicotine, tobacco-specific *N*-nitrosamines (TSNAs) and pH that exist among these heterogeneous products.
- This paper is one of the first to examine nicotine, pH and toxic constituents across a diverse spectrum of oral tobacco products distributed globally. Because these diverse products were characterised in a single laboratory, the differences in concentration levels are meaningful and can be readily used to compare product categories. The observed wide concentration ranges of nicotine, unionised nicotine, TSNAs and pH values suggest that the impact on addictiveness, toxicity, or carcinogenicity of a given product type are not uniform and the oral tobacco products should not be lumped into a single category.

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Competing interests JEH serves as a consultant through Pinney Associates to GlaxoSmithKline Consumer Healthcare on an exclusive basis regarding matters relating to smoking cessation, has a financial interest in a potential new nicotine replacement product, and has provided expert testimony against the tobacco industry. The other authors declare they have no competing interests.

Contributors SBS was project manager, performed FT/IR analysis, 30% of pH analysis, 40% of nicotine analyses and was author of the first draft. GNC was study originator, coordinated sample acquisition and was involved in writing/editing of the paper. LZ performed 100% of TSNA analyses and was involved in editing the paper. LTJ performed 60% of the nicotine analysis and 30% of pH analyses. JEH was involved with extensive writing/editing of the paper. PR performed sample acquisition and extensive writing/editing of the paper. TL performed 40% of pH analysis, sample preparation for 40% of nicotine analysis, and extensive editing of the paper. OAA-Y performed extensive writing/editing of the paper. DLA was involved with writing/editing of the paper. CHW was involved with writing/editing of the paper and statistical analysis.

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