

# Applying toxicological risk assessment principles to constituents of smokeless tobacco products: implications for product regulation

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## ABSTRACT

**Objective** To determine how information on chemical constituents of different smokeless tobacco products (STPs) may be used in cancer risk assessment for regulatory purposes.

**Methods** This study investigated select STP constituents potentially associated with significant cancer risk by applying a known toxicological risk assessment framework. Cancer risk estimates were obtained for selected constituents of STPs and a medicinal nicotine gum formulation with comparable toxicity information and also median concentration data on the GothiaTek analytes. The calculated cancer risk was considered 'unacceptable' if it exceeded the US Environmental Protection Agency's (USEPA's) benchmark of an 'acceptable' cancer risk of  $10E-6$ .

**Results** The cancer risk estimates derived from daily use of 10 g of STPs meeting the industry-set GothiaTek limits exceed the levels generally considered 'acceptable' by the USEPA at least 8000 times. Except for the medicinal nicotine tested, all the STP types, including the relatively lower tobacco specific nitrosamine (TSNA)-containing snus, were found to carry an 'unacceptable' cancer risk. The calculated cancer risks associated with the snus and the US moist snuff products were, respectively, at least 1000 times and 6000 times greater than the minimum acceptable. TSNA and cadmium are associated with the largest estimated cancer risks for all the STPs evaluated.

**Conclusions** This study's findings provide an empirical risk assessment that could guide STP regulation using an existing toxicological assessment framework. The study findings question the scientific rationale of the industry-set standards and highlight the need for regulatory actions to reduce specific toxicants in all STPs.

## INTRODUCTION

The use of oral and nasal smokeless tobacco products (STPs) including snuff, is common worldwide, but is highest on the Indian subcontinent. Although epidemiological data from the USA and India show that an elevated risk of oral cancer is associated with oral STPs, these findings have not been confirmed by studies in northern Europe.<sup>1</sup> However, a significantly increased risk for oesophageal and pancreatic cancer has been associated with oral snuff use in studies undertaken in the USA and northern Europe.<sup>1</sup> Snuff inhalation has also been reported to be associated with nasal sinus and nasopharyngeal cancer in some parts of Africa.<sup>2-5</sup> A case-control study in India demonstrated snuff use to be more common among patients with cancers of the oesophagus, hypopharynx or oropharynx than among controls.<sup>4</sup> In

a case-control study of lung cancer from Morocco,<sup>5</sup> a twofold increase in relative risk was observed for those who reported that they had ever inhaled snuff regularly. A recent study also found an increased risk for high-grade cervical squamous intraepithelial lesions (an immediate precursor lesion of cervical cancer) among a population of African women using smokeless tobacco.<sup>6</sup> Major agencies have indeed concluded that smokeless tobacco is carcinogenic to humans.<sup>4,7</sup>

However, some have argued that some newer oral STPs called snus (a Swedish oral moist STP), do not pose significant cancer risk because they contain lower levels of carcinogenic tobacco-specific nitrosamines (TSNAs). It has therefore been suggested that there is no justification for the use of a warning label with a risk phrase: 'causes cancer'.<sup>8</sup>

It has also been suggested that unlike the traditional STPs, snus that have been manufactured using the GothiaTek technique (with pasteurised tobacco instead of fire-cured tobacco, among other technologies) meets the Swedish Match company's so-called GothiaTek standard and therefore pose a significantly lower risk of cancer and should be considered a potentially reduced exposure product (PREP).<sup>9</sup> However, these newer, relatively lower TSNA snus products have been on the market for too short a time for there to be any convincing epidemiological support for the presence or absence of a lower cancer risk among users of these products as compared to users of STPs not manufactured to the GothiaTek standards. Indeed, it will take several years to conclude any epidemiological study to confirm the cancer risks associated with these new products. It therefore remains unclear, as defined by any objective risk assessment, whether on one hand the currently marketed brands of STPs not manufactured to the GothiaTek standard are likely to be more hazardous than ones that are manufactured to that standard and whether, on the other hand, the products manufactured to that standard are likely to be substantively more hazardous than the currently regulated medicinal nicotine products.

