October 27, 2017

Gerard J. Roerty, Jr.
Vice President, General Counsel & Secretary
SWEDISH MATCH USA, INC.
Two James Center
1021 East Cary Street, Suite 1600
Richmond, VA 23219
Phone: 804-787-5100
e-mail: Gerry.Roerty@Swedishmatch.com

FOOD AND DRUG ADMINISTRATION
CENTER FOR TOBACCO PRODUCTS
Document Control Center
Building 71, Room G335
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

SUBJECT: PERIODIC REPORT for STN PM0000012

Dear Sir or Madam:

Swedish Match USA, Inc. ("Swedish Match" or "we") writes in regard to FDA's Marketing Order PM0000012 for General Portion Original Large ("PM0000012"), included below as **Attachment A.2017-PM0000012**.

Per requirements under section 910(f) of the FD&C Act, we are submitting a Postmarket Annual Report ("Report") for PM0000012 beginning October 2016 so that FDA may determine whether continued marketing of the tobacco product is appropriate for the protection of public health or whether there are or may be grounds for withdrawing or temporarily suspending the Marketing Order.

Periodic Report for the following tobacco product:

STN	PM0000012
Tobacco Product Name	General Portion Original Large
Applicant	Swedish Match
Date of Report	10/31/2017
Reporting Period	10/1/2016 – 9/30/2017
Marketing Order Status USA	In market date is 4/6/2016
Marketing Status Outside USA	Commercially distributed in Sweden. No sales in EU member states. All other sales as governing law permits.

We set forth below our response to each Agency request enumerated in the Marketing Order. As directed by FDA, we are providing this single submission in response to the Marketing Order.

Swedish Match submits this Report with the confidence that continued marketing of the tobacco product is appropriate for the protection of public health.

Swedish Match submits that this submission and the information we are supplying in connection with this Report, are trade secret, proprietary information that is protected under state and federal law from public disclosure. This information should therefore be handled in accordance with the security procedures adopted by FDA in connection with enforcement of the FD&C Act.

If further information is required, please contact us.

Sincerely yours,

(b) (6)

Gerard J. Roerty, Jr.
Vice President, General Counsel & Secretary

Document attachments:

Attachment A.2017-PM0000012 – Marketing Order PM0000012
Attachment 2A1.2017-PM0000012.pptx – General Snus PMTA Post-Surveillance Market Research *(in Microsoft PowerPoint format)*
Attachment 2A2.2017-PM0000012 – SM Market Tracker (FY16-User Groups) *(in Microsoft Excel format)*
Attachment 2A3.2017-PM0000012 – SM Market Tracker (FY15 vs. FY16) *(in Microsoft Excel format)*
Attachment 2B.2017-PM0000012 – Full Text Articles of Relevant New Publications
Attachment 2C.2017-PM0000012 – Summary of Consumer Complaints (Adverse Experiences)
Attachment 2D.2017-PM0000012 – Summary of Sales and Distribution Data
Attachment 3A.2017-PM0000012 – Certification Statement
Attachment 4A.2017-PM0000012 – Summary of Manufacturing Deviations
Attachment 5A.2017-PM0000012 – Full Color Copies of Advertising

Swedish Match Reply to section III. Periodic Reporting Information Request:

The information requested in the Marketing Order, Periodic Reporting, is reproduced below in bold type followed by Swedish Match's reply.

III.1. A single submission with a cover letter that includes the following text in your subject line PERIOD REPORT FOR STN: PM0000012. The cover letter should include the STN and corresponding name, applicant name, date of report, reporting period, and marketing order status outside the United States.

Swedish Match Reply to III.1. for PM0000012:

Please see cover letter above.

III.2. A summary of how the tobacco product continues to be appropriate for the protection of the public health which includes:

a. A status report of ongoing studies and a summary of completed studies about the tobacco product conducted by, or on behalf of, the applicant;

b. A summary of significant findings on publications not previously reported and include full articles. Any new scientific data (published or otherwise) should also be reported on the likelihood of product use by current users of tobacco products within the same tobacco product category, current users of tobacco products in other tobacco product categories, former users of any tobacco product, and youth and young adults;

c. A summary of adverse experiences with this tobacco product reported to you, providing a listing and analysis (accompanied by a statement of any changes to the reference risk information and a summary of important risks, including the nature, frequency, and potential risk factors) of all adverse experiences including those serious and unexpected adverse experiences reported previously.

d. A summary of sales and distribution of the tobacco product: Total U.S. sales reported in dollars, units, and volume with breakdowns by US census region, major retail markets, and channels in which the product is sold (e.g., convenience stores, food and drug markets, big box retailers, internet/online sales, tobacco specialty shops);

e. Data on current product users. Data should be collected about new users, current users, those who have switched tobacco products, and multiple product users. The results should be broken down by key demographic variables including age, gender, and race/ethnicity. Also, any change in the intended target market for the product should be reported. The data described above may include sales data and post-marketing analysis.

Swedish Match Reply to III.2.a. for PM0000012:

The following is included in this Report as a summary of research relative to General snus:

1. A PowerPoint deck that highlights some key data points regarding General Snus. **(See Attachment 2A1.2017-PM0000012.)** The slides break out into two primary sections:
    a. Health perceptions. Information about how people see risks of snus in comparison to other products, primarily cigarettes.
    b. Usage information. Information about incidence of snus usage in the U.S., along with a couple of points regarding supplemental usage and the sophistication of the General user.
2. (b) (4) (b) (4) Included in this annual report is:
    a. Cross-tabulated data from the full year 2016 market tracker. The banner table is divided by tobacco user groups. **(See Attachment 2A2.2017-PM0000012.)**
    b. Cross-tabulated data comparing FY 2016 to FY 2015 (market tracker). Again, tobacco user groups are used to divide the metrics (along with year). **(See Attachment 2A3.2017-PM0000012.)**

(b) (4)

Swedish Match Reply to III.2.b. for PM0000012:

Swedish Match is supplying a summary of publications not previously reported (see Table 2b-1 below). Full text articles are available in **Attachment 2B.2017-PM0000012**. (b) (4)
(b) (4)

120 **Table 2b-1. Summary of publications.**

Item #	Publication Citation
1.	Araghi M., Galanti, M.R., Lundberg, M., Lager, A., Engström, G., Alfredsson, L., Knutsson, A., Norberg, M., Sund, M., Wennberg., Lagerros, Y.T., Bellocco, R., Pedersen., N.L., Östergren, P., Magnusson, C. (2017): Smokeless tobacco (snus) use and colorectal cancer incidence and survival: Results from nine pooled cohorts. Scandinavian Journal of Public Health (1-8). • Focus is on potential link between snus use and pancreatic cancer: 424,152 male participants (9 cohorts, including the Swedish Construction Workers Cohort mentioned below) in Swedish Collaboration on Health Effects of Snus Use were followed up for risk of pancreatic cancer. • Compared to never snus use, current snus use was not associated with risk of pancreatic cancer (HR 0.96, 95% CI 0.83 – 1.11) after adjustment for smoking. • Tobacco smoke constituents associated with combustion (other than nicotine or its metabolites) may explain the increased risk of pancreatic cancer from smoking.
2.	Byhamre, M.L., Gustafsson, P.E., Jansson, J.H., Wennberg, M., Hammarstrom, A., Wennberg, P. (2017): Snus use during the life-course and risk of the metabolic syndrome and its components. Scandinavian Journal of Public Health, 1-8 (online). • Authors investigate the association between life-course exposure to snus and prevalence of the metabolic syndrome and its components in adulthood: 880 participants in a population-based cohort in Northern Sweden. • No association noted between exclusive snus use (at ages 16, 21, 30, and 43) and the presence of metabolic syndrome at age 43. • Though snus use was associated with central obesity, raised triglycerides and impaired fasting glucose/diabetes mellitus type 2, adjustments for smoking or dual use of cigarettes/snus indicate a possible association (relative to exclusive snus use).
3.	Carlsson, S., Andersson, T., Araghi, M., Galanti, R., Lager, A., Lundberg, M., Nilsson, P., Norberg, M., Pederson, N.L., Trolle-Lagerros, Y., Magnusson, C. (2017): Smokeless tobacco (snus) is associated with an increased risk of type 2 diabetes: results from five pooled cohorts. Journal of Internal Medicine (doi: 10.1111/joim.12592). • Authors explore possible association between snus use and development of Type 2 Diabetes through participants in five cohorts (n = 54531 never smokers). • Relative to never users, snus user was associated with Type 2 Diabetes (HR 1.15, 95% CI: 1.00–1.32). The HR increased with each additional box of snus consumed per week with the heaviest users (over 7 boxes a week) carrying the highest risk (1.68). • The authors cite the possibility that snus users may have started smoking post-baseline tobacco use assessment and also cite self-reporting of confounding factors as both limitations of the study.

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Item #	Publication Citation
4.	Carpenter, M.J., Wahlquist, A.E., Burris, J.L., Gray, K.M., Garrett-Mayer, E., Cummings, K.M., Alberg, A.J. (2017): Snus undermines quit attempts but not abstinence: a randomised clinical trial among US smokers, Tobacco Control, 26: 202-209. • Longitudinal study (n = 1236 cigarette smokers) assessed self-reported quit attempts while using snus over course of one year • Compared to control participants, smokers in the snus group were less likely to make any quit attempt (RR=0.83; 95% CI 0.70 to 1.00) or any day long quit attempt (RR=0.77; 95% CI 0.63 to 0.95). • Methods used U.S. snus product (Camel Snus Winterchill or Robust) – because this is a different product category than SM's Swedish Snus, this paper is not relevant for our product.
5.[1]	Choi et al. (2017) ("Trends in Awareness, Use of, and Beliefs About Electronic Cigarette and Snus Among a Longitudinal Cohort of US Midwest Young Adults") and Choi et al. (2017) (Sources and Number of Coupons for Cigarettes and Snus Received by a Cohort of Young Adults) both not relevant as they deal with perceptions of U.S. snus from a too early time frame.
6.	Greene, T., Rogers, S., Franzen, A. & Gentry, R. (2016): A critical review of the literature to conduct a toxicity assessment for oral exposure to methyl salicylate, Critical Reviews in Toxicology, 47: 98-120. • Critical assessment of proposed or current ADIs (Acceptable Daily Intakes) established by worldwide health organizations regarding methyl salicylate. • Review suggests that existing ADIs/NOAELs/etc. are too low with problematic sources of information – the authors propose an ADI of 11/mg/kg/day. • Snus is not specifically mentioned in the discussion.
7.	Gudnadottir et al. (2017) ***or*** Gudnadottir, A. Y., Olafsdottir, I.S., Middelveld, R., Ekerljung L., Forsberg, B., Franklin K., Lindberg, E., Janson, C. (2017): An investigation on the use of snus and its association with respiratory and sleep-related symptoms: a cross-sectional population study. BMJ Open (7e015486. doi: 10.1136/bmjopen-2016-015486). • Focus is on any potential effects of snus use on asthma, respiratory symptoms and sleep-related problems: cross-sectional study of 26,697 Swedish participants responding to a postal questionnaire sent to allergy patients. • The current use of snus in never-smokers was associated with an increased risk of asthma (OR 1.51 95% CI 1.28 – 1.77), asthmatic symptoms, chronic bronchitis and chronic rhinosinusitis relative to ex-snus users. • The authors suggest that the lack of a longitudinal study and the smaller than expected response would be "beneficial in order to indicate whether causation is present between snus use and these symptoms or only a connection" (pg. 6).

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[1] Assessed for relevance: not relevant. Thus, not attached.

