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Commentary

# Use of rodent data for cancer risk assessment of smokeless tobacco in the regulatory context

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ABSTRACT

To support risk management decisions, information from different fields has been integrated in this presentation to provide a realistic quantitative cancer risk assessment of smokeless tobacco.

Smoking among Swedish men is currently below 10%, while about 20% use a special smokeless tobacco (snus) as a substitute for cigarettes. Epidemiological data and molecular biomarkers demonstrate that rodent bioassays with tobacco specific nitrosamines (TSNA) overestimate cancer risk from snus by more than one order of magnitude. The underlying reasons are discussed.

DNA damage constitutes a necessary, although not sufficient prerequisite for cancer initiation. Individuals who have not used tobacco exhibit DNA lesions identical with those induced by TSNA. No increase above this adduct background can be shown from snus, and extensive epidemiological studies in Sweden have failed to demonstrate elevated cancer risks even in long term users.

A "bench mark" for acceptable risk of 1/10(6) derived from rodent data has been suggested when regulating snus. By relating similarly derived estimates for some food contaminants, the implementation even of a limit of 1/10(4) may be unrealistic. The management of smokeless tobacco products has rarely been based on a scientifically sound risk assessment, where attention is given to the outstandingly higher hazards associated with smoking.



## 1. Main objectives

- For assessing the impact on cancer incidence from the use of smokeless tobacco, Swedish males probably represents the most appropriate population for study. In this context the presentation aims, *inter alia*, at describing the characteristics and use of Swedish snus, a special type of low nitrosamine oral tobacco.
- Data from bioassays in rodents exposed to carcinogenic tobacco specific nitrosamines (TSNA) have been extensively used to define human risk. To assess the concordance of such data with Swedish epidemiological findings represents a primary objective.
- The key role of DNA lesions in the carcinogenic action of TSNA is generally acknowledged. Given the fact that information on such biomarkers has been inadequately exploited in epidemiology, this author aims at using such data for quantitative cancer risk assessment of different smokeless tobacco products.
- The ultimate objective is to assess regulatory agencies' risk assessments of smokeless tobacco in relation to actual risk, as well as in the wider context of carcinogenic impurities in foods.

## 2. Background

In Europe, North America, Japan, Australia, and New Zealand tobacco-related disease, notably cancers, is one of the single most important avoidable risk factors, where an average of about 25% percent of male deaths has been attributed to smoking (Ramström and Wikmans, 2014). In the last decades mortality from cigarette smoking is rapidly increasing also in a country like China, where lung cancer has become the leading cause of cancer mortality

*Abbreviations:* CPF, oral carcinogen potency factor; ELCR, excess lifetime cancer risk; ETS, environmental tobacco smoke; HPB, 4-hydroxy-1-(3-pyridyl)-1-butanone; ICH, International Conference on Harmonization; MGMT, $O^6$-methylguanine DNA methyltransferase; 7N-meGua, 7N-methylguanine; NDMA, nitrosodimethylamine; NNAL, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butan-1-ol; NNK, (4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone); NNN, N'-nitrosonornicotine; NPYR, N-nitrosopyrrolidine; O6-mGua, $O^6$-methylguanine; PAH, polycyclic aromatic hydrocarbons; POB, pyridyloxobutyl; TSNA, tobacco specific nitrosamines.

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(Zheng et al., 2016).

*Use of smokeless tobacco in Sweden* - Sweden demonstrates a unique pattern. Thus, the prevalence of cigarette smoking in men aged 15 years and above fell from about 40% in the late 1960s down to 15% in 2002 (Forey et al., 2002; Foulds et al., 2003), and is now about 8%, whereas among women 11% smoke (SPH, 2016a). This can be explained largely by the use of "snus", a special type of smokeless tobacco, traditionally prepared from heat-processed non-fermented tobacco as a replacement for cigarettes. Daily use of snus has decreased somewhat from 22% in 2004 (SPH, 2016a) to 18% in 2015 among adult men, but remained unchanged at approximately 3–4% among women (SPH, 2016a). In northern Sweden exclusive use of snus increased from 18% in 1986/1990 to 27% in 2004 (Stegmayr et al., 2005). This implies that the total nicotine intake of Swedish men has remained fairly stable over the last decades. The Swedish Government Food Authority reported, that the average daily consumption among users was 14 g moist snus, i.e. about 7g on a dry weight basis (Österdahl and Slorach, 1985). Concurrent with the shift from cigarettes to snus, smoking related diseases have been significantly reduced (Foulds et al., 2003; Lee, 2013).

*Previous studies on potential cancer risks associated with snus* - Studies of three cohorts and eight case-control studies on the Scandinavian users of smokeless tobacco have been published. Apart from a study from *Norway* (Bofetta et al., 2005), all were conducted in Sweden, where several extensive and adequately conducted studies failed to demonstrate an elevated cancer risk from snus (Lewin et al., 1998; Schildt et al., 1998; Rosenquist et al., 2005). Based on one of the most comprehensive cancer registries in the world, two large population-based case–referent studies included about 2.5 million person-years at risk in the Stockholm county, the southern healthcare region and in the four northern counties. Whereas the cancer risk in smokers and high consumers of alcohol was strikingly elevated, the outcome was negative with respect to users of snus (Lewin et al., 1998; Schildt et al., 1998) in spite of the fact that the levels of TSNA in Swedish products were considerably higher in previous decades (see below). With respect to oropharyngeal squamous cell carcinoma, poor oral hygiene, inadequate dental status, as well as high-risk human papillomavirus infection have been identified as significant confounding risk factors (Rosenquist, 2005).