Toxicological risk assessment principles have recently been applied to cigarette smoke chemical constituents<sup>10,11</sup> and brands, including those that are categorised as PREPs.<sup>12</sup> The information obtained from such a risk assessment has recently been used to propose toxicants in cigarette smoke emissions to prioritise for a reduction of toxicants by regulatory authorities.<sup>13</sup> The current study therefore applies a risk assessment methodology developed by the US Environmental Protection Agency (USEPA) to estimate the cancer risk associated with exposure

to selected STP constituents and a commonly used medicinal nicotine gum formulation. The findings of this study are intended to form the basis for scientific discussion on an appropriate regulatory approach for smokeless tobacco products.

## METHODS

The current analysis computed the cancer risk for select STP constituents using the most comprehensive and comparable concentration data available for various STP types analysed in the same laboratory<sup>14</sup> and for a set of compounds (GothiaTek analytes) for which limits were set and published by the industry as manufacturing standards.<sup>15</sup> In order to compare the risk estimates derived for STPs with those for a regulated nicotine product, using the same laboratory, the level of similar constituents in a medicinal nicotine gum formulation [2 mg Nicorette gum (Original); GlaxoSmithKline plc, UK], was also determined. These data on STPs and medicinal nicotine gum constituent concentrations were combined with the constituents' toxicity information, as obtained from the University of California's (Berkeley) carcinogenic potency database (CPDB).<sup>16</sup> Consequently, STP constituents with cancer risk estimates that exceed levels of  $10E-6$  were identified for an adult STP user of 16 g wet weight<sup>17 18</sup> or 10 g dry weight product equivalent per day for 30 years. The USEPA uses the  $10E-4$  to  $10E-6$  range (1 in 10 000 to 1 in 1 000 000) as a 'target range' within which the agency strives to manage the risks posed by potentially hazardous substances. Estimates that are within this range require regulatory actions that can be determined based on a combination of factors, such as the size of the exposed population, the need for the continued availability of the product, the extent to which the potentially hazardous constituent cannot be avoided and the technological feasibility of achieving reductions.<sup>19</sup>

The risk calculations in the current study were limited to selected constituents for which the comparable carcinogenic potency is available in the CPDB and which have been classified as being at least reasonably anticipated to be a human carcinogen. The CPDB is a systematic and unifying analysis of chronic, long-term animal cancer tests. It includes results reported in the general literature up to 2001 and technical reports of the National Cancer Institute/National Toxicology program in the USA up to 2004.<sup>16</sup> The CPDB standardises the published literature and creates an easily accessible research resource that can be used to address a variety of research and regulatory issues regarding carcinogenesis.<sup>16</sup> The measure of carcinogenic potency used ( $TD_{50}$ ) is that chronic dose rate in mg/kg body weight/day that would induce tumours in half the test animals at the end of a standard lifespan for the species. A low  $TD_{50}$  value indicates a more potent carcinogen, whereas a high value indicates a less potent one. It is however, to be noted that the extrapolation of animal data to man may over estimate the cancer risk estimates in humans. Nevertheless, it has also been recently argued that judging from the comparability of levels of certain carcinogens in haemoglobin (ie, haemoglobin adducts), the quantitative extrapolation of risk estimates from rat to human could be considered justified.<sup>8</sup>

The potential for cancer effects was consequently estimated by calculating the incremental probability that an individual will develop cancer over a lifetime as a result of chronic exposure to a particular substance (that is, above baseline lifetime risk). Recognising differences in the route of administration between cigarette (inhalation) and STP constituents (oral absorption or

ingestion), the equation used for estimating cancer risk was modified as follows:

$$\text{Incremental lifetime cancer risk} = \text{ADE}_{\text{lifetime}} \times \text{CPF}$$

Where  $\text{ADE}_{\text{lifetime}}$  = lifetime average daily oral exposure (mg/kg body weight/day) and CPF = cancer potency factor ((mg/kg body weight/day)<sup>-1</sup>).