Item #	Publication Citation
8.	Gunnerbeck, A. (2017): Thesis - Prenatal nicotine exposure and effects on the health of the newborn, Karolinska Institutet (http://hdl.handle.net/10616/45575). • Focus is on whether nicotine (relative to components of combustion) cause adverse effects on the health of the newborn: four studies based on self-reported information of snuff use from the Swedish Medical Birth Register. • Results indicated increased risk of neonatal apnea, oral cleft malformations, and extremely preterm birth. • No forms of tobacco or nicotine are safe to use in pregnancy .
9.	Lie, T.M., Bomme, M., Hveem, K., Hanson, J.M., & Ness-Jensen, E. (2017): Snus and risk of gastroesophageal reflux. A population-based case-control study: the HUNT study, Scandinavian Journal of Gastroenterology, 52:2, 193-198, DOI: 10.1080/00365521.2016.1245775. • Study assesses whether snus increases the risk of gastroesophageal reflux symptoms (GERS): uses self-reported results from population based study of all residents of Nord-Trøndelag County, Norway (2006–2009). • Relative to never users of snus, daily snus users had a reduced risk of GERS (OR 0.77, 95% CI 0.64–0.93); previous snus users and those using <2 boxes of snus/month had an increased risk (OR 1.20, 95% CI 1.00–1.46 and OR 1.41, 95% CI 1.02–1.96, respectively). • Authors conclude that the overall result of the study indicates that daily snus use seems to protect against GERS (while some subgroups had an increased risk) – they suggest reverse causality as an explanation: individuals experiencing GERS while using snus quit using snus while daily snus users are individuals who may not be prone to GERS. • Authors note previous article that indicates a protective effect of snus regarding GERS (Kalixanda study) with which their study contrasts.
10.	Lund, K.E., Vedoy, T.F., & Bauld, L. (2016): Do never smokers make up an increasing share of snus users as cigarette smoking declines? Changes in smoking status among male snus users in Norway 2003-15: Addiction, 112: 2, 340-348. • Examination of how relative size of male ever snus users varies over time as compared to other tobacco form users: 2067 Norwegian males from cross-sectional national survey. • Attempt to quantify the transformation of a population from smoking to snus use (which exists much lower on the continuum of risk in terms of tobacco specific nitrosomines). • Authors concluded that in Norway, as smoking declines rapidly and never-smokers increases, the majority of snus users remain former smokers (e.g. they are not never-users of tobacco products or dual users of cigarettes/snus).

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Item #	Publication Citation
11.	Meier, E., Vogel, R.I., Carmella, S., Paiano, V., Hecht, S.S., Hatsukami, D.K. (2017): Polycyclic Aramoatic Hydrocarbon Biomarker Levels among Smokers who Switch to Oral Nicotine, Tobacco Regulatory Science, 3(2): 204-209 (DOI: https://doi.org/10.18001/TRS.3.2.8). • Study assessed levels of the urinary polycyclic aromatic hydrocarbon (PAH) biomarker *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) in cigarette smokers after switching to snus. • Cigarette smokers (N = 308, 46.4% women) were randomized to receive snus or nicotine gum and were instructed to use only their assigned product for 12 weeks, then urine samples for analysis of PheT were collected at baseline and 4 weeks after receiving the test product. • While all participants saw a reduction of the biomarker, those who completely switched to gum or snus saw the largest reduction.
12.	Nordenstam, F., Lundell, B., Cohen, G., Tessma, M.K., Raaschou, P., Wickstrom, R. (2017): Prenatal Exposure to Snus Alters Heart Rate Variability in the Infant, Nicotine & Tobacco Research, 797-803 (doi: 10.1093/ntr/ntx035). • Study assesses cotinine concentrations in infants (n = 56, from mothers who used snus or cigarettes and did not use tobacco) 2 months after birth to assess whether nicotine affected autonomic cardiac regulation in the infant, as measured by heart rate variability (HRV) and the low frequency and high frequency ratio (LF/HF ratio). • Results indicated that LF/HF ratio was higher in snus (mean 3.31; 95% CI 2.78–3.83) and smoke (3.51;2.54–4.47) compared to controls (2.15; 1.76–2.54, p = .002) and that there was no difference in results between snus or cigarette source (e.g. nicotine was common causal agent). • Authors concluded that early prenatal exposure to snus impacts autonomic cardiac regulation ("a fetal "re-programing" of the developing autonomic nervous system")(pg. 1).
13.[2]	O'Connor et al. (2017) ***or*** O'Connor, R.J., Lindgren, B.R., Schneller, L.M., Shields, P.G., and Hatsukami, D.K. (2017). • Focus is on U.S. snus products (Camel Snus Winterchill and Robust) – not relevant for our product.
14.	Rasouli, B., Andersson, T., Carlsson, P.O., Grill, V., Groop, L., Martinell, M., Midthjell, K., Storm, P., Tuomi, T., Carlsson, S. (2017): Use of Swedish smokeless tobacco (snus) and the risk of Type 2 diabetes and latent autoimmune diabetes of adulthood (LADA), Diabetic Medicine (DOI: 10.1111/dme.13179). • Authors explored any possible links between snus use and the risk of Type 2 Diabetes or Latent Autoimmune Diabetes of Adulthood (LADA) using Swedish population and cohort data (from multiple sources). • No association between snus use and Type 2 diabetes or LADA was seen in the Swedish data (Type 2 diabetes: OR 1.00 95% CI, 0.47 to 2.11; LADA: 1.01 95% CI, 0.45 to 2.29). • Authors suggest that any link between diabetes and tobacco use may not result from nicotine but other constituents in cigarette smoke.

[2] Assessed for relevance: not relevant. Thus, not attached.

Item #	Publication Citation
15.	Saebo, G. (2017): Cigarettes, snus and status: differences in lifestyle of different tobacco user groups in Norway, Health Sociology Review, 26:2, 175-189 (DOI: 10.1080/14461242.2016.1197043). • Discusses status differences (informed by Bourdieu, 1984) as reflected in Norwegian consumption of varying tobacco products: data from a qualitative 2007 questionnaire of representative sample of population age 15 and up. • Concludes that different tobacco products are consumed as part of different lifestyles in Norway and different tobacco products are judged in relation to the social contexts and lifestyles in which they occur. • Thinking about different "types" of users of the same product (e.g. daily users, occasional users, social users, etc.) means considering them in contrast to each other, not as a single unit. • Snus users are characterized by "signs of youthfulness" (e.g. "technology competence and an active social life") (see pg. 184). • Snus use can be seen as both youthful and as a boundary exploring zone existing between smoking of all forms and non-use of tobacco products.
16.[3]	Schneller, L.M., Lindgren, B.R., Shields, P.G., Hatsukami, D.K., O'Connor, R.J. (2017). • Focus is on U.S. snus products (Camel Snus Winterchill and Robust) – not relevant for our product.
17.[4]	Stein, J.S., Wilson A.G., Koffarnus, M.N., Judd, M.C., Bickel, W.K. (2016): Naturalistic assessment of demand for cigarettes, snus and nicotine gum, Psychopharmacology (DOI 10.1007/s00213-016-4455-y). • Behavioral economic measurement: sets up a faux purchasing experiment whereby cigarette smokers can purchase nicotine gum or snus at variable levels. • Methods used U.S. snus product (Camel Snus Winterchill) – because this is a different product category than SM's Swedish Snus, this paper is not relevant for our product. • Article not relevant to questions posed in PMTA report.

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[3] Assessed for relevance: not relevant. Thus, not attached.

[4] Ibid.

Item #	Publication Citation
18.	The Snus Commission's third report – May 2017 (2017). • Used WHO compilation of statistics surrounding tobacco-related mortality (males, age 30+) at the EU level: Sweden has the lowest tobacco-related mortality rate of all EU countries relative to its population size (despite daily tobacco consumption being comparable). • 355,000 lives per year could have been saved if snus had not been banned by the EU and if the other EU countries had matched Sweden's tobacco-related mortality rate. • The Commission conclusions most relevant to this report: o Due to the positive role snus plays in terms of Swedish public health, authors propose that European politicians reconsider the ban on snus in the EU. o Because snus has a significantly lower impact on someone's health than cigarettes, the Swedish government should promote snus within the EU and EU citizens should be offered accurate information regarding the health effects of snus.
19.	Scheffels, J. and Lund, I. (2017): Cute as candy: a qualitative study of perceptions of snus branding and package design among youth in Norway. BMJ Open 2017; 7e012837. doi: 10.1136/bmjopen02016-012837. • Focus is on how youth in Norway perceive snus branding and package design and the role of this marketing material on perceptions of appeal and harm of snus. • 6 focus groups (4-7 participants) for 15-17 year olds were shown snus packaging. o Identity development (e.g. masculinity) and belonging could be confirmed through use of snus and display of packaging on the self. o Boys identified dark General packaging as more harmful vs. lighter colors. o Packaging shape matters: girls liked feminine colors and designs that made the packaging appear more like candy or make-up. • Branding and packaging work like advertising and can communicate information about product strength and harm (lighter colors vs. darker colors). • Authors promote a standardization of colors and package design to reduce potential appeal to youth and prevent dissemination of the above noted information.
20.[5]	Timberlake, D.S., Nikitin, D., Johnson, N.J. and Altekruse, S.F. (2017): A longitudinal study of smokeless tobacco use and mortality in the United States, International Journal of Cancer, 2017 Jul 15; 141(2):264-270. doi: 10.1002/ijc.30736. • 349,282 population of smokeless tobacco (SLT) users from National Longitudinal Mortality Study who completed a single Tobacco Use Supplement to the Current Population Survey between the years 1985 – 2011. • Relative to never tobacco users, current SLT users did not have elevated mortality risks from all cancers combined but did have higher mortality risk for coronary heart disease. • Not relevant: Timberlake specifically says the results do not pertain to snus (pg. 14), but can be informative for future MRTP applications similar to Swedish Match's application, but related to moist snuff tobacco.

[5] Assessed for relevance: not relevant. Thus, not attached.

Item #	Publication Citation
21.	Wilson, K.M., Markt, S.C., Fang F., Nordenvall, C., Rider, J.R., Ye, W., Adami, H., Stattin, P., Nyrèn, O. and Mucci, L.A. (2016): Snus use, smoking and survival among prostate cancer patients, International Journal of Cancer, 2016 Dec 15; 139(12):2753-2759. doi: 10.1002/ijc.30411. • 336,381 Swedish construction workers were assessed in a cohort for prostate cancer mortality and were classified as "never users of any tobacco", "ever users of snus only", "ever smokers only" and "ever users of both snus and smokers" based on their initial visit in the 1970s. • Compared to never users of tobacco, exclusive snus users had increased risks for prostate cancer mortality (HR 1.24, 95% CI: 1.03 – 1.49) and total mortality (HR 1.19, 95% CI: 1.04 – 1.37). • As the researchers noted in their limitations section, the results are suggestive, but a single assessment of tobacco use recorded an average of 20 years before cancer diagnosis leaves room for "substantial misclassification of tobacco use closer to and after the cancer diagnosis" (pg. 2757) and, further, prevents the answering of additional questions of public health, such as whether quitting is eventually associated with lower prostate cancer mortality.
22.	Yang, F., Pedersen, N.L., Ye, W., Liu, Z., Norberg, M., Forsgren, L., Lagerros, Y.T., Bellocco, R., Alfredsson, L., Knutsson, A., Jansson, J., Wennberg, P., Galanti M.R., Lager, A.C.J., Araghi, M., Lundberg, M., Magnusson, C., and Wirdefeldt, K. (2016): Moist smokeless tobacco (Snus) use and risk of Parkinson's disease, International Journal of Epidemiology, Volume 46, Issue 3, 1 June 2017, Pages 872-880, https://doi.org/10.1093/ije/dyw294. • Focus is on potential link between tobacco use and Parkinson's disease: 348,601 men from seven cohorts were followed over 16.1 years. • Ever-snus users (non-smokers) had a 60% lower Parkinson's disease risk relative to never-snus users: HR 0.41, 95% CI 0.28 – 0.61. ○ Current snus users: HR 0.38, 95% CI 0.23 – 0.63 ○ Moderate-heavy users: HR 0.41, 95% CI 0.19 – 0.90 ○ Long-term snus users: HR 0.44, 95% CI 0.24 – 0.83 • Nicotine or other components of tobacco leaves may influence the development of Parkinson's disease.

Swedish Match Reply to III.2.c. for PM0000012:

Swedish Match did not receive any reports of serious or unexpected adverse experiences, as defined on page 3 of the Marketing Order for PM0000012, relative to this tobacco product for the reporting period 10/1/2016 through 9/30/2017. There have been no changes to the reference risk information as was described in the PMTA.

We are supplying a summary of consumer complaints (all other reported adverse experiences) relative to this tobacco product for the reporting period 10/1/2016 through 9/30/2017 in **Attachment 2C.2017-PM0000012**.

Swedish Match Reply to III.2.d. for PM0000012:

Swedish Match is supplying a summary of sales and distribution data for the reporting period 10/1/2016 through 9/30/2017 in **Attachment 2D.2017-PM0000012**. This information includes total U.S. sales reported in dollars and units (i.e. number of cans), and volume (i.e. net weight multiplied by units) with breakdowns by US census region and retail markets and channels in which the product is sold (e.g. convenience stores, food and drug markets, big box retailers, internet/online sales, tobacco specialty shops).

Swedish Match Reply to III.2.e. for PM0000012:

Other than the research (provided above at III.2.a) and sales and distribution data (provided above at III.2.d.) supplied in attachments referenced above, there is no current product user data for the reporting period 10/1/2016 through 9/30/2017 for this product. Likewise, there has been no change in the intended target market for this product for the reporting period 10/1/2016 through 9/30/2017.