Although one study (Roosaar et al., 2008) reported a significantly increased risk for oropharyngeal cancer, six other studies did not, a combined relative risk estimate from the seven studies being 0.97 (CI 0.68–1.37) (Lee, 2011). The never smoker estimate 1.01 (CI 0.71–1.45), based on four studies, is also negative. These results are supported by long-term follow-up of 1115 individuals with "snuff-dippers lesion" (Axéll et al., 1976), which observed no oral cancers at the sites of lesions seen initially. Several recent studies from North America found no increased risk of oropharyngeal cancer associated with snus use (Lee and Hamling, 2009).

The allegation that Swedish snus causes pancreatic cancer has been widely disseminated (IARC, 2007; Song et al., 2010; SPH, 2016b; USFDA, 2017), and is based on two Scandinavian studies. The first of these investigations comprised Norwegian users of "skrå", a tobacco of unknown quality. An increase in pancreatic cancer (RR 1.67; CI 1.2–2.5) was only found for current smokers who reported use of snuff when enlisted for the study, and there was no adequate follow up of tobacco habits. No increase was found for never smokers (RR 0.85, CI 0.24–3.07 (Bofetta et al., 2005). A retrospective study of construction workers by Luo et al. (2007) in contrast reported a relative risk of 2.0 (CI 1.2–3.3) for never smokers, but not for the whole population (RR 0.9, CI 0.7–1.2). The most obvious flaw for these studies was that confounding from alcohol abuse as well as diabetes, both of which are major risk factors, had not been adjusted for. For alcohol an odds ratio of 5.4 (adjusted for smoking) was reported for frequent vs. no use (Heuch et al., 1983). Another possible confounder is the consumption of grilled or barbecued meat. In a study where, in contrast to the aforementioned Scandinavian studies, the possible impact from diabetes and alcohol was taken into account, for high consumers an odds ratio of 2.19 (CI 1.4–3.4) was found (Anderson et al., 2002). Supporting this observation, DNA adducts derived from heterocyclic carcinogenic aromatic amines generated in such meats (PhIP-DNA adducts) were detected in cancer tissues from patients with pancreatic adenocarcinoma, with an associated OR of 3.4 (CI 1.5–7.5, $p = 0.002$) for individuals with a higher level of PhIP-DNA adducts (Zhu et al., 2006).

If we accept the risk estimates claimed for snuff use to be valid, and which are of a magnitude comparable to that for smokers (Fuchs et al., 1996), this would significantly impact the total incidence/mortality in pancreatic cancer for a population where about 20% of the grown up males use snus. This is not so. Although the use of snus is about 5 times lower in women than men, the mortality in pancreatic cancer is virtually the same (IARC, 2013). In a subsequent evaluation conducted by the *International pancreatic Cancer Case-Control Consortium,* and based on 11 case-control studies involving 6056 cases and 11,338 controls, no association between smokeless tobacco and pancreatic cancer was found (Bertuccio et al., 2011), a finding which was confirmed in a second independent meta-analysis (Lee, 2011).

Still, oral moist snuff may induce keratosis, hyperplasia and associated injuries in the oral cavity, which usually are reversible (Andersson and Axéll, 1989). We are currently exploring an approach to suppress these pathological reactions by product modification (Nilsson et al., 2016).

*Carcinogens in smokeless tobacco* - In smokeless tobacco products marketed in North America, Germany, UK (nasal snuff) and Scandinavia, the nitrosamines NNK (*4*-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone) and NNN (N'-nitrosonornicotine) are by far the most important carcinogens. Oral tobacco products used in Africa and Asia have different risk profiles due to very high levels of TSNA (up to 13,000 μg/g), and some products from South East Asia often contain other carcinogens, like areca alkaloid derived carcinogens (Prokopczyk et al., 1987; IARC, 1985a, 2007). In contrast, modern Swedish "snus" has at the present time typically a TSNA content of 1.6 μg/g dry weight (Österdahl et al., 2004; Rutqvist et al., 2011).

Tobacco also contains anti-mutagenic and anti-carcinogenic compounds. When NNK and NNN were swabbed in the oral cavity of the rat with and without an extract from US oral tobacco, the yield of oral tumors was drastically reduced in presence of the extract (Hecht et al., 1986).

Rodent bioassays have revealed the carcinogenic potential of NNK and NNN as well as provided insight of the metabolism and molecular mechanisms involved (reviewed by Hecht, 1998; Hecht et al., 2016). The metabolic transformations of NNK mediated by CYP450 enzymes generate short lived diazo-hydroxides which react with DNA to produce mainly *N7*-methylguanine, $O^6$-methylguanine (O6-mGua) and smaller amounts of $O^4$-methylthymine. Reduction of the carbonyl group of NNK leads to the formation of carcinogenic 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butan-1-ol (NNAL), which together with its glucuronides are useful biomarkers of NNK exposure. By a second pathway pyridylhydroxybutyl and pyridyloxobutyl (POB) adducts are introduced in DNA and proteins like hemoglobin. Mild acid or alkaline hydrolysis of these adducts releases 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) which can be determined e.g. by GC/MS after derivatization. Oxidative metabolism of NNN produces POB adducts, but no methylated DNA adducts.