The cancer potency factor (CPF) is the lifetime cancer risk estimated to result from continuous exposure to a substance at a concentration of 1 mg/kg body weight. The lifetime average daily exposure (ADE) is estimated by adjusting the ADE according to adult body weight (assumed to be 70 kg), the number of years of snuff use (assumed to be 30 years) and the average lifetime (assumed to be 70 years). The equation for estimating the lifetime ADE is the following:

$$\text{ADE}_{\text{lifetime}} = \frac{\text{ADE} \times \text{Number of years snuffing}}{\text{Average lifetime}}$$

As with the assumptions used in previously published assessments for cigarette products,<sup>10-12</sup> the above equation is based on the assumption that 100% of the toxicant is transferred and is thus potentially biologically available, as would be typical for any conservative risk assessment calculation. Given that medicinal nicotine gum is only indicated for use as a nicotine replacement for a period of 12 weeks<sup>20</sup> during quit attempts and considering that it has been reported that it takes an average quitter four quit attempts before success,<sup>21</sup> in this study, a lifetime exposure of 48 weeks (about 1 year) was assumed to compute the cancer risk that may be associated with the use of medicinal nicotine gum. An average 10 pieces or 10 g of gum daily was assumed to be used throughout this period.

## Sensitivity analysis

If one assumes that 60% of the total compound is extracted in the mouth<sup>17</sup> and 60% of this would be absorbed (in order words, eventually 36% of the constituents are potentially bioavailable), then one can adjust the daily dose from a product meeting the GothiaTek standard accordingly, as depicted in the last column of Table 1. However, based on the literature on their bioavailability in human, an absorption fraction of 6% was assumed for the heavy metals: cadmium<sup>22</sup> and lead.<sup>23</sup> It was also assumed that 85% of each of the two major TSNA compounds (*N*'-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)) is bioavailable.<sup>24</sup>

Furthermore, considering that of the carcinogenic polycyclic aromatic hydrocarbons (PAHs), only comparable benzo(a)pyrene (BaP) concentration data was available, we computed BaP equivalents using the toxic equivalency factors (TEFs)<sup>25</sup> for each of the other carcinogenic PAHs that have been detected in STPs.<sup>26</sup> From this published PAH data that included BaP, we determined that the other PAHs would be expected to contribute an additional BaP equivalent, giving an effective BaP equivalent dose of twice the BaP concentration data reported by Rickert *et al.*<sup>14</sup> Given that we also had only the cancer potency for BaP in the CPDB, we assumed a cancer potency ratio (ie, carcinogenic potency of mixture of PAHs: carcinogenic potency of BaP as a single substance) of 1, considering that the cancer potency ratio reported for oral application of a mixture of PAHs was in the range of 0.7-1.2.<sup>27</sup> In order to make all the calculated risks comparable, the risk associated with the use of medicinal nicotine gum was also computed for 30 years as if it were to be used on a long-term basis.

**Table 1** Calculated cancer risk for selected smokeless tobacco product (STP) constituents meeting GothiaTek standards

Compound	GothiaTek limit (ng/g dry weight)	Laboratory‡ method detection limit (ng/g dry weight)	Compound TD <sub>50</sub> (mg/kg body weight/day)	Cancer potency factor ((mg/kg body weight/day) <sup>-1</sup> )	Cancer risk estimate (100% transfer)	Cancer risk estimate (reduced percentage transfer)
TSNA*	10000	230§	0.0999	10.1	6.2×10 <sup>-3</sup>	5.3×10 <sup>-3</sup>
BaP (BaPeq)†	20 (40)†	0.04	0.956	1.1	2.7×10 <sup>-6</sup>	1.6×10 <sup>-7</sup>
Cadmium	1000	43.3	0.0217	46.1	2.8×10 <sup>-3</sup>	1.7×10 <sup>-4</sup>
Lead	2000	37.9	46.6	0.02	2.5×10 <sup>-6</sup>	1.5×10 <sup>-7</sup>
Arsenic	500	25	No comparable CPDB data			
Chromium	3000	11.9	No comparable CPDB data			
Product total risk					9.0×10 <sup>-3</sup>	8.1×10 <sup>-3</sup>

\*TD<sub>50</sub> (chronic dose rate in mg/kg body weight/day, which would induce tumours in half the test animals at the end of a standard lifespan for the species) for NNK and NNN was used as a conservative estimate of risk for the composite of TSNAs and 85% bioavailability was assumed for each when calculating reduced percentage transfer.<sup>24</sup>

†Cancer risk estimates were based on concentrations using BaP equivalents (BaPeq) to represent contributions from other carcinogenic PAHs.

