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CLINICAL & BASIC RESEARCH

Analysis of Tobacco-Specific Nitrosamines in the Common Smokeless Tobacco *Afzal* in Oman

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تحليل نيتروزامينات التبغ وبالأخص التبغ غير المدخن "أفضل" الأكثر شيوعاً في عمان

نوال المخينية، طاهر باعمر، الصادق الطيب، عائشة الشحية

abstract: ***Objectives:*** There is a lack of awareness regarding the carcinogenicity of *Afzal*, an illegal smokeless tobacco product (STP) widely used among the Omani youth. Previous research has shown that certain types of tobacco-specific nitrosamines (TSNAs) are associated with oral and lung cancers. This study therefore aimed to assess levels of four common TSNAs in a randomly selected sample of *Afzal*. ***Methods:*** This study was carried out at Sultan Qaboos University in Muscat, Oman, between April and September 2013. A random sample of *Afzal* was analysed for four types of TSNAs using high-performance liquid chromatography-tandem mass spectrometry. The four types of TSNAs analysed were 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB). As a reference product, a sample of laboratory-manufactured American moist snuff (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) was also used to confirm the accuracy and precision of the analysis. ***Results:*** The analysis revealed total TSNA levels of 3.573 µg/g in the *Afzal* sample. Mean levels of NNN, NNK, NAT and NAB were 1.205, 1.015, 0.809 and 0.545 µg/g, respectively. ***Conclusion:*** Levels of the two most abundant TSNAs (NNN and NNK) found in the *Afzal* sample exceeded international regulatory limits. *Afzal* users therefore need to be educated regarding the potential health risks associated with their STP use. Stricter implementation of current legislation is recommended to reduce the availability and usage of *Afzal* in Oman.

Keywords: Smokeless Tobaccos; Carcinogenesis; Nitrosamines; Tandem Mass Spectrometry; Liquid Chromatography; Oman.

الملخص: الهدف: هناك حاجة إلى نشر الوعي بمضار التبغ غير المدخن" أفضل" وما يحتويه من مواد مسرطنة. يعتبر "افضل" أحد انواع التبغ غير المدخن المنتشر بين الشباب والمراهقين في سلطنة عمان. وقد ذكرت بحوث سابقة إرتباط بعض أنواع النيتروزامينات بسرطان الفم والرئة. هدفت هذه الدراسة إلى تقييم إمكانية تواجد 4 أنواع من النيتروزامينات من خلال عينة عشوائية من تبغ افضل. **الطريقة:** نفذت هذه الدراسة في جامعة السلطان قابوس في مسقط، سلطنة عمان، في الفترة من إبريل إلى سبتمبر 2013م. تم تحليل أربعة نماذج شائعة من النتيروزامينات المختصة بالتبغ في العينة العشوائية من مادة أفضل عن طريق تقنية الأداء العالي للتخطيط اللوني السائل بالمقياس الترادفي الطيفي للكتلة. وهذه النيتروزامينات المختصة بالتبغ هي: 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)، N-nitrosonornicotine (NNN)، N-nitrosoanatabine (NAT)، و N-nitrosoanabasine (NAB). ولتأكيد دقة التحليل تم الاستعانة بعينة معيارية من مركز السيطرة على الأمراض والوقاية منها في اتلانتا، جورجيا، الولايات المتحدة الأمريكية. **النتائج:** أظهرت النتائج وجود النيتروزامينات المختصة بالتبغ بكمية كلية تعادل 3.573 ميكروجرام/ جرام والترتيب حسب الوفرة هو: (NNN = 1.205)، (NNK = 1.015)، (NAT = 0.809)، (NAB = 0.545). **الخلاصة:** إن مستوى النيتروزامينات (NNN و NNK) في التبغ افضل قد تتعدى المستويات المعيارية العالمية. ولذا فإن مستخدمي مادة الأفضل بحاجة للتثقيف عن المخاطر الصحية الحرجة وكميات المواد المسرطنة في هذا المنتج جنبا إلى جنب مع الدعم الحكومي وسن قوانين أكثر صرامة لمنع بيع مادة "الأفضل" في سلطنة عمان.