III.3. A description of each change made to the manufacturing, facilities or controls during the reporting period, including:

a. A comparison of each change to what was described in the PMTA;

b. The rationale for making each change; and

c. A certification that the reported change did not result in any modification (including a change in design, any component, any part, or any constituent, including a smoke constituent, or in the content, delivery or form of nicotine, or any other additive or ingredient) of the tobacco product; the basis for concluding that each change did not result in any modification to the final product.

Swedish Match Reply to III.3. for PM0000012:

Swedish Match made a change to the manufacturing for this product during the reporting period 10/1/16 through 9/30/16. Specifically, the addition of propylene glycol added during packing (snus packing) was stopped and the addition of propylene glycol at an earlier stage of processing (snus processing) was started. The reported change is indicated in RED on figure 3.1 below. The quantity of propylene glycol in the final product (34mg/pouch) is the same as was described in the PMTA (PM0000012). Based upon the forgoing and chemical, sensory evaluations and full length storage (real time shelf life) study, Swedish Match determines the final tobacco product has not been modified.

The impetus for making the reported change was to reduce waste in the process. The resulting product has the same characteristics as were described in the PMTA i.e., has the same per weight composition, design features, heating source, and all other features. Except for the reported change, the product is otherwise identical to what was described in the PMTA.

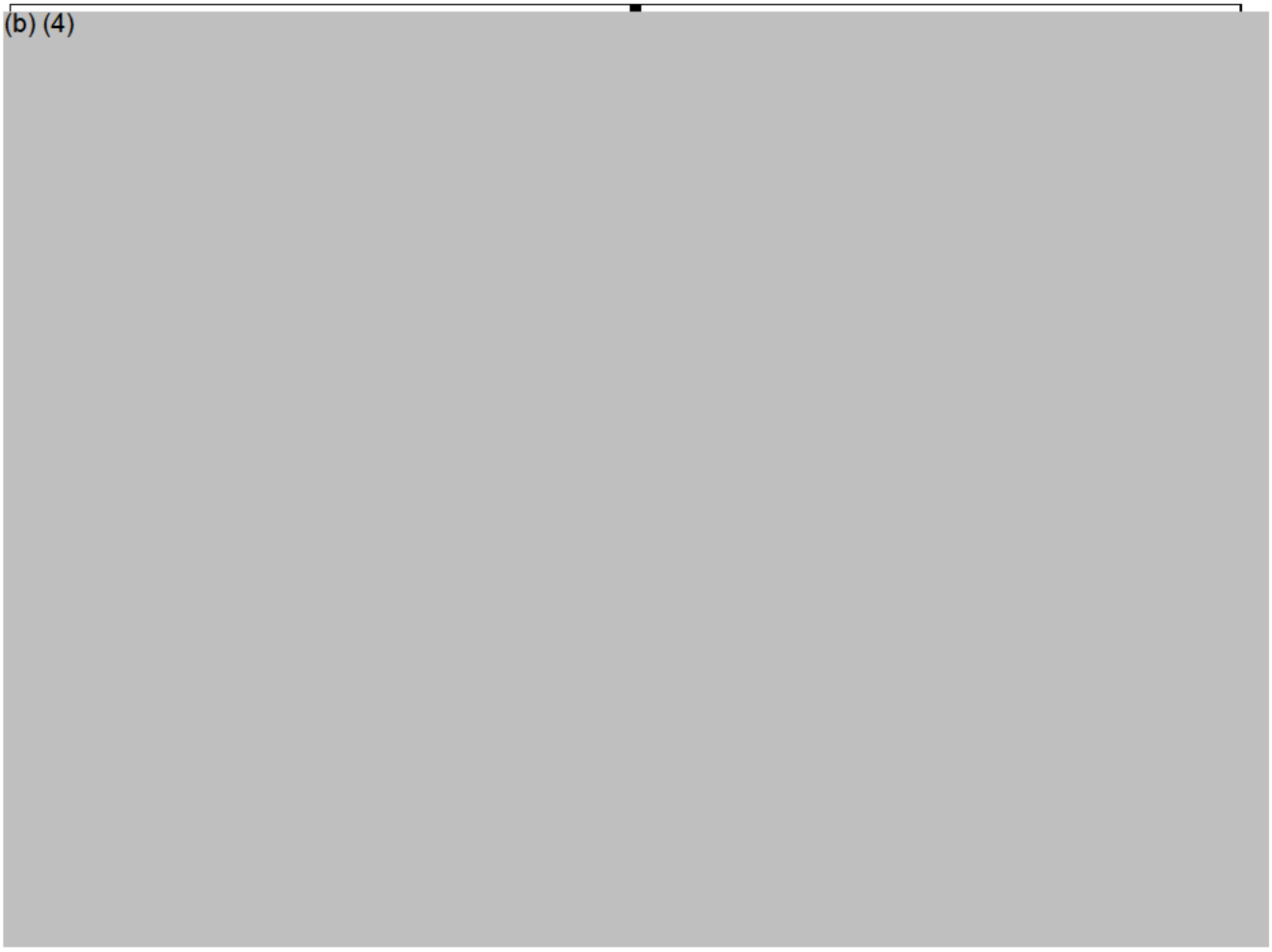
The reported change does not result in any modification (including a change in design, any component, any part, any constituent, or in the content, delivery or form of nicotine, or any other additive or ingredient) of the final tobacco product.

The reported change to the existing product does not result in a "new tobacco product" and therefore does not give rise to the FD&C Act's premarket approval provisions. In this circumstance a PMTA, SE or Exemption report is not necessary since the resulting product is not a new tobacco product.

A certification statement is attached at **Attachment 3A.2017-PM0000012.**

Figure 3.1. Fig 1. Snus Processing & Fig. 2 Snus packing

(b) (4)



III.4. A summary of all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution and indicate a deviation that may affect the characteristics of the final product.

Swedish Match Reply to III.4. for PM0000012:

Swedish Match is supplying a summary of all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution and indicated any deviation(s) that may affect the characteristics of the final product for the reporting period 10/1/2016 through 9/30/2017 in **Attachment 4A.2017-PM0000012**. This product had no manufacturing deviations for the reporting period 10/1/2016 through 9/30/2017.

III.5. Full-color copies of all advertising for the tobacco product that has not been previously submitted, along with the original date the advertisements were first disseminated and the date the advertisements were discontinued; and

Swedish Match Reply to III.5. for PM0000012:

Swedish Match is supplying full-color copies of all advertising for this tobacco product, for the reporting period 10/1/2016 through 9/30/2017, in **Attachment 5A.2017-PM0000012**. First disseminated and discontinuation dates are indicated next to the advertisement. Advertisements are still in market unless a discontinuation date is indicated.

III.6. In all annual reports, include a description of any or all labeling changes and submit revised full color final printed labeling.

a. The labeling should include all the panels, be presented in the actual size and color with legible text.

b. For the first annual report only, submit all final printed labeling (actual labeling for each required warning distributed with the product); include labels, inserts/onserts, instructions, and other accompanying information or materials for this product.

Swedish Match Reply to III.6. for PM0000012:

There have been no changes to any labels for this product. Thus, no revised full color final printed labeling is included (as required in II.6.a., above).

As this is the second annual PMTA Report for this product, we are not required to submit actual physical labels for this product (as required in II.6.b., above).

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Center for Tobacco Products
10903 New Hampshire Avenue
Silver Spring, MD 20993

MARKETING ORDER

Swedish Match North America, Inc.
Attention: Gerard Roerty, Jr., Vice President, General Counsel & Secretary
Two James Center
1021 East Cary Street, Suite 1600
Richmond, VA 23219
via Certified Mail

FDA Submission Tracking Number (STN): PM0000012

Dear Mr. Roerty:

The Food and Drug Administration (FDA) completed the review of your Premarket Tobacco Product Application (PMTA) submitted under section 910(b) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), for the following tobacco product:

Applicant:	Swedish Match North America, Inc.
Tobacco Product Name:[1]	General Portion Original Large
Tobacco Product Category:	Smokeless Tobacco
Tobacco Product Sub-Category:	Portioned Snus
Package Type:	Plastic Can
Package Quantity:	24.0 g
Characterizing Flavor:	None
Portion Count:	24 pouches
Portion Mass:	1000 mg
Portion Length:	33 mm
Portion Width:	18 mm
Portion Thickness:	
Tobacco Cut Size:[2]	(b) (4)

Based on our review of your PMTA, we find permitting the new tobacco product specified above to be marketed is appropriate for the protection of public health, and that you have met the other requirements of section 910(c) of the FD&C Act. Under the provisions of section 910, you may introduce or deliver for introduction into interstate commerce the new tobacco product specified above with the enclosed labeling.

[1] Brand/sub-brand or other commercial name used in commercial distribution
[2] The applicant provided fraction scale weight buckets to characterize the tobacco cut size. Therefore, the tobacco cut size cannot be represented with a single value and corresponding range limit.

RECORD RETENTION

Under section 910(f) of the FD&C Act, we are requiring in this order that you retain the records listed below for a period of not less than 4 years from the date of distribution of the last batch of the new tobacco product specified above. These records must be legible, in the English language, and available for inspection and copying by officers or employees duly designated by the Secretary, upon request:

- PMTA submitted prior to product order
- Postmarket reports/postmarket status reports submitted to FDA, including adverse experiences and all relevant documentation associated with the experience
- Correspondence with FDA pertaining to authorized product
- Nonclinical or clinical study documentation including:
    - study protocols (including statistical analysis plan);
    - amendments showing the dates and reasons for each protocol revision;
    - Institutional Review Board (IRB) or Independent Ethics Committee (IEC) approvals;
    - Informed consent forms;
    - Correspondence with study monitors/investigators/contract research organizations/sponsors/IRB/IEC;
    - Investigator financial disclosure statements;
    - Progress reports;
    - Monitoring reports;
    - Adverse experience reports;
    - Case report forms/subject diaries/medical records/laboratory reports;
    - Subject data line listings/observations records;
    - Test article accountability records;
    - Study results/protocol summaries/study reports; and
    - Certifications and amendments to certifications.
- Records pertaining to the manufacture, in process and release testing, process (including any changes to the process, facility, or controls), packaging, and storage of product
- Records pertaining to the sale, marketing, distribution, or other disposition of the product
- Specimens of all labeling, labels, inserts/onserts, instru.ctions, and other accompanying information
- Hazard analysis

POSTMARKET REPORTS

I. Serious and Unexpected Adverse Experiences Reporting

Under section 910(f) of the FD&C Act, we are requiring in this order that you report to the FDA all adverse experiences that are both serious and unexpected and your analysis of the association between the adverse experience and the tobacco product **within 15 calendar days** after the report is received by you. These experiences may become known to you through a response to a customer complaint, request, or suggestion made as a result of an adverse experience, tobacco product defect, or failure reported to you; or identified in the literature or media. Your information should be submitted with a cover letter that includes the following text in the subject line: **SERIOUS UNEXPECTED ADVERSE EXPERIENCE REPORT for STN PM0000012**.

For purposes of reporting under this order, serious adverse experience means an adverse experience that results in any of the following outcomes:

- Death;
- A life-threatening adverse event;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- A congenital anomaly/birth defect; or
- Any other adverse experience that, based upon appropriate medical judgment, may jeopardize the health of a person and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

For purposes of reporting under this order, unexpected adverse experience means an adverse experience occurring in one or more persons in which the nature, severity, or frequency of the experience is not consistent with:

- The known or foreseeable risks associated with the use or exposure to the tobacco product as described in the PMTA and other relevant sources of information, such as postmarket reports and studies;
- The expected natural progression of any underlying disease, disorder, or condition of the persons(s) experiencing the adverse experience and the person's predisposing risk factor profile for the adverse experience; or
- The results of nonclinical laboratory studies.

II. Manufacturing Deviations

You should promptly investigate all manufacturing deviations, including but not limited to those associated with processing, testing, packing, labeling, storage, holding and distribution. For products that have been distributed, if the deviation may negatively impact public health, you must promptly identify and report that deviation to the Center for Tobacco Products. See instructions below for submitting your regulatory correspondence.