‡These are detection limits for the selected constituents in processed tobacco as reported by the laboratory that tested the STPs used in this study.

§This was based on the analysis of the tobacco 'as received'.

BaP, benzo(a)pyrene; CPDB, carcinogenic potency database; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N'-nitrososnicotine; PAH, polycyclic aromatic hydrocarbon; TSNA, tobacco specific nitrosamine.

## RESULTS

The calculated risk estimates for products meeting the GothiaTek limits varied between 8.1×10<sup>-3</sup> and 9.0×10<sup>-3</sup>, depending on the assumption on the amount of constituents that is potentially bioavailable (table 1), but remained considerably higher than the 10<sup>-6</sup> to 10<sup>-4</sup> range advocated by the USEPA. Although, the lowest cancer risk was associated with the constituents of low-moisture snuff (1.2×10<sup>-3</sup>), none of the smokeless tobacco types that were assessed met USEPA benchmark criteria for 'acceptable' risk. The calculated cancer risk for medicinal nicotine gum was however well below the 'significant' cancer risk level (table 2).

## DISCUSSION

The current study demonstrates that medicinal nicotine gum is the only product analysed that meets the USEPA benchmark for an 'acceptable' cancer risk. Under the key assumptions made in the current analysis, TSNA is the main component in any cancer risk associated with STPs. It would require a considerable decrease in exposures to the TSNA and cadmium in the STPs to bring the risk that they pose down to the 'acceptable risk' benchmark of the USEPA, that is, a level equivalent to 2 ng/g and 1 ng/g for TSNA and cadmium, respectively. However,

considering that the lowest reported TSNA levels reported in the recent literature was 223 ng/g for tobacco leaf<sup>25</sup> and about 2000 ng/g in the typical snus, it is unlikely that such a reduction to 'acceptable' cancer risk levels can be attained in these tobacco products under the existing manufacturing processes. There is nevertheless evidence from this study and elsewhere that suggests that there are methods for reducing TSNAs and cadmium in tobacco<sup>29</sup> to much lower levels than in many of these products studied.

While it is desirable to lower the level of toxicants in these products to the lowest levels possible, consideration has to be given to the practical level for enforcement of any action levels that may be set by a regulatory agency for a particular product. Notwithstanding this limitation, it is clear that the GothiaTek performance specification for the manufacture of STPs surely poses an 'unacceptable' cancer risk. Products meeting such standards should therefore, for a start, be required to reduce their levels at least to TSNA levels in the modern snus-type products<sup>14 15</sup> (that is, TSNA not higher than 2000 ng/g or an equivalent of a fivefold decrease from the current GothiaTek limit), while working towards further reductions to levels that would be considered to pose an 'acceptable' risk or to levels below the detection limits of any reliable laboratory test methodology.