مفتاح الكلمات: منتجات التبغ غير المدخن؛ أفضل؛ تكون السرطان؛ النيتروزامينات؛ الأداء العالي للتخطيط اللوني السائل بالمقياس الترادفي الطيفي للكتلة؛ عمان.

Advances in Knowledge

- *This study analysed the composition of a random sample of the common Omani smokeless tobacco product Afzal. It revealed high levels of certain tobacco-specific nitrosamines, including 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone and N-nitrosonornicotine.*

Application to Patient Care

- *The results of this study may be utilised by healthcare policy-makers in Oman to help raise awareness among Afzal users about the potential dangers of this smokeless tobacco product.*

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Smokeless tobacco products (STPs) represent a significant health risk and have been associated with oral and pancreatic cancers, oral lesions, coronary artery and peripheral vascular disease and adverse pregnancy outcomes.[1] Approximately 28 carcinogens have been identified in STPs so far.[2] Tobacco-specific nitrosamines (TSNAs) are considered a potent class of carcinogens in STPs.[2] During the STP curing process, TSNAs form in the leaves and increase if the tobacco is subsequently fermented.[3] Levels of TSNAs are also dependent on other factors, such as the basic pH level and the nitrite/nitrate content of the product.[4] The moisture content of the product and the related increase in microbial action are other causes of increased TSNA content in STPs.[3] There are four primary alkaloids in tobacco—nicotine, nornicotine, anatabine and anabasine—each of which can be nitrosated by nitrite to form the following TSNAs: 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosonornicotine (NNN), N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB), respectively.[4] The International Agency for Research on Cancer classifies NNN and NNK as group one carcinogens.[5] In contrast, levels of NAT and NAB are lower in tobacco and their carcinogenicity is thought to be much weaker than NNN or NNK.[4] Although NAB has been classified as a weak oesophageal carcinogen in rats, both NAB and NAT are classified as group three carcinogens, indicating that they are not classifiable as a human carcinogen.[5,6] However, the Food and Drug Administration in the USA considers nitrosamines as carcinogens which are not safe at any level.[7]

Different types of STPs have shown wide variations in their components and are therefore not equal in their delivery of carcinogenic TSNAs into the human body.[8] The World Health Organization (WHO) reports that Swedish *snus* has the lowest level of nitrosamine—the most dangerous carcinogen—among STPs available on the global market.[9] In contrast, the highest levels of TSNAs have been detected in Sudanese *toombak*.[10] In the USA, the three most popular brands of snuff were found to have high concentrations of TSNAs.[11] *Afzal* is a STP widely used by youth and teenagers in Oman.[12,13] This may be perhaps due to its ease of use during manual work compared to smoked tobacco, its low cost and the limited public awareness of its harmful effects.[3] High levels of certain heavy metals and cancer-enhancing anions in *Afzal* have previously been reported.[12,13] This study aimed to analyse the levels of TSNAs in a random sample of *Afzal* in order to provide further evidence of the potential carcinogenic risk of this product.

Methods

This study was carried out at the Sultan Qaboos University (SQU) in Muscat, Oman, between April and September 2013. A single package of 4.00 kg of *Afzal* was purchased by the researchers from one source in order to maintain uniformity throughout the study. The *Afzal* sample was labelled, pH and moisture levels were tested and the sample was refrigerated as previously described.[12,13] The analysis was undertaken within six months of manufacture of the product. In order to enhance the quality of the data and to confirm the accuracy and precision of the analysis, a sample of American moist snuff was utilised as a reference. The certified reference product-2 (CRP-2) is purposely manufactured for laboratory analysis by the Tobacco and Volatiles Branch of the Division of Laboratory Sciences at the Centers for Disease Control and Prevention ([CDC] Atlanta, Georgia, USA) and is not commercially available. This manufactured product is useful for comparing reference values obtained from different international analytical laboratories.[14,15] Samples of CRP-2 were thus prepared and analysed using the same methods as the *Afzal* samples [Figure 1]. Simultaneously, the CRP-2 samples were dealt with in the analysis as a different STP sample matrix in order to prove the validity of the method used for other STPs.