III. Periodic Reporting

Under section 910(f) of the FD&C Act, we are requiring in this order that you submit, on an annual basis, beginning October 2016, unless otherwise notified, the following information in a postmarketing annual report to help FDA determine whether continued marketing of your tobacco product is appropriate for the protection of public health or whether there are or may be grounds for withdrawing or temporarily suspending such order. For the 12- month reporting period, the report must include:

1. A single submission with a cover letter that includes the following text in your subject line: **PERIODIC REPORT for STN PM0000012**. The cover letter should include the STN and corresponding tobacco product name, applicant name, date of report, reporting period, and marketing order status outside the United States.
2. A summary of how the tobacco product continues to be appropriate for the protection of the public health which includes:
    a. A status report of ongoing studies and a summary of completed studies about the tobacco product conducted by, or on behalf of, the applicant;

   b. A summary of significant findings on publications not previously reported and include full articles. Any new scientific data (published or otherwise) should also be reported on the likelihood of product use by current users of tobacco products within the same tobacco product category, current users of tobacco products in other tobacco product categories, former users of any tobacco product, and youth and young adults;
   c. A summary of adverse experiences with this tobacco product reported to you, providing a listing and analysis (accompanied by a statement of any changes to the reference risk information and a summary of important risks, including the nature, frequency, and potential risk factors) of all adverse experiences including those serious and unexpected adverse experiences reported previously.
   d. A summary of sales and distribution of the tobacco product: Total U.S. sales reported in dollars, units, and volume with breakdowns by US census region, major retail markets, and channels in which the product is sold (e.g., convenience stores, food and drug markets, big box retailers, internet/online sales, tobacco specialty shops);
   e. Data on current product users. Data should be collected about new users, current users, those who have switched tobacco products, and multiple product users. The results should be broken down by key demographic variables including age, gender, and race/ethnicity. Also, any change in the intended target market for the product should be reported. The data described above may include sales data and post-marketing analysis.
3. A description of each change made to the manufacturing, facilities or controls during the reporting period, including:
   a. A comparison of each change to what was described in the PMTA;
   b. The rationale for making each change; and
   c. A certification that the reported change did not result in any modification (including a change in design, any component, any part, or any constituent, including a smoke constituent, or in the content, delivery or form of nicotine, or any other additive or ingredient) of the tobacco product; the basis for concluding that each change did not result in any modification to the final product.
4. A summary of all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution and indicate a deviation that may affect the characteristics of the final product.
5. Full-color copies of all advertising for the tobacco product that has not been previously submitted, along with the original date the advertisements were first disseminated and the date the advertisements were discontinued; and
6. In all annual reports, include a description of any or all labeling changes and submit revised full color final printed labeling.
   a. The labeling should include all the panels, be presented in the actual size and color with legible text.
   b. For the first annual report only, submit all final printed labeling (actual labeling for each required warning distributed with the product); include labels, inserts/onserts, instructions, and other accompanying information or materials for this product.

In accordance with 40 CFR 1506.6, we will make your environmental assessment publicly available.

This order authorizing the marketing of this new tobacco product does not mean FDA "approved" the new tobacco product specified above; therefore, you may not make any express or implied statement or representation directed to consumers that conveys, or misleads or would mislead consumers into believing, among other things, that the new tobacco product specified above is "approved" by FDA. See Section 301(tt) of the FD&C Act. This marketing order is subject to withdrawal or temporary suspension under section 910(d) of the FD&C Act.

We remind you that all regulated tobacco products, including the new tobacco product specified above, are subject to the requirements of Chapter IX of the FD&C Act and its regulations. These requirements currently include, but are not limited to, annual registration, listing of products, listing of ingredients, reporting of harmful and potentially harmful constituents, and payment of user fees. There are also packaging, labeling, and advertising requirements with which you must comply. It is your responsibility to ensure the tobacco product specified above complies with all applicable statutory and regulatory requirements. FDA will monitor your compliance with these applicable statutes and regulations.

If you discontinue the manufacture, preparation, compounding or processing for commercial distribution this tobacco product and later decide to reintroduce the product into the market, please contact the Office of Science to discuss if additional information is necessary.

For more information on your responsibilities under the FD&C Act, we encourage you to visit our website at http://www.fda.gov/TobaccoProducts. You may also obtain information by contacting FDA's Center for Tobacco Products at 1-877-CTP-1373, AskCTP@fda.hhs.gov, or SmallBiz.Tobacco@fda.hhs.gov.

We remind you all regulatory correspondence can be submitted via the FDA Electronic Submission Gateway (http://www.fda.gov/esg) using eSubmitter or by mail to:

Food and Drug Administration
Center for Tobacco Products
Document Control Center
Building 71, Room G335
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

We are unable to accept regulatory submissions by electronic mail.

If you have questions, you may contact Asia Brown, MHSA, Regulatory Health Project Manager, at (240) 402-3833.

Sincerely,

Digitally signed by David Ashley -S
Date: 2015.11.10 06:00:57 -05'00'

David L. Ashley, Ph.D.
RADM, US Public Health Service
Director
Office of Science
Center for Tobacco Products

Enclosure

General Portion Original Large Labeling







Accepted Article

Use of moist oral snuff (snus) and pancreatic cancer: pooled analysis of nine prospective observational studies

Marzieh Araghi,[1] Maria Rosaria Galanti,[1,2] Michael Lundberg,[1] Anton Lager,[1,2] Gunnar Engström,[3] Lars Alfredsson,[4] Anders Knutsson,[5] Margareta Norberg,[6] Malin Sund,[7] Patrik Wennberg,[8] Ylva Trolle Lagerros,[9,10] Rino Bellocco,[11,12] Nancy L. Pedersen,[12] Per-Olof Östergren,[13] Cecilia Magnusson[1,2]

[1]*Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden*
[2]*Centre for Epidemiology and Community Medicine, Stockholm Health Care District, Stockholm County Council, Sweden*
[3]*Department of Clinical Sciences, Lund University, Lund, Sweden*
[4]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden*
[5]*Department of Health Sciences, Mid Sweden University, Sundsvall, Sweden*
[6]*Department of Public Health and Clinical Medicine, Epidemiology and Global Health, Umeå University, Umeå, Sweden*
[7]*Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden*
[8]*Division of Family Medicine, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden*
[9]*Department of Medicine, Clinical Epidemiology Unit, Karolinska Institutet, Stockholm, Sweden*
[10]*Department of Medicine, Clinic of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital Huddinge, Stockholm, Sweden*
[11]*Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan, Italy*
[12]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*
[13]*Social Medicine and Global Health, Department of Clinical Sciences, Lund University, Lund, Sweden*

Corresponding author:

Dr. Marzieh Araghi. Department of Public Health Sciences, Karolinska Institutet Tomtebodavägen 18A, SE-171 77 Stockholm. (email: marzieh.araghi@ki.se).

Novelty & Impact Statements

The study adds to the still ambiguous data regarding snus use and pancreatic cancer development. Swedish snus use does not appear to be implicated in the development of pancreatic cancer in men. Tobacco smoke constituents other than nicotine or its metabolites may account for the relationship between smoking and pancreatic cancer.

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While smoking is a well-established risk factor for pancreatic cancer, the effect of smokeless tobacco is less well understood. We used pooled individual data from the Swedish Collaboration on Health Effects of Snus Use to assess the association between Swedish snus use and the risk of pancreatic cancer. A total of 424,152 male participants from nine cohort studies were followed up for risk of pancreatic cancer through linkage to health registers. We used shared frailty models with random effects at the study level, to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) adjusted for confounding factors. During 9,276,054 person-years of observation, 1,447 men developed pancreatic cancer. Compared to never snus use, current snus use was not associated with risk of pancreatic cancer (HR 0.96, 95% CI 0.83 – 1.11) after adjustment for smoking. Swedish snus use does not appear to be implicated in the development of pancreatic cancer in men. Tobacco smoke constituents other than nicotine or its metabolites may account for the relationship between smoking and pancreatic cancer.

Key words: pancreatic cancer, incidence, snus

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Pancreatic cancer is one of the most lethal malignancies.[1] In the year 2012, in Europe, there were 78,700 new cases and 77,900 deaths.[2] Pancreatic cancer incidence rates are higher in the Nordic countries and Central Europe than in other parts of the world.[3] Although the aetiology of pancreatic cancer remains poorly understood, cigarette smoking is a well-established and modifiable risk factor.[1] A meta-analysis of 82 studies demonstrated that current and former smoking was associated with a 74% and a 20% increased risk of pancreatic cancer, respectively.[4] The mechanism explaining the increased risk of pancreatic cancer with cigarette smoking is unclear, but a role of nicotine or its metabolites cannot be ruled out.[5]

Smokeless tobacco yields lower exposure to tobacco carcinogens compared with smoking, because it does not undergo combustion, but delivers an equivalent dose of nicotine.[6] Smokeless tobacco products vary with respect to packaging, modality of use and known impact on cancer risks.[7] Snus is a moist tobacco for oral use that is common in Scandinavian countries, where its use is increasing rapidly among young people.[8] According to national public health surveys, about 18% of Swedish men and 27% of Norwegian young men are daily users.[9] With the exception of Sweden, the sale of snus is currently banned in the European Union (EU).[10] Swedish snus is known to deliver lower levels of carcinogenic tobacco-specific nitrosamines (TSNA) than North American smokeless tobacco products.[7]

Evidence regarding smokeless tobacco use and pancreatic risk is inconsistent.[11-15] The International Agency for Research on Cancer (IARC) and European Community Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) on the health effects of smokeless tobacco, in 2007 and 2008, respectively, concluded that smokeless tobacco products are carcinogenic to humans and the pancreas has been identified as a main target organ.[11, 12] A meta-analysis of 6 studies (including two Scandinavian studies of Swedish snus) by Boffetta and colleagues,[13] also demonstrated a moderate risk increase, which emanated

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from the two included studies on snus use.[13] Two recent meta-analyses[14, 15] of mainly European and North American case–control studies, however, did not find any association.

Assessing the effects of smokeless tobacco products may provide insights into carcinogenic mechanisms of smoking. Similar associations as for smoking would make a role of carcinogens not associated to combustion more likely. Hence, studies of snus are relevant not only to inform users and public health policy about the health consequences of snus, but also on the long-term safety of nicotine (often administered as nicotine replacement therapy or via e-cigarettes).

The Swedish Collaboration on Health Effects of Snus Use consists of a group of Swedish investigators, who have conducted prospective studies where data on snus use has been collected.[16] The collaboration involves data from nine Swedish cohort studies,[17-25] of which only one[17] had published data on snus use and pancreatic cancer. We here take advantage of this large pooling project to investigate the impact of snus use on pancreatic cancer risk.

Material and Methods

Contributing studies and data collection

We used data from nine prospective cohort studies, including participants of varying ages, recruited at different time periods from diverse geographic regions across Sweden with information on both snus use and tobacco smoking. Exclusion criteria were pancreatic cancer prior to study enrolment, age less than 18 years or missing information on body mass index (BMI) (Figure 1). Since snus use is rare in women;[9] the study was restricted to men. Details on study design and data collection procedures of the individual studies have been reported elsewhere.[17-25]

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Each cohort study provided individual participant data, and data harmonization and analyses were implemented centrally. The specific studies were approved by their respective regional ethical vetting boards, and approval for the pooling project was granted by the Stockholm Regional Ethical Review Board (registration number 2009/971-31/3).

Pancreatic cancer cases were identified from the Swedish Cancer Register[26] established in 1958.[26] Since the Swedish Cancer Register does not accept notifications from death certificates only, and therefore incompletely record pancreatic cancer because of their poor prognosis,[27, 28] we complemented our case ascertainment with data from the Cause of Death Register. The Cause of Death Register covers all deaths in Sweden since 1961, and includes ICD-codes for the main and contributory causes of death.[29] Linkages were performed using the national registration number, an unique personal identifier assigned to all Swedish residents,[29] and pancreatic cancer cases were identified by the ICD-7 code 157 and ICD-10 code C25.

Information on tobacco use was collected at baseline using self-administered questionnaires in eight studies[17-20, 22-25] and by a structured telephone interview in one study.[21] All studies contributed information on current snus use, and seven on former snus use[17, 19-21, 23-25] while amount and duration of snus use was available from seven[17-21, 24, 25] and five studies,[17-19, 24, 25] respectively. Covariates collected at baseline, included body mass index (BMI),[30] alcohol intake,[31] physical activity level,[32] and type 2 diabetes.[33]

Information on height and weight, whether it was self-reported[20-23] or measured by health professionals,[17-19, 24, 25] was collected in all studies. Information on alcohol consumption was retrieved from all studies, except one.[17] Physical activity data was collected from seven studies,[19-25] and diagnosis of type 2 diabetes were identified in five studies[18, 20, 21, 23, 24] using several sources including self-reported data, record-linkage to the National Patient Register[34]

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(ICD-9 code 250 and ICD-10 code E11 and E14) and Prescribed Drug Register (as antidiabetics, code A10 according the Anatomic Therapeutic Chemical classification system).[35]

Smoking and snus use was categorized into never, former and current users (where non-current snus use was treated as never use in the studies[18, 22] that did not have information on former snus use). Current snus use was further categorized according to amount consumed per week (<4 cans, 4-6 cans, ≥7 cans) and duration (<5 years, 5-<10 years, 10-<15 years, 15-<20 years, ≥20 years) of use. Such information for smoking status was not available. Never-users of snus constituted the reference group.