**Table 2** Cancer risk of broad types of smokeless tobacco products for which comparable data is available

Smokeless tobacco type	Median pH	Category	Median concentration (ng/g) and cancer risk estimates*				
			TSNA	BaPeq	Cadmium	Lead	Total
Swedish snus (n=2)	7.4	Level	2309	3.68	980	238	
		Risk estimate	1.2×10 <sup>-3</sup>	9.0×10 <sup>-8</sup>	1.7×10 <sup>-4</sup>	1.8×10 <sup>-8</sup>	1.4×10 <sup>-3</sup>
Low-moisture snuff (n=6)	9.5	Level	2025	34	320	659	
		Risk estimate	1.1×10 <sup>-3</sup>	8.2×10 <sup>-7</sup>	5.4×10 <sup>-5</sup>	4.8×10 <sup>-8</sup>	1.2×10 <sup>-3</sup>
US-style chewing tobacco (n=2)	5.4	Level	2013	BDL	503	333	
		Risk estimate	1.1×10 <sup>-3</sup>	—	8.5×10 <sup>-5</sup>	2.5×10 <sup>-8</sup>	1.2×10 <sup>-4</sup>
Indian chew (Manikchand Gutka)†	8.3	Level	797	276	BDL	NQ	
		Risk estimate	4.3×10 <sup>-4</sup>	8.8×10 <sup>-6</sup>	—	—	†
US loose moist snuff (n=15)	7.5	Level	11675	140	933	340	
		Risk estimate	6.4×10 <sup>-3</sup>	3.4×10 <sup>-6</sup>	1.6×10 <sup>-4</sup>	2.5×10 <sup>-8</sup>	6.6×10 <sup>-3</sup>
US pouch moist snuff (n=4)	7.6	Level	11667	70	1018	394	
		Risk estimate	6.4×10 <sup>-3</sup>	1.7×10 <sup>-6</sup>	1.9×10 <sup>-4</sup>	2.9×10 <sup>-8</sup>	6.6×10 <sup>-3</sup>
Medicinal Nicotine gum (Nicorette)	9.3	Level	BDL	1.72	NQ	NQ	
		Risk estimate	—	3.6×10 <sup>-9</sup>	—	—	3.6×10 <sup>-9</sup>

\*Based on data from Rickert *et al.*<sup>14</sup> and the constituent reduced percentage transfer assumptions (sensitivity analysis).

†Contains areca nut for which the carcinogen data is not provided, so that the risk estimate is likely to be grossly underestimated.

BaP, benzo(a)pyrene; BDL, below detection limit; NQ, below the limit of quantification; TSNA, tobacco specific nitrosamine.

## Research paper

Cadmium is not as commonly cited in the literature as BaP, which is an important carcinogen in STPs, but consistent with the results obtained from cigarettes in a toxicant analysis,<sup>10</sup> the current analysis showed that the potential risks arising from exposure to cadmium in any of the STPs tested was much higher than that arising from exposure to BaP, a representative carcinogenic PAH. It is pertinent to note that cadmium has also been suggested to be a cause of human pancreatic cancer.<sup>50</sup> Some of the effects of exposure to cadmium in laboratory animals also include renal tubular damage, placental necrosis, structural and functional liver damage, osteomalacia, testicular tumours, anaemia, hypertension, pulmonary oedema, chronic pulmonary emphysema and induced deficiencies of iron, copper and zinc.<sup>51</sup> However, cadmium has not received the level of regulatory scientific attention that it actually deserves compared to the attention paid to other chemical constituents in STPs with regard to potential health risks. To illustrate this further, if a 70 kg adult uses 10 g dry weight equivalent of STPs containing 1000 ng/g of cadmium, this will result in an exposure equivalent to 0.0006 mg/kg body weight/day, which is higher than the reference dose (0.0005 mg/kg) specified for chronic oral ingestion that may result in significant proteinuria.<sup>32</sup> Therefore, the use of snuff among those predisposed to absorbing more cadmium (eg, those with iron deficiency<sup>22</sup> or those using snuff at a higher intensity), may result in 'significant' non-cancer health risks that can be attributable to the cadmium content of snuff. This further shows that the GothiaTek specified performance levels are not acceptable as 'health standards' for the manufacture of STPs.

Fire curing of tobacco in the STPs in the USA, possibly explains the consistent finding of relatively high levels of BaP (a product of combustion) in these products.<sup>14 28 33 34</sup> Nevertheless, the findings of the current study suggest that with the exception of the Indian chewing tobacco and US loose and pouch moist snuff, the BaP levels seen in the other snuff types were at levels that do meet the criteria for an 'acceptable' cancer risk. Nevertheless, BaP is an avoidable constituent of STPs, therefore it should be ultimately eliminated starting with an enforceable regulatory limit as recently suggested by the WHO's tobacco regulation study group.<sup>35</sup>

Considering that STPs are a complex mixture of different compounds, it was not surprising that the relatively low risk levels associated with the levels of BaP and lead in these STPs on their own did not translate to reducing the overall significant composite cancer risk estimate derived for these products. The current analysis nonetheless suggests that the high cancer risk demonstrated in epidemiological studies with the use of the STPs in India<sup>36</sup> may be attributable to other additives or toxicants (eg, in areca nuts) in these products other than those examined in the current analysis. It has been suggested that oral products with high pH levels that also contain areca nut or catechu can produce reactive free radicals that damage DNA and can contribute to oral cancer formation.<sup>37</sup> This observation also supports the view that when one constituent is lowered as may be required by regulatory action, it should not be assumed that this guarantees product safety.