Afzal and CRP-2 samples were dried separately in accordance with standard protocols from the CDC to determine moisture content in STPs.[16] Approximately 15.00 g each of *Afzal* and CRP-2 were weighed separately in pre-weighed moisture dishes and placed uncovered in an oven at 99 ± 1 °C for three hours. The samples were then removed from the oven, covered and cooled in a desiccator at room temperature for

Figure 1: Photograph of the reference product* (left) and the common smokeless tobacco *Afzal* (right) samples. These samples were analysed via high-performance liquid chromatography-tandem mass spectrometry to determine tobacco-specific nitrosamine levels.

**American moist snuff product purposely manufactured for laboratory analysis by the Tobacco and Volatiles Branch of the Division of Laboratory Sciences at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA).*

Table 1: Accuracy, precision, reliability and linearity values for each type of tobacco-specific nitrosamine analysed in the *Afzal* samples

Value	NAB	NAT	NNK	NNN
Average in µg/g	10.480	83.400	23.270	89.690
SD	1.110	0.650	0.280	0.970
RSD in %	10.550	0.780	1.220	1.080
Accuracy in %	104.840	92.670	93.090	99.660
R-value	0.986	0.986	0.971	0.994
LOD in ppb	3.320	1.960	0.850	2.910
LOQ in ppb	11.060	6.530	2.850	9.710

NAB = N-nitrosoanabasine; NAT = N-nitrosoanatabine; NNK = 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = N-nitrosonornicotine; SD = standard deviation; RSD = relative standard deviation; LOD = limit of detection; ppb = parts per billion; LOQ = limit of quantification.

approximately 30 minutes to avoid drawing moisture from the air. After drying, the samples were ground separately to form a homogenised powder.

The TSNA analysis was performed according to methods described by Lawler *et al*. with minor modifications.[8] Three *Afzal* samples and a single CRP-2 sample of 0.50 g each were transferred to 50 mL volumetric flasks and extracted using 10 mL of 100 mM aqueous ammonium acetate buffer. The samples were then shaken using an incubator shaker (KS 4000 i control shaker, IKA® Werke GmbH & Co, Staufen im Breisgau, Germany) at 250 revolutions per minute (rpm) for one hour. Each extract was air-filtered twice with filter paper (Whatman® grade 42 filtration paper, Sigma-Aldrich Corp., St. Louis, Missouri, USA) followed by a 0.22 µm pore-sized nylon syringe filter (Whatman® GD/X syringe filter, Sigma-Aldrich Corp.). Analytical standards of NNN, NNK, NAT and NAB were purchased (Sigma-Aldrich Corp.) Methanol dilution was used to prepare five different concentrations of each analyte (10, 30, 50, 70 and 100 parts per billion [ppb]). Standard calibration curves were plotted for each of the TSNAs. For accuracy and precision, the relative standard deviation (RSD), limit of detection (LOD), limit of quantification (LOQ) and r-value of 10 replicate injections of 10 ppb each of the standards were calculated.

Table 2: Concentrations of tobacco-specific nitrosamine levels in three *Afzal* samples and one reference product* sample as determined by high-performance liquid chromatography-tandem mass spectrometry

Sample	TSNA levels in µg/g				
	NNK	NNN	NAB	NAT	Total
Afzal 1	1.018	1.178	0.624	0.807	3.627
Afzal 2	1.017	1.221	0.524	0.818	3.580
Afzal 3	1.009	1.216	0.486	0.802	3.513
Mean *Afzal* ± SD	1.015 ± 0.004	1.205 ± 0.019	0.545 ± 0.058	0.809 ± 0.007	3.573 ± 0.047
Mean CRP-2 ± SD	0.465 ± 0.280	1.793 ± 0.970	0.209 ± 1.110	1.668 ± 0.650	4.135 ± 0.752

TSNA = tobacco-specific nitrosamine; NNK = 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = N-nitrosonornicotine; NAB = N-nitrosoanabasine; NAT = N-nitrosoanatabine; SD = standard deviation; CRP-2 = certified reference product-2.