Each cohort member contributed person-time from the date of entering into the study until the date of pancreatic cancer diagnosis, death, or the end of the study, whichever came first. Shared frailty models with random effects at the study level were used to estimate hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) of pancreatic cancer in relation to tobacco use, using attained age (in years) as the time scale. The shared frailty model is an extension of the Cox proportional hazards model and accounts for within study correlation by incorporating shared random effects. We tested for heterogeneity among included studies, using a Q test and I^2 statistics.[36] In addition to the inherent adjustment for age, all models were adjusted for body mass index (BMI, kg/m^2), calculated as body weight in (kilograms) by the height (in meters) squared and used as a continuous variable, and smoking (categorized as never, former or current smoking). Evaluation of the proportional hazards assumption with Schoenfeld's global test[37] revealed that smoking status variable did not satisfy the proportional hazards assumption. Modelling was therefore performed using an extended Cox regression analysis, with inclusion of smoking as a time-varying covariate.

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All tests were 2-sided, and we considered $p<0.05$ to be statistically significant. Stata statistical software (Version 13.0, Stata Corporation, and College Station, TX, USA) was used for all analyses.

We conducted sensitivity analysis according to the following scenarios: a) Using data from Cancer Register only. b) Excluding the Construction Workers Cohort, this dominates the results of our main analyses because of size. c) Adjusting for alcohol consumption (grams/week), physical activity ('less than 2 hours of light activity per week', 'more than 2 hours of light activity per week', '1 to 2 hours of at least moderate physical activity per week', and 'more than 2 hours of at least moderate physical activity per week'), and type 2 diabetes (yes/no) in the subset of studies where this information was available.[18, 20, 21, 23, 24] d) Excluding cohorts[18, 22] with no available information on former snus use, thus enabling correct classification of former snus use. e) Restriction to never smokers, as an alternative approach to control for the potential confounding effect of tobacco smoking.

Results

Figure 1 shows the numbers of study participants meeting the inclusion criteria. Characteristics of the 418,448 men constituting our analytical sample, yielding 9,276,054 person-years of observation, are shown in Table 1. Period of recruitment and duration of follow-up ranged from 1978 to 2013 and from 5 to 35 years, respectively. Average age at entry was 40 years (range 18 – 99). A total of 1,423 incident cases of pancreatic cancer, including 424 solely identified from the Cause of Death Register, occurred during follow-up. At time of entry, 30% of study participants had ever used snus.

Our main analyses, including the full analytical sample and adjusting for smoking status, did not support any relationship between snus use and pancreatic cancer risk (HR 0.93, 95% CI 0.82 – 1.06, comparing ever to never snus users). Additionally, there was no indication that

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current snus use, regardless of its duration or intensity, affected the risk (Table 2). Study specific HRs of pancreatic cancer for current snus users as compared to never-snus users are shown in Figure 2. We observed a moderate degree of heterogeneity between studies (I^2 statistics 63%).

Sensitivity analysis

Table 3 presents the results from sensitivity analyses. The estimates did change when using data from Cancer Register only. Excluding the Construction Workers Cohort, the HR for pancreatic cancer in current snus users was 1.30 (95% CI 0.97 – 1.73) after adjustment for BMI and smoking status. In the subset of studies where further covariate information was available, additional adjustment for alcohol consumption, physical activity and interaction between alcohol consumption and smoking, yield the corresponding HR of 1.32 (95% CI 0.84 – 2.08), and a similar result was produced with adjustment for diabetes (data not shown). Lastly, when the analytical sample was restricted to never smokers, the adjusted HR of pancreatic cancer in current snus users was 1.07 (95% CI 0.77 – 1.50).

Discussion

Findings from this large pooling project, including nine prospective cohort studies and 1,423 incident cases, did not support any relationship between snus use and risk of pancreatic cancer in men regardless of timing, duration or intensity of use.

We judge prior evidence regarding the association between smokeless tobacco use and pancreatic cancer to be inconsistent.[11-15] This is in spite of conclusions from prior and well recognized reviews, including the IARC Monograph 89,[11] a SCENIHR report,[12] and a meta-analysis by Boffetta and colleagues.[13] IARC Monograph 89[11] based its review on four studies, including three from the US and one from Norway; a US cohort study[38] reporting a relative risk (RR) of 1.70 (95% CI 0.90 – 3.19) for ever users of smokeless tobacco after adjustment

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for smoking, a US case-control study[39] showing an association with chewing tobacco but not snuff, another US case-control study,[40] finding no overall association, but an apparent positive trend in risk with amount used. Lastly, a prospective cohort study of 10,136 Norwegian men recruited in 1966 and followed up to 2001,[41] reported no association with snus use in never smokers but an increased risk overall after adjustment for smoking (RR 1.67, 95% CI 1.12 – 2.50). The SCENIHR report additionally considered data from the Swedish Construction Workers Cohort,[17] which demonstrated an increased risk for snus use among never smokers (RR 2.00, 95% CI 1.20 – 3.30) but not any overall association (RR 0.90, 95% CI 0.70 – 1.20, with adjustment for smoking). In contrast to the Norwegian study, Boffetta and colleagues[13] meta-analysed the six studies cited above, resulting in a summary RR of 1.60 (95% CI 1.10 – 2.20) for any use of smokeless tobacco, but only based the analysis on the positive associations, i.e. the increased risk in never smokers from the Swedish Construction Workers Cohort[17] and overall increased risk in the Norway Cohorts Study,[41, 42] while ignoring the reciprocal null associations. Further reviews, a pooled analysis by Bertuccio and colleagues[14] of eleven case–control and a meta-analysis by Lee and Hamling,[15] did not support any association between smokeless tobacco use and pancreatic cancer.

Although snus use was not associated with risk in smoking-adjusted models in the Swedish Construction Workers Cohort, with follow-up until 2004,[17] a twofold increased risk for ever or current snus users was observed when the sample was restricted to never smokers. We could replicate this result in our analyses, but the association was weaker and no longer statistically significant when the follow-up was extended until 2013 (HR 1.34, 95% CI 0.90 – 1.99). Restricting to never smokers, we stratified our analyses of the Construction Workers Cohort according to calendar period of follow-up. The HR in current snus users was 1.98 (0.97 – 4.03, based on 4 exposed cases) with follow-up from 1978 through 1994, but 1.11 (0.68 – 1.79, based on 27 exposed cases) in 1995-2013. The reason for this discrepancy is

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unclear, but may be due to increasing misclassification of exposure with longer follow-up (diluting any true associations), or to chance. The latter notion is sustained by our sensitivity analysis, excluding the Construction Workers Cohort, which was not in support of any strong relationship between snus and pancreatic cancer risk.

Our study has several strengths. Its prospective design minimises recall and selection bias, often afflicting retrospective studies. The latter bias is particularly problematic in studies of pancreatic cancer, since its high lethality imposes on case recruitment. Our study is also the largest to date, and we were hence able to explore dose-response relationships. In order to control for confounding by smoking, we used two approaches; multivariate modelling including current and former smoking as covariates, and restriction of the study population to never-smokers – both supporting a null association. In contrast to Boffetta and colleagues[41] and Luo and colleagues,[17] we had the opportunity to control for alcohol consumption, level of physical activity as well as diabetes, and again the main findings did not change. We used both Swedish Cancer Register and the Cause of Death Register to identify cancer cases, thus maximizing case ascertainment.[26, 43] Information on smoking and snus use was, however, self-reported, and only assessed at baseline. Although self-reports show high concordance with serum cotinine levels (a biomarker of smoking status) in cross-sectional data,[44] tobacco habits may change over the life-course. Yet, Swedish data shows 70% of snus users at baseline continued use after ten years.[45] Misclassification of tobacco use may nevertheless have biased our estimates of associations towards the null.

Tobacco smoking is a strong risk factor for pancreatic cancer.[46] Tobacco smoke contains high doses of carcinogenic TSNAs, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN), which may have specificity for the pancreas.[47] NNK metabolites can bind to DNA to form adducts and induce activating point mutations in the RAS gene,[48] which are thought to be the most common genetic alterations in the progression

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of pancreatic cancer.[49] Nitrosamine levels are substantially lower in Swedish snus than in tobacco smoke, as well as than in other types of smokeless tobacco products,[50] strengthening the plausibility of a null association between snus use and pancreatic cancer.

Our findings, from the largest sample to date, do not support a role of snus use in the development of pancreatic cancer in men. They, furthermore, point to tobacco smoke constituents other than nicotine or its metabolites, i.e. carcinogens associated with combustion, as the causal agent explaining the increased risk of pancreatic cancer in smokers.

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Table 1. Baseline characteristics of study participants in the Swedish Collaboration of Health Effects of Snus Use.

Study	Study population	Data collection	Period recruitment	Male participants (N)	Person years of follow-up (N)	Mean age at recruitment (years)	Cases (N)	Current snus users (%)	Information available regarding snus use		
									Duration	Amount	Former use
Construction Worker Cohort (CWC)[17]	Construction workers, national	Questionnaire	1978-1993	273,604	7,699,364	34	1,025	26	Yes	Yes	Yes
Malmö diet and Cancer Study (MDCS)[18]	Population-based, Malmö City	Questionnaire	1991-1996	11,217	207,444	59	63	7	No	Yes	No
Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)[19]	Population-based, Norrbotten and Västerbotten Counties	Questionnaire	1986-2004	4,474	57,109	48	14	23	Yes	Yes	Yes
National March Cohort (NMC)[20]	Participants in a charity walk, national	Questionnaire	1997	13,305	181,464	52	54	10	Yes	Yes	Yes
Screening Across the Lifespan Twin Study (SALT)[21]	Twins born in Sweden between 1926–1958, national	Structured telephone interview	1998-2002	17,923	174,124	56	85	15	Yes	Yes	Yes
Scania Public Health Cohort (Scania_PHC)[22]	Population-based, Scania County	Questionnaire	1999	5,837	54,647	48	3	20	No	No	No
Stockholm Public Health Cohort (Sthlm_PHC)[23]	Population-based, Stockholm County	Questionnaire	2002-2010	37,807	185,581	49	42	18	No	No	Yes
Västerbotten Intervention Programme (VIP)[24]	Population-based, Västerbotten County	Questionnaire	1992-2013	47,181	616,045	47	129	27	Yes	Yes	Yes
Work, Lipids and Fibrinogen Study (WOLF)[25]	Employees, Västernorrland, Jämtland, and Stockholm Counties	Questionnaire	1992-1997	7,100	100,276	42	8	23	Yes	Yes	Yes
All studies			1978-2013	418,448	9,276,054	40	1,423	24			

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Table 2. Pooled hazard ratios and 95% confidence intervals for pancreatic cancer in relation to snus use.

Use of snus at baseline	Number of cases	Hazard ratio[a]	95% confidence interval
Never-users	1,103		Ref.
Ever users	321	0.93	(0.82 – 1.06)
Former users	93	0.88	(0.71 – 1.10)
Current users	227	0.96	(0.83 – 1.11)
Amount (cans/week)[b]			
<4	91	0.87	(0.70– 1.08)
4-6	83	1.16	(0.93 – 1.46)
≥7	48	0.87	(0.65 – 1.17)
Duration (years)[b]			
<5	27	0.82	(0.56 – 1.21)
5-<10	38	1.00	(0.72 – 1.39)
10-<15	41	0.99	(0.72 – 1.36)
15-<20	27	0.98	(0.67 – 1.44)
≥20	78	0.95	(0.75 – 1.19)

[a] All hazard ratio estimates were adjusted for attained age, smoking (never, former, and current) and body mass index.
[b] Among current snus users only.

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Table 3. Pooled hazard ratios (HR) and 95% confidence intervals (CI) of pancreatic cancer in relation to snus use from sensitivity analyses.