### Study limitations

It should be noted that only a limited number of STPs and STP constituents were included in the calculations in the current study, thus the estimates reported here are to be considered conservative estimates of actual cancer risk. For example, only one product from India was analysed, yet other products may contain higher levels of toxic constituents

than that reported here. Similarly, from the category of PAHs, only BaP was included in our calculations. However, BaP is the most potent of the range of carcinogenic PAHs in STP. Furthermore, we have used an appropriate multiplier to account for the exclusion of other carcinogenic PAHs; therefore the inclusion of other PAHs is not likely to change the results significantly.

It is also possible that the true bioavailability of chemical constituents in STPs may have been underestimated or overestimated, given that bioavailability may differ by compound and that, over time, users may increase the intensity of use and correspondingly increase their exposure to these constituents.<sup>38</sup> Also, as previously indicated, risk estimates derived from the extrapolation from animal exposure data over a relatively short period to human exposure, needs to be interpreted with caution. However, even if an adjustment is made for such potential overestimation as suggested by others,<sup>8</sup> the cancer risk estimates associated with the STPs evaluated in the current study will remain significantly above the minimum acceptable.

It also to be noted that the potential role of endogenous nitrosation that may increase the availability of TSNA during the use of any of the products tested,<sup>39 40</sup> particularly those with pH at 9.5 (a level that promotes endogenous nitrosation) has not been accounted for,<sup>40</sup> nor has the role of portion package materials (pouch) or any other STP constituent in either reducing or enhancing the cancer risk of certain STP brands been included.

These limitations notwithstanding, the model presented in the current analysis is likely to remain a much closer match to epidemiological observations than the similar estimates derived for cigarette constituents.<sup>12</sup> Indeed, a better match is expected between the calculated risk estimates based on exposure assumptions for smokeless tobacco than for smoking, as there is less variability in the constituent transfer and thus the bioavailability of smokeless tobacco constituents as a function of the user's consumption behaviour.

As with other risk assessment reports, the risk estimates in the current study are based on a number of assumptions. However, we believe these were reasonable assumptions and this is not likely to significantly change the conclusions reached in the current analysis given that conservative estimates of constituent transfer were used as opposed to complete transfer (which is normally employed in such risk estimate computations for regulatory purposes).

### What this paper adds

- ▶ It remains unclear how several published reports on smokeless tobacco product (STP) constituents, including the industry-set 'GothiaTek manufacturing standards', might be used in providing scientific basis for STP regulation.
- ▶ Guided by the cancer risk assessment methodology developed by the US Environmental Protection Agency (USEPA), this study demonstrated that, except for the medicinal nicotine gum tested, all the STPs including those meeting the GothiaTek standards carry an 'unacceptable' cancer risk as determined by the benchmark of 'acceptable' risk of  $<10E-6$ . The current study findings highlight the need for regulatory actions to lower specific toxicants in STPs as a matter of priority.



## Conclusions

The risk assessment framework presented in the current analysis provides a useful scientific basis to begin the work of considering the toxicant-specific aspects of health risks of using smokeless tobacco. The results obtained here provide a guide for prioritising chemical hazards in STPs in terms of the actions required to reduce the potential cancer risk from STP use. The current study findings strongly suggest that all smokeless tobacco products currently on the market carry an 'unacceptable' risk for cancer and therefore require regulatory actions. In particular, the findings highlight the urgent need for calling on manufacturers to take steps to reduce the concentrations of these major carcinogens. The findings also demonstrated that there is not enough justification for any regulatory agency to consider the GothiaTek limits suitable as a health risk standard for regulatory purposes.

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## Applying toxicological risk assessment principles to constituents of smokeless tobacco products: implications for product regulation

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