**American moist snuff product purposely manufactured for laboratory analysis by the Tobacco and Volatiles Branch of the Division of Laboratory Sciences at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA).*

Table 3: Comparison of certified reference tobacco-specific nitrosamine levels[15] with levels determined in the present study in one reference product* sample

	TSNA levels in µg/g	
	Reference levels	Present study
NAT	1.460–2.230	1.668
NNK	0.370–0.580	0.465
NNN	1.440–2.120	1.793
NAB	0.120–0.210	0.209
Total	3.380–5.000	4.135

TSNA = tobacco-specific nitrosamine; NAT = N-nitrosoanatabine; NNK = 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = N-nitrosonornicotine; NAB = N-nitrosoanabasine.

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Analytical separation of the *Afzal* and the CRP-2 samples was performed using a high-performance liquid chromatography (HPLC) system (1290 Infinity LC System, Agilent Technologies, Santa Clara, California, USA) with a Zorbax SB C18 1.8 µm, 2.1 x 50 mm stationary phase column (Agilent Technologies). Eluent A (aqueous phase) was a five mM ammonium acetate solution, whereas eluent B (organic phase) was comprised of acetonitrile and water at a ratio of 70:30, along with 5 mM of ammonium acetate. The column temperature was kept at 40 °C and the flow rate was constant at 0.4 mL/minute. The eluents were run in a gradient manner with a running time of five minutes. The detector used was a 6460 Triple Quadrupole LC/MS (Agilent Technologies). All chromatographic data were processed using MassHunter Workstation software B.06.00 (Agilent Technologies). All chemicals used in the analysis were from Sigma-Aldrich Corp. and were of analytical grade. Deionised water from a Milli-Q® Integral Water Purification System (EMD Millipore Corp., Bedford, Massachusetts, USA) was used throughout the analysis. Machine detection limits were calculated for each of the analysed TSNAs.