Type of analysis	Use of snus at baseline					
	Ever users		Former users		Current users	
	n	HR(95% CI)	n	HR(95% CI)	n	HR(95% CI)
Cases from Cancer Register only	250	0.97 (0.84 – 1 12)	73	0.95 (0.75 – 1.22)	177	0.98 (0.83 – 1.15)
Excluding Construction Workers Cohort	98	1.13 (0.87 – 1.45)	33	0.90 (0.61 – 1.31)	65	1.30 (0.97 – 1.73)
Controlling for additional potential confounders[a]	92	1.12 (0.76 – 1.63)	33	0.90 (0.51 – 1.59)	59	1.32 (0.84 – 2.08)
Excluding cohorts with no information on former snus use	315	0.93 (0.81 – 1.06)	93	0.88 (0.70 – 1.09)	222	0.95 (0.82 – 1.10)
Restriction to never smokers	50	1.04 (0.77 – 1.42)	9	0.92 (0.47 – 1.80)	41	1.07 (0.77 – 1.50)

[a]Additional adjustment for alcohol consumption, physical activity and interaction between alcohol consumption and smoking among the studies where this information was available (MONICA, NMC, SALT, Scania_PHC, Sthlm_PHC, VIP and WOLF).

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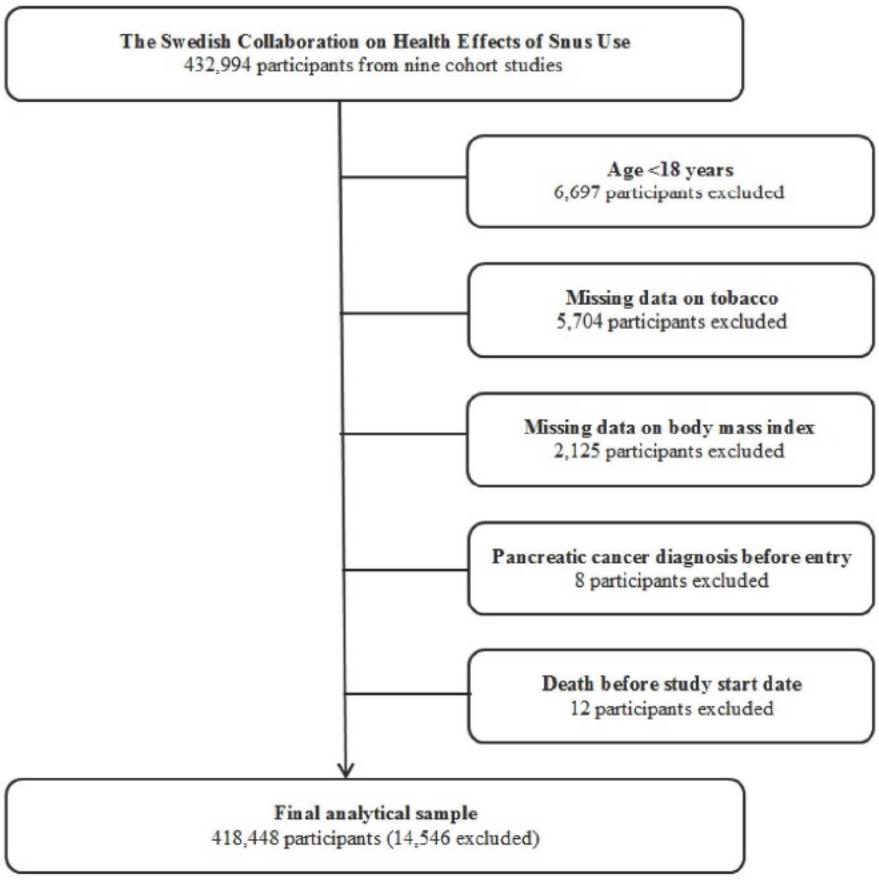


Figure 1. Derivation of the analytical sample.

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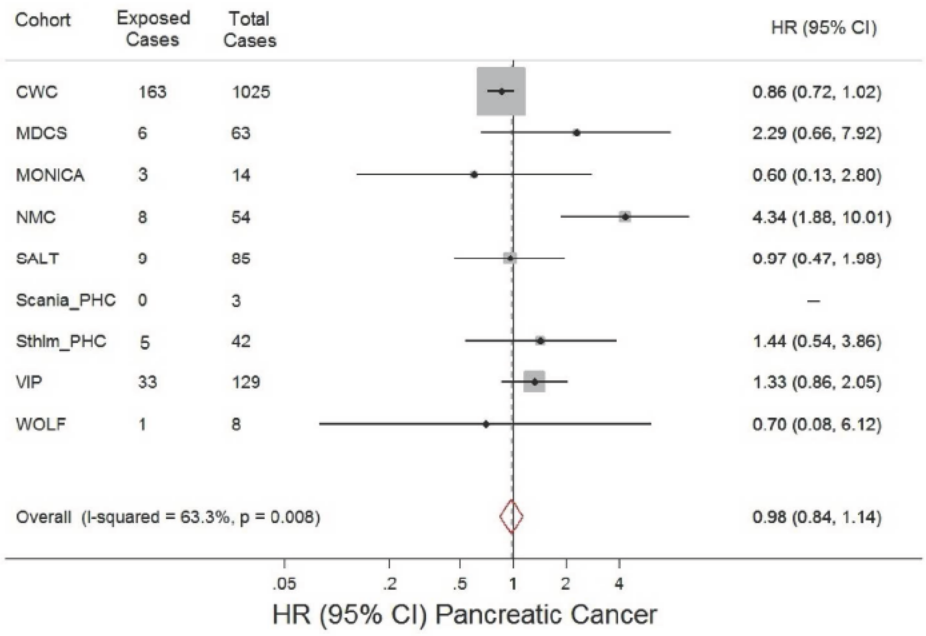


Figure 2. Study-specific hazard ratios (HR) and 95 percent confidence intervals (95% CI) of pancreatic cancer among current-snus users versus never-snus users."

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ORIGINAL ARTICLE

Snus use during the life-course and risk of the metabolic syndrome and its components

MARJA LISA BYHAMRE[1], PER E GUSTAFSSON[2], JAN-HÅKAN JANSSON[3], MARIA WENNBERG[4], ANNE HAMMARSTRÖM[5] & PATRIK WENNBERG[1]

[1]Department of Public Health and Clinical Medicine, Family Medicine, Umeå University, Sweden, [2]Department of Public Health and Clinical Medicine, Epidemiology and Global Health, Umeå University, Sweden, [3]Department of Public Health and Clinical Medicine, Skellefteå Research Unit, Umeå University, Sweden, [4]Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Sweden, and [5]Department of Public Health and Caring Sciences, Uppsala University, Sweden

Abstract

Objective: We aimed to investigate the association between life-course exposure to snus and prevalence of the metabolic syndrome and its components in adulthood. *Design and method:* Tobacco habits at baseline (age 16) and three follow-ups (ages 21, 30 and 43) were assessed among 880 participants in a population-based cohort in Northern Sweden. Presence of the metabolic syndrome at age 43 was ascertained using the International Diabetes Federation criteria. Odds ratios and CIs for risk of the metabolic syndrome and its components by snus use at 16, 21, 30 and 43 years were calculated using logistic regression. Cumulative snus use was defined as number of life periods (1–4) with current snus use. *Results:* At age 43, 164 participants (18.6%) were current snus users. We found no association between exclusive snus use at the ages of 16, 21, 30 and 43 years and the metabolic syndrome at age 43 years. Snus use (among non-smokers) was associated with raised triglycerides and high blood pressure in crude analysis, but not in multivariable models. There was no association between cumulative snus use and risk of the metabolic syndrome. Cumulative snus use was associated with central obesity, raised triglycerides and impaired fasting glucose/diabetes mellitus type 2 in crude analyses, but not after adjustments. ***Conclusions:*** **The health consequences of snus exposure from adolescence to mid-adulthood do not seem to include increased risk of the metabolic syndrome or its components. The cardio-metabolic risk of dual exposure to snus and cigarettes may warrant further attention.**

Key Words: *Swedish moist snuff, snus, smokeless tobacco, metabolic syndrome, metsy, obesity, hypertension, triglycerides, life-course*

Background

Snus is a moist smokeless tobacco product. Most common in Sweden, with 18% of the male population being daily users, snus is also becoming increasingly popular worldwide [1]. The scientific evidence hitherto produced on health effects from snus use is generally weak [2]. With increasing use, and the suggestion to promote snus as a strategy for smoking cessation [3], there is need for more knowledge in this field.

Snus has been suggested as a possible risk factor for developing the metabolic syndrome, a concept including risk factors strongly associated with increased morbidity and mortality in cardiovascular diseases, diabetes mellitus type two (T2DM) and cancer [4]. It also increases overall mortality [4]. The metabolic syndrome includes central obesity, hypertension, T2DM or raised fasting glucose, and abnormal serum lipids [5].

Correspondence: Marja Lisa Byhamre, Department of Public Health and Clinical Medicine, Family Medicine, Umeå University, SE-901 85 Umeå, Sweden. E-mail: marja_lisa.byhamre@umu.se

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Increased risk of obesity – the driving force behind the metabolic syndrome – has been shown in four studies on snus use [6-9]. A recent large observational study also showed an association between snus use and T2DM [10]. Further, nicotine from snus stimulates the sympathetic nervous system, resulting in immediately increased blood pressure and heart rate [11]. These effects will persist during active snus use, which averages 13–15 hours per day [2]. An association between snus use and the metabolic syndrome, if present, could partly explain the higher cardiovascular and overall mortality for snus users that has been observed in the last few years [12-15]. However, the possible association between snus use and the metabolic syndrome has only been evaluated in two studies, with inconclusive results [7,16]. These studies have included former smokers in the analyses, which may lead to residual confounding. They are also limited by cross-sectional design [16] or a follow-up of 10 years [7], while tobacco habits often shift substantially over time [2]. Regarding associations between snus and lipid levels, previous research is divergent [17].

Health hazards caused by long-term snus use are of special interest, as cardio-metabolic risk accumulates over decades. Life-course epidemiology may provide applicable theories for studying health effects of different exposures throughout life. Two important concepts are 'sensitive period' and 'accumulation of risk'. The sensitive period theory states that humans may be subject to biological programming early in life, so that exposure during this period may have an independent impact on later life health. [18] Accordingly, it could be hypothesized that adolescents exposed to snus are more prone to long-term hormonal and metabolic effects, and therefore suffer more damage from nicotine than adults. In contrast, the 'accumulation of risk' model assumes that health risks instead accumulate with long-term exposure [18], such as snus use across the life-course. We have previously shown that the metabolic syndrome indeed may be partially rooted in behavioural conditions during adolescence [19] and accumulated throughout life [20], but to our knowledge, the life-course effects of snus use on the metabolic syndrome have not been investigated previously.

This study follows a population for 27 years, from adolescence to mid-adulthood, with repeated measurements of tobacco habits. We aim to investigate the association between life-course exposure to snus and prevalence of the metabolic syndrome and its components in adulthood. Specifically, we aim (a) to describe tobacco use over life-course; (b) to evaluate whether snus use during certain life periods is associated with the development of the metabolic syndrome at age 43 (sensitive period model); and (c) to investigate whether accumulated exposure to snus during adolescence and mid-adulthood is associated with increased risk of the metabolic syndrome and its components at age 43 years (cumulative risk model).

Method

Participants

Data were derived from the 27-year prospective Northern Swedish cohort [21]. The sample consists of all students in the municipality of Luleå who in 1981 attained the 9th grade (age 16) of Swedish compulsory school (n=1083). Follow-ups have been performed in 1983, 1986, 1995 and 2008 (at ages 18, 21, 30 and 43). In 2008, 94% of the baseline sample still alive participated in the study (n=1001). The sample has been found to be representative of the corresponding age cohort of Sweden in demographic comparisons [21]. The Regional Ethical Review Board in Umeå, Sweden, approved the study.

The present study is based on data collected in 1981, 1986, 1995 and 2008. From the original study population, participants with missing information on metabolic syndrome at age 43 (n=107) were excluded. Another 14 were excluded due to missing information on tobacco habits, in total excluding 121 participants. Hence, analyses were based on 880 individuals, representing 88% of those still participating in 2008.

Procedures

Participants completed self-administered questionnaires at baseline and follow-ups [21]. Weight and height at age 16 were measured by school nurses. At age 43, participants underwent a health examination by trained medical personnel at local health care centres [19]. This included blood pressure measured according to the WHO MONICA manual [22], and venous blood sampling after overnight fast. These were analysed for glucose, high-density lipoprotein cholesterol (HDL-C) and triglycerides. The coefficient of variance at high/low concentration was 1.5/1.2% for glucose, 2.8/2.8% for HDL-C and 1.7/1.5% for triglycerides.