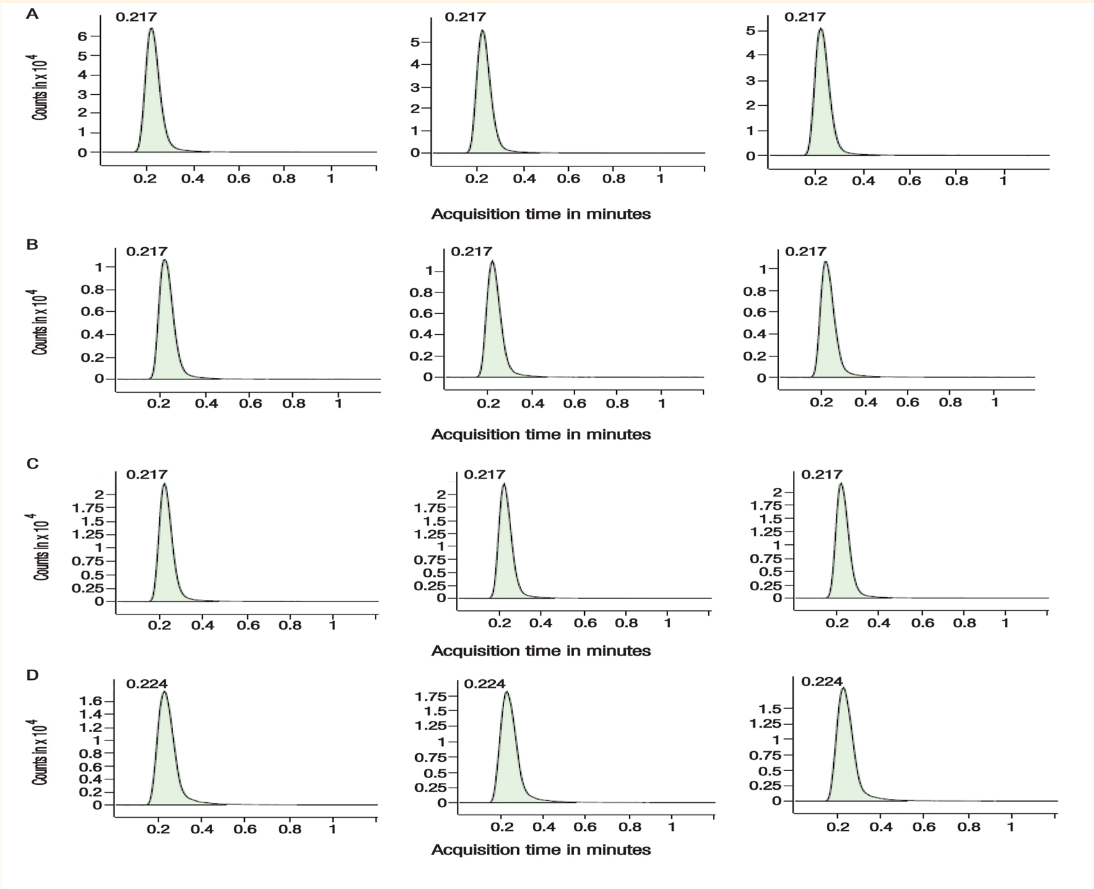

Figure 2A–D: High-performance liquid chromatography-tandem mass spectrometry chromatograms of (**A**) N-nitrosoanabasine, (**B**) N-nitrosoanatabine, (**C**) 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone and (**D**) N-nitrosonornicotine in each of the three *Afzal* samples.

Statistical analysis was performed using Excel spreadsheet software, Version 2010 (Microsoft Corp., Redmond, Washington, USA). As this study was a chemical analysis of two forms of STPs, it did not require ethical approval.

Results

The method used for the analysis of the *Afzal* samples showed high sensitivity and reliable validity, as indicated by the LOD, LOQ, RSD and other accuracy and linearity values of the calibration curves for all four TSNA types [Table 1]. Concentrations of all four TSNAs were detected in both the *Afzal* and the CRP-2 samples [Table 2]. TSNA levels for each of the three *Afzal* samples were very similar, reflecting the precision of the analysis. In the *Afzal* samples, mean analyte levels of NNN, NNK, NAT and NAB were 1.205 ± 0.019 μg/g, 1.015 ± 0.004 μg/g, 0.809 ± 0.007 μg/g and 0.545 ± 0.047 μg/g, respectively. For the CRP-2

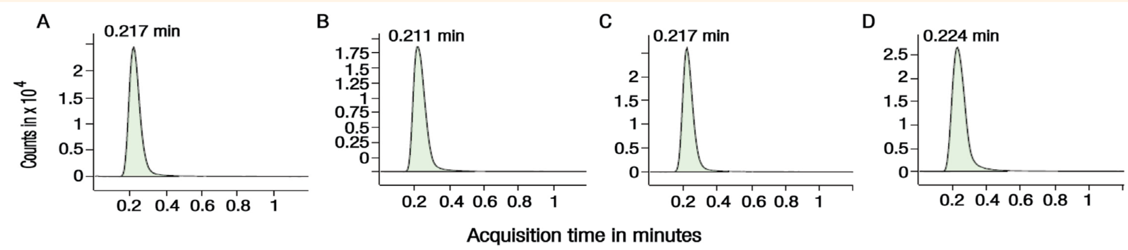

Figure 3A–D: High-performance liquid chromatography-tandem mass spectrometry chromatograms of (**A**) N-nitrosoanabasine, (**B**) N-nitrosoanatabine, (**C**) 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone and (**D**) N-nitrosonornicotine in one reference product* sample.

**American moist snuff product purposely manufactured for laboratory analysis by the Tobacco and Volatiles Branch of the Division of Laboratory Sciences at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA).*

sample, mean levels of the same TSNAs were 1.793 ± 0.970 µg/g, 0.465 ± 0.280 µg/g, 1.668 ± 0.650 µg/g and 0.209 ± 1.110 µg/g, respectively. Levels of NNN were highest while NAB levels were lowest for both the *Afzal* and the CRP-2 samples. Overall, the CRP-2 sample contained higher mean total TSNA levels (4.135 µg/g) than the *Afzal* sample (3.573 µg/g).

The observed TSNA values for the CRP-2 sample were in line with certified reference values provided by the CDC,[15] validating the results of the analysis [Table 3]. Chromatograms of the analysed TSNAs in all three *Afzal* samples were very similar, confirming the presence of the same analytes in each sample [Figure 2]. Chromatograms of TSNAs in the CRP-2 sample were comparable to those of *Afzal* samples, although there were slight differences between each analyte [Figure 3]. These chromatograms of TSNAs in CRP-2 showed comparable results, reflecting the effectiveness of the method used for the new STP sample matrix.