Measures

The metabolic syndrome was defined according to the International Diabetes Federation [5]: (a) central obesity, defined as waist circumference ⩾80 cm for women and ⩾94 cm for men, and (b) at least two of

the following: (i) low serum HDL-C (<1.29 mmol/l for women and <1.03 mmol/l for men), or specific therapy for that lipid abnormality; (ii) high serum triglycerides (⩾1.7 mmol/l), or specific treatment for that lipid abnormality; (iii) high blood pressure (⩾130 mm Hg systolic and/or ⩾85 mm Hg diastolic) or antihypertensive medication; and (iv) raised fasting glucose levels (⩾5.6 mmol/l) (impaired fasting glucose, IFG) or diagnosed T2DM.

Results from the age 43 health examination were used to define presence of the metabolic syndrome in each participant. Information on medication and pre-existing conditions was self-reported. Individuals reporting diabetes mellitus at age 43 (n=21) were considered as having T2DM, excluding those reporting diabetes at age 30 (n=4, who also reported having diabetes at age 21), who were considered as having type 1 diabetes. Because of lack of specific therapy for individual serum lipid abnormalities, those treated with lipid-lowering medicines and having normal triglycerides and HDL-C in blood samples were regarded as having normal serum lipids (n=8).

Information on current and former tobacco use was self-reported at baseline and at each follow-up and divided into nine categories (Supplementary Table 1): (1) never tobacco users, (2) current snus users who had never smoked, (3) current snus users who had previously smoked, (4) current smokers who had never used snus, (5) current smokers who had previously used snus, (6) current snus users who also currently smoke, (7) former snus users who had never smoked, (8) former smokers who had never used snus, and (9) former users of both snus and cigarettes, in accordance with previous assessments [23]. Former tobacco use reported at age 16 was considered non-significant and therefore regarded as never-use.

When reviewing the self-reports, it was evident that previous tobacco use was sometimes ignored or forgotten (assuming that current exposure is more accurately reported than previous exposure). Therefore, the dataset was adjusted according to previously stated tobacco use. For example, if a person stated 'current snus use, no smoking' in 1986, and then reported never-use of tobacco in 1995 and 2007, the last two follow-ups were categorized as 'former snus use, never smoking'. We adjusted 154 individuals' responses regarding former tobacco use.

For the sensitive period model, we performed analyses on the nine categories of tobacco use for baseline and each follow-up.

The cumulative model analyses were operationalized as the number of life periods (1 to 4) an individual reported current snus use. All snus users, irrespective of smoking habits, were included in the analyses due to low numbers of pure snus users (n from 9 to 38). Similarly, cumulative smoking was defined as number of life periods smoking.

Body mass index (BMI) at age 16 was calculated from the school nurses' measurements. Family history of diabetes mellitus was self-reported at age 16. Socioeconomic status in adolescence was based on parental occupation as reported at age 16. Using the classification of Statistics Sweden, they were divided into three groups. The two upper groups were combined into a socially favourable category, while the group 'manual workers' was defined as a disadvantaged category, following previous classification [19,24]. Annual alcohol consumption at age 43 was estimated from self-reports on typical frequency and quantity. It was coded as high or low using the sex-specific 80th percentile as cut-off. Physical activity during the last 12 months at age 43 was reported with six response options: daily, several times per week, once a week, several times per month, once a month, and seldom. The last three categories were collapsed based on low numbers [24].

Statistical analyses

Statistical analyses were performed using SPSS Statistics, version 19 (SPSS, Chicago, Illinois, USA). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the metabolic syndrome and its individual components (central obesity, low HDL-C, high triglycerides, high blood pressure, IFG/T2DM). Health exams at age 16 were insufficient to determine exact presence of the metabolic syndrome. Therefore, we could not exclude prevalent cases at baseline. However, only five of the 880 participants had a BMI ⩾30 at age 16, making them eligible for diagnosis of the metabolic syndrome [5]. This number was considered too low to alter the results significantly.

Covariates with significant (p>0.05) association with the metabolic syndrome: sex, BMI and socioeconomic status at 16 years, family history of diabetes mellitus, alcohol consumption and physical activity at 43 years, were adjusted for in all multivariable models. In the cumulative model, we also adjusted for cumulative smoking. BMI after 16 years was considered part of a possible causal chain and hence not adjusted for.

The 121 participants who were excluded from the original population did not differ significantly from those included regarding gender distribution, current snus use, smoking rates at 16 and 43 years, SES and BMI at age 16, family history of diabetes mellitus, or physical activity level at age 43. They had significantly higher alcohol consumption at age 43 (p<0.001) and

Table I Descriptive statistics for participants of the Northern Swedish cohort.

	n	Never tobacco users at age 16	Current users of snus who have never smoked at age 16	Current smokers who have never used snus at age 16	Current dual users of snus and cigarettes at age 16	*p* for overall difference
		(Group 1, *n*=572)	(Group 2, *n*=81)	(Group 4, *n*=174)	(Group 6, *n*=53)	
Assessed at age 16						
Male sex	457	303 (53.0%)	68 (84.0%)	51 (29.3%)	35 (66.0%)	**0.000**
Body Mass Index (kg/m^2)	877	19.85 (2.72)	19.67 (2.43)	20.06 (2.60)	20.20 (2.98)	0.708
Socioeconomic disadvantage	333	203 (35.5%)	35 (43.2%)	76 (43.7%)	19 (35.8%)	0.195
Family history of diabetes mellitus	34	18 (3.1%)	1 (1.2%)	12 (6.9%)	3 (5.7%)	0.068
	n	Never tobacco users at age 43	Current users of snus who have never smoked at age 43	Current smokers who have never used snus at age 43	Current dual users of snus and cigarettes at age 43	*p* for overall difference
		(Group 1, *n*=308)	(Group 2, *n*=37)	(Group 4, *n*=88)	(Group 6, *n*=60)	
Assessed at age 43						
Metabolic syndrome	237	71 (23.1%)	12 (32.4%)	25 (28.4%)	20 (33.3%)	0.247
Physical inactivity (<once a week)	364	110 (35.7%)	17 (45.9%)	48 (54.5%)	38 (63.3%)	**0.000**
Alcohol consumption (cl/year)	880	272.95 (1155.91)	444.75 (452.89)	291.87 (413.71)	1034.61 (2737.09)	**0.000**

Reported values are number of participants (% of tobacco use group) for categorical variables, and mean (standard deviation) for continuous variables.
p-values calculated using chi square test or Kruskal–Wallis test.

smoked to a greater extent at age 21 (p=0.006) and 30 (p=0.007).

Among the 880 included participants, individuals with missing data on covariates (n=15) did not differ significantly from those with complete data regarding gender distribution, prevalence of the metabolic syndrome or ever-use of snus or cigarettes.

We found no significant interaction between snus use and sex on prevalence of the metabolic syndrome. Therefore, our results were not stratified by sex. Also, the number of never-smoking women using snus was too small (n from 4 to 13) to allow analyses for women separately, and separate analyses for men did not alter the conclusions.

Results

Table I shows characteristics of the cohort at baseline and the 2008 follow-up. The number of snus users that had never smoked was 81 at age 16 (9.2% of cohort), 53 at age 21 (6.0%), 57 at age 30 (6.5%) and 37 at age 43 (4.2%).

There were significantly more male snus users than female at baseline and all follow-ups. At age 43 the numbers were 7% and 1.2%, respectively (p<0.001).

At age 43, alcohol consumption was significantly higher among all categories of tobacco users than among never-users of tobacco, and 81 women (19.1%) and 156 men (34.1%) met the metabolic syndrome criteria.

Figure 1 shows tobacco use among the 880 participating subjects at baseline and follow-ups. The number of snus users increased with age. For smoking, numbers peaked at 21 years and then dropped steadily. Dual use showed a stable prevalence over the life-course.

Figure 2 shows the proportion of exclusive snus users among all snus users and exclusive smokers among all smokers. At all follow-ups, the proportion of exclusive snus users was significantly lower compared with the proportion of exclusive smokers (p<0.001), but not at baseline (p=0.094).

We found no significant risk increase compared with never-users of tobacco for metabolic syndrome, central obesity or low HDL-C for exclusive snus users at any age in the sensitive period analyses (Table II). In crude analyses, there was increased risk of high triglycerides (ages 16, 21, 30), high blood pressure (ages 21, 30, 43) and IFG/T2DM (age 21) for current exclusive snus users; however, none of these results remained significant after adjusting for confounders.

For cumulative snus use, there was a significantly increased risk of the metabolic syndrome at age 43 for those who had used snus during two life periods in crude analysis compared with never tobacco users,

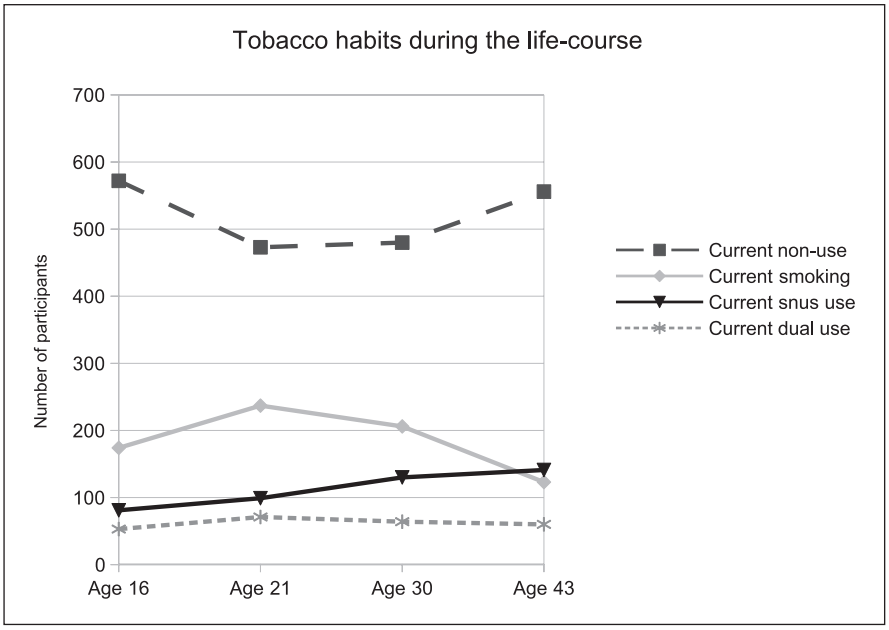


Figure 1. Self-reported current tobacco use at baseline and three follow-ups.

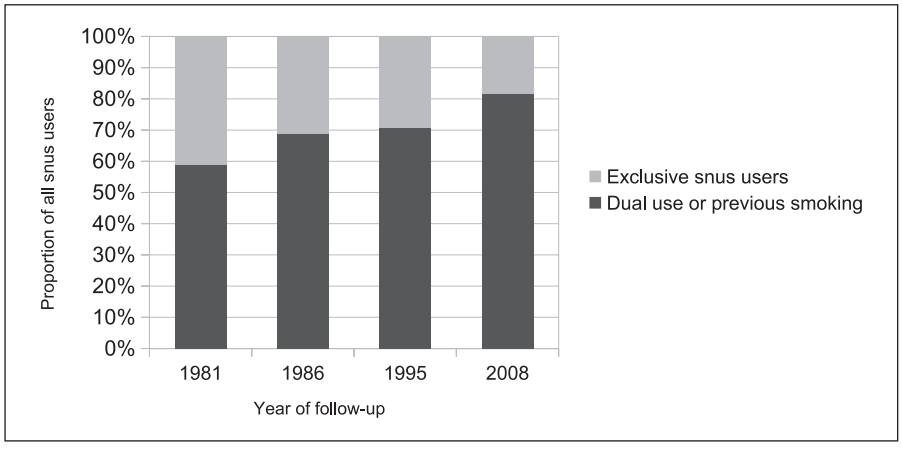


Figure 2(a). Proportion of exclusive snus users among all snus users.

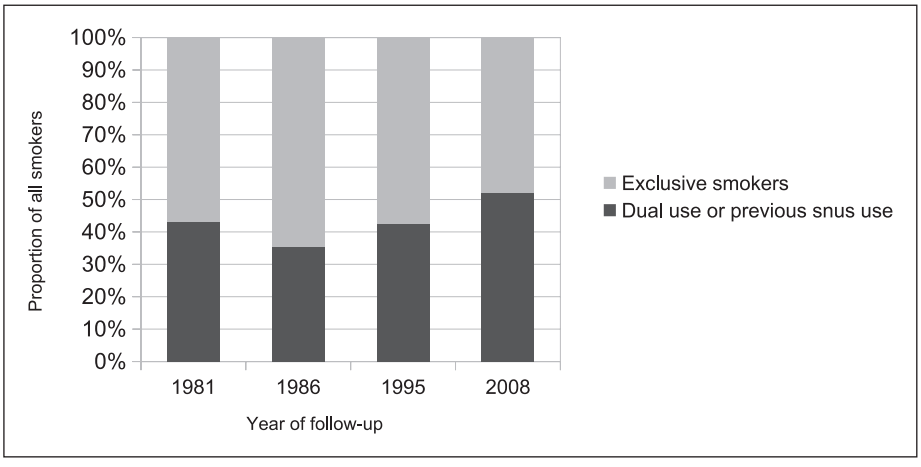


Figure 2(b). Proportion of exclusive smokers among all smokers. Reports from baseline and follow-ups.