Discussion

Nitrosamines have been strongly associated with several human and animal cancers.[4] While nitrosamines are present in many foods and items which come into contact with food in amounts ranging from 1–10 ng/g, the highest exposure to humans comes from tobacco use.[17] These chemical compounds have been detected in the saliva of tobacco chewers and are believed to form in the digestive system.[17] All STPs demonstrate a wide diversity in their chemical compositions which result from variations in geographical location, additives used and manufacturing methods.[4] In order to avoid potentially diverse chemical compositions, one random sample of *Afzal* was chosen to conduct several chemical and toxicological tests. The presence of TSNAs in the *Afzal* samples was confirmed using a modified version of Lawler *et al.*'s method.[8]

Levels of nitrosamines in green tobacco leaves are negligible; however, several factors may foster the formation of TSNAs in STPS, including the damping, curing and fermentation processes, storage temperatures, high nitrate/nitrite content, alkaline pH and high moisture levels.[18,19] Most of these factors are present in *Afzal*. As reported previously, samples of *Afzal* were observed to have an alkaline pH (10.460), high moisture levels (52%) and a high nitrate concentration (8,792.200 µg/g).[12] In general, *Afzal* is stored illicitly in local STP shops or retail outlets where there is likely to be poor hygiene, a lack of air circulation and high temperatures which dramatically increase in the summer. Shi *et al*. confirmed that STP storage temperatures of above 30 °C increased TSNA formation significantly, as did high levels of nitrate.[20] A study by the WHO revealed that storage at room temperature increases TSNA concentrations in STPs due to microbial action.[9] Different manufacturing processes also play a role in TSNA level variations. High TSNA levels in American moist snuff and low TSNA levels in Swedish *snus* have been reported to be due to the fermentation process in the former and heat treatment or a pasteurisation-like process in the latter.[9] Heat treatment gives rise to more sterile products by killing the bacteria implicated in the formation of nitrosamines, while fermentation leads to a high concentration of nitrites.[21] *Afzal* is manufactured using a fermentation process; this may therefore facilitate the formation of TSNAs. The high TSNA content in *toombak* has been attributed to its high nitrate content, alkaline pH and its manufacture via fermentation.[10]

In the current study, high levels of two specific forms of TSNAs—NNN and NNK—were noted. Measurements of NNK and NNN show significant variation from one country to another. Levels of NNN and NNK levels in *Afzal* noted in the current study were lower than those found in traditional American STPs (135.000 µg/g and 17.800 µg/g, respectively) or dry weight *toombak* (3,085 µg/g and 7,870 µg/g, respectively);[4] however, these levels may nevertheless increase in *Afzal* blends due to the effect of the aforementioned factors that contribute to TSNA formation. Stepanov *et al*. reported that NNN and NNK levels in Indian *khaini* and *zarda* range between 1.740–76.900 µg/g and 0.080–28.400 µg/g, respectively, while lower ranges are reported for *gutkha* (0.090–1.090 µg/g and 0.040–0.430 µg/g, respectively).[22] In the current study, levels of NNN in *Afzal* were much higher than those observed in *gutkha*, but were lower than those found in *khaini* and *zarda*. In terms of NNK levels, the results of the current study were within the same range as those found in *khaini* and *zarda*, but were higher than that of *gutkha*.[21] The NNK and NNN levels in *toombak* (516.000 µg/g and 368.000 µg/g, respectively) were markedly higher than dry weight levels of Bangladeshi *zarda* (3.840 µg/g and 28.600 µg/g, respectively).[22] Low levels of NNN were also noted in Indian tobacco products (18.600 µg/g).[23]

Concentrations of NNN and NNK in American moist snuff have been reported to be 42.600 µg/g and 9.950 µg/g, respectively.[23] Hearn *et al*. found that Alaskan *Iq'mik* had NNN and NNK dry weight levels of 2.700 µg/g and 0.340 µg/g, respectively.[24] Handmade Pakistani *gutkha* has a lower total TSNA content compared with handmade *gutkha*.[23] New STPs in the USA have decreased mean total dry weight TSNA levels (2.610 µg/g) in comparison to traditional STPs

(7.420 μg/g); the same is true for specific NNN and NNK levels (2.050 versus 4.410 μg/g and 0.231 versus 1.200 μg/g, respectively).[25] Nevertheless, these NNN and NNK levels are still 100–1,000 times higher than nitrosamine levels reported in food and beer.[26]

Both NNN and NNK are thought to be potent carcinogens.[5] Balbo *et al*. reported a significant association between NNN in drinking water and the development of oesophageal and oral cancers in rats.[27] In addition, NNK has been found to increase the risk of lung cancer.[28] The NNN and NNK compounds are also known to form haemoglobin adducts in humans as well as in experimental animals.[29,30] The use of *toombak*, which has high NNN and NNK levels as mentioned above, has been associated with cancers of the oral cavity.[31,32] Many factors may influence the uptake or absorption of TSNAs in the oral cavity. TSNAs are highly water-soluble; therefore, as the moisture content of a STP increases, the levels of soluble TSNAs may also rise, resulting in enhanced absorption by the oral mucosa.[33] The authors of the current study strongly believe that TSNAs are not safe at any level. As a result, it is strongly recommended that existing legislation in Oman regarding the illegal sale of *Afzal* be more strictly enforced. Furthermore, there is a need for national public health awareness programmes regarding the potential carcinogenic effects of *Afzal*.

For Swedish *snus*, maximum permissible levels of carcinogenic substances are laid out by the GOTHIATEK® standard (Swedish Match, Stockholm, Sweden).[34] According to this, the maximum permissible combined NNN and NNK level is 1.000 μg/g.[34] Between 1983 and 2004, manufacturers of Swedish *snus* gradually decreased total TSNA levels to approximately 2.000 μg/g.[21] A recent study revealed that the total TSNA level in an unused 1.000 g pouch of *snus* was approximately 0.830 μg/g, with quantifiable levels of NAT, NNK, NNN and NAB (0.268 μg/g, 0.191 μg/g, 0.344 μg/g and 0.025 μg/g, respectively).[33] Therefore, total TSNA levels in the *Afzal* sample analysed in the current study were five-fold higher than the recommended GOTHIATEK® limits. Specific TSNA levels in *Afzal* were six-fold, 2.4-fold, 5.2-fold and 8.4-fold higher than the NAT, NNK, NNN and NAB levels, respectively, in *snus*. As such, TSNA levels in *Afzal* can be considered alarmingly high. The WHO recommends regulatory limits on the concentrations of selected carcinogens in tobacco products, including TSNAs.[9] Their recommendations permit <2.00 μg/g of combined NNN and NNK levels on a dry weight basis.[9] Combined levels of these two TSNAs in the studied *Afzal* sample (2.22 μg/g) exceeded this limit. This may have a negative effect on the health of *Afzal* users with frequent unmonitored use. Moreover, *Afzal* users may be exposed to higher levels of TSNAs as a result of varying self-determined pinch sizes; pinch sizes of *Afzal* are usually greater than the portions provided in packets of the more monitored and controlled Swedish *snus*.[12,13,33,34]

It is important to bear in mind that the results obtained by the current study and previous research on *Afzal* were derived from a single package of *Afzal* blend.[12,13] The findings of future studies may therefore differ due to variations in the additives and manufacturing processes used.

Conclusion

This study revealed the presence of carcinogenic TSNAs in a single random sample of *Afzal*. Despite being present at relatively low levels in *Afzal* as compared to other STPs, two of the most potent carcinogenic TSNAs—NNN and NNK—still exceeded the regulatory limits proposed by the WHO. Total TSNA levels were five-fold higher than the limits recommended by the GOTHIATEK® standard. These findings indicate that *Afzal* consumption may pose serious health risks for users. Consequently, existing legislation on the sale and availability of this STP should be enforced more rigorously. Increased public health education regarding the potential carcinogenic effects of *Afzal* is recommended.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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