Table II Metabolic risk for current snus users who have never smoked, evaluated at different ages (sensitive period model).

	Metabolic syndrome		Central obesity		Raised triglycerides		Low HDL-C		High blood pressure		Impaired fasting glucose or T2DM	
	Crude	Multivariable*	Crude	Multivariable*	Crude	Multivariable*	Crude	Multivariable*	Crude	Multivariable*	Crude	Multivariable*
Age 16 (1981) *n*=81	1.17 0.70–1.96	0.95 0.54–1.65	1.28 0.79–2.07	1.40 0.83–2.35	**1.83 1.11–3.01**	1.38 0.81–2.37	1.15 0.69–1.92	1.23 0.72–2.12	1.45 0.91–2.32	1.08 0.66–1.77	1.55 0.87–2.74	1.08 0.59–1.97
Age 21 (1986) *n*=53	1.68 0.91–3.08	1.15 0.60–2.21	1.18 0.66–2.13	1.24 0.65–2.34	**2.05 1.11–3.79**	1.27 0.66–2.45	0.83 0.43–1.61	0.84 0.41–1.70	**2.08 1.16–3.72**	1.31 0.71–2.42	**2.20 1.12–4.32**	1.28 0.63–2.62
Age 30 (1995) *n*=57	1.51 0.82–2.80	1.01 0.52–1.99	1.20 0.68–2.13	1.15 0.61–2.15	**2.21 1.20–4.06**	1.37 0.71–2.63	0.61 0.30–1.23	0.53 0.25–1.12	**2.37 1.33–4.19**	1.61 0.88–2.96	1.81 0.90–3.62	1.01 0.48–2.11
Age 43 (2008) *n*=37	1.60 0.78–3.35	1.15 0.52–2.51	1.76 0.85–3.62	1.65 0.76–3.58	1.78 0.83–3.80	1.10 0.49–2.45	0.72 0.32–1.64	0.69 0.29–1.66	**2.06 1.04–4.09**	1.41 0.69–2.89	0.71 0.24–2.09	0.38 0.12–1.16

Reported values are odds ratios and 95% confidence intervals.
Logistic regression was used to estimate odds ratios and 95% confidence intervals.
*The multivariable model is adjusted for sex, cumulative smoking, BMI at 16 years, socioeconomic status at 16 years, family history of diabetes mellitus, alcohol consumption at 43 years and physical activity level at 43 years.

and *p* for trend was significant. The results did not remain significant after adjustments. Table III shows the results of these analyses using different multivariable models.

Supplementary Table 2 shows cumulative snus use and risk of the components of the metabolic syndrome compared with never-use of tobacco. An association with central obesity was observed in crude analyses for 1 and 4 life periods of snus use. High triglycerides were associated with snus use for 2, 3 and 4 life periods. High blood pressure was associated with snus use for 4 periods. IFG/T2DM was associated with 2 and 3 life periods of snus use. These associations did not remain significant in the fully adjusted models. No association between snus use and low HDL-C was found.

Discussion

Our main finding was that exposure to snus in different life periods and accumulated exposure to snus from adolescence until adulthood was not associated with the development of the metabolic syndrome, or its components, at age 43. Individuals who had used snus were found to have an increased OR for high triglycerides, raised blood pressure and IFG/T2DM at age 43 compared with non-tobacco users, but the associations did not remain significant after adjustment for confounders. This indicates that differences between non-tobacco users and snus users regarding the potential confounders, rather than snus use itself, may explain the associations.

Another main finding of this study was that the use of snus slowly increased from adolescence to adulthood, and that the majority of snus users at some point also had smoked. The increasing prevalence of snus use may be attributable to (a) increased snus use in the Swedish population during the study period [1], (b) smokers in the cohort shifting to snus use and (c) snus use being a more stable habit than, for example, smoking. The latter is supported by previous studies [25,26]. Smoking drastically declines from age 21 onwards, which could be an expression of the well-known negative health effects of cigarette smoking. Furthermore, the proportion of exclusive snus users among all snus users was low, a finding that has been observed in previous studies [8,9,27, 28]. Our results confirm that snus users often smoke at some point during life.

Two previous studies have investigated the relationship between snus use and the metabolic syndrome. A 10-year prospective Swedish study by Norberg et al., including over 16,000 individuals, reported an increased risk of the metabolic syndrome in heavy

Table III Cumulative snus use and risk of the metabolic syndrome.

	Model A*	Model B•	Model C→	Model D#
Never tobacco use	1.00	1.00	1.00	1.00
n=308				
Snus use 1 period	1.40	1.37	1.13	1.08
n=122	0.87–2.24	0.85–2.20	0.64–2.00	0.59–1.96
Snus use 2 periods	**1.64**	1.45	1.12	1.11
n=97	**1.00–2.71**	0.87–2.41	0.59–2.13	0.57–2.17
Snus use 3 periods	1.63	1.29	1.09	1.01
n=64	0.91–2.93	0.71–2.35	0.56–2.13	0.50–2.06
Snus use 4 periods	1.72	1.31	1.04	0.91
n=47	0.89–3.33	0.67–2.58	0.49–2.24	0.40–2.05
***p* for trend**	**0.018**	0.216	0.826	0.660

Reported values are odds ratios and 95% confidence intervals.
Logistic regression was used to estimate odds ratios and 95% confidence intervals.
*Model A: Crude
•Model B: Adjusted for sex.
→Model C: Adjusted for sex and smoking.
#Model E: Adjusted for sex, smoking, BMI at 16 years, socioeconomic status at 16 years, family history of diabetes mellitus, alcohol consumption at 43 years and physical activity level at 43 years.

snus users (>4 cans/week) [7]. In a cross-sectional study, Wändell et al. found no association between snus use and the metabolic syndrome among the 1850 middle-aged male participants [16]. Our study adds to this previous research by showing that exposure to snus from adolescence to adulthood does not seem to increase the risk of the metabolic syndrome, even with long-term use. Altogether, these three studies indicate that snus use is not a major contributor to the development of the metabolic syndrome, although an association in heavy snus users cannot be excluded.

The results from previous research on the association between snus use and risk of the metabolic syndrome components hypertension, T2DM and raised triglyceride levels are also divergent [17]. In the study by Norberg et al. [7], raised triglycerides were associated with heavy snus use. Also, an increased risk of T2DM for snus users was recently shown in a large sample study by Carlsson et al. [10]. Our study does not support these associations independently of confounders, although a type II statistical error cannot be excluded.

However, the question of increased risk of obesity or central obesity deserves special attention, as an increased risk for snus users has been shown in four previous studies [6-9]. A methodological problem in all of these studies is that ex-smokers among snus users have not been excluded. Hence, the observed association with obesity may be an effect of smoking cessation, which is a known reason for weight gain [29], and perhaps not attributable to snus use. This is supported by a cross-sectional study performed on over 800 men, which concluded that abdominal obesity in snus users is in fact limited to former smokers [27].

Methodological considerations

The main strength of this study is that it uses a cohort with long follow-up time and repeated measurements of tobacco use, and a very high retention rate. This gives us opportunity to follow individuals and assess their tobacco habits and other lifestyle factors in relationship to the outcomes, and minimizes possible recall bias. It also largely allows us to separate exclusive snus users from previous smokers, so that all remaining effects can be attributed to snus use. One of the main limitations is the size of the cohort; it is too small to allow analyses stratified by sex or by amount of snus used. It may also be that the age of 43 is too young to have attained the full effect of snus use on metabolic risk factors. Further, we cannot rule out some residual confounding, for example from changes in socioeconomic status over the life-course.

We have distinguished between two models of life-course epidemiology, which are conceptually distinct. Still, there are well-known methodological difficulties in disentangling the models from each other [30]. As snus use is highly addictive, it is possible that the two models operate in concert. Those who start using snus at an early age will also have increased risk for accumulation of snus use during life. However, we found that only a minority (*n*=47) actually reported snus use at all four follow-ups. This means that although all of those in the group with highest accumulation started early with snus, the reverse is not true; not all who used snus in adolescence kept up the habit throughout the life-course. The distinction between the two models was necessary to empirically differentiate whether there actually is a graded accumulation of risk with increased life-course periods of snus use, or if snus use at a specific life-course period matters for later health.

Further research

It can be argued that exclusive snus users are not representative of snus users in general, as a majority of these have also smoked. Therefore, we suggest studies on metabolic risk and dual use of snus and cigarettes. Moreover, the trends of dynamic tobacco patterns among snus users give rise to questions that demand further studies on shifts in tobacco use during life-course.

Conclusions

Snus use in this cohort shows an increasing prevalence from 16 to 43 years of age, and dual use or former smoking is common among current snus users. However, snus use over the life-course does not seem to be an independent risk factor for developing the metabolic syndrome or any of its components in middle age.

Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Smokeless tobacco (snus) is associated with an increased risk of type 2 diabetes: results from five pooled cohorts

S. Carlsson[1], T. Andersson[1,2], M. Araghi[3], R. Galanti[3,4], A. Lager[3,4], M. Lundberg[3,4], P. Nilsson[5], M. Norberg[6], N. L. Pedersen[7], Y. Trolle-Lagerros[8] & C. Magnusson[3,4]

From the [1]Institute of Environmental Medicine, Karolinska Institutet; [2]Center for Occupational and Environmental Medicine, Stockholm County Council; [3]Department of Public Health Sciences, Karolinska Institutet; [4]Centre for Epidemiology and Community Medicine, Stockholm County Council, Stockholm; [5]Department of Clinical Sciences, Lund University, Skåne University Hospital, Malmö; [6]Epidemiology and Global Health, Department of Public Health and Clinical Medicine, Umeå University, Umeå; [7]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet; and [8]Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Abstract. Carlsson S, Andersson T, Araghi M, Galanti R, Lager A, Lundberg M, Nilsson P, Norberg M, Pedersen NL, Trolle-Lagerros Y, Magnusson C (Karolinska Institutet; Stockholm County Council; Stockholm; Skåne University Hospital, Malmö; Umeå University, Umeå; Sweden). Smokeless tobacco (snus) is associated with an increased risk of type 2 diabetes: results from five pooled cohorts. *J Intern Med* 2017; **281**: 398–406.

Background. Smoking and nicotine exposure increase insulin resistance and the risk of type 2 diabetes. Swedish smokeless tobacco (snus) is high in nicotine, and its use is prevalent in Scandinavian countries, but few studies have investigated snus use in relation to diabetes risk.

Objective. To explore the association between snus use and risk of type 2 diabetes using pooled data from five cohorts.

Methods. Analyses were based on prospective studies conducted between 1990 and 2013 including 54 531 never-smoking men and 2441 incident cases of type 2 diabetes identified through screening, self-reporting and hospital and prescription registries. Hazard ratios (HRs) and 95% confidence intervals (CIs) were assessed and adjusted for age, body mass index, educational level, alcohol consumption and physical activity.

Results. Compared to never users, the HR of type 2 diabetes was 1.15 (95% CI: 1.00–1.32) in current users of snus. In individuals consuming 5–6 boxes per week, the HR was 1.42 (95% CI: 1.07–1.87); in those consuming ≥7 boxes per week, the HR was 1.68 (95% CI: 1.17–2.41). Each additional box of snus consumed per week yielded an HR of 1.08 (95% CI: 1.01–1.16).

Conclusion. Our findings indicate that high consumption of snus is a risk factor for type 2 diabetes. The risk was similar to that in smokers, implying that smokers will not reduce their risk of type 2 diabetes by changing to snus use. The results also support the notion that nicotine increases the risk of type 2 diabetes.

Keywords: meta-analysis, public health, tobacco, type 2 diabetes.

Introduction

Snus is a Swedish smokeless tobacco product that is commonly used in Scandinavia, and 11% of the Swedish population are daily users [1]. It is high in nicotine, but the content of other potentially toxic compounds including nitrosamines is low compared to cigarettes and to other smokeless tobacco products [2, 3]. The sale of snus is banned in the European Union, but Swedish snus is sold in the USA and consumption is also high in Norway. Snus is arguably a less harmful tobacco product than cigarettes [4] as its association with cardiovascular disease and cancer is much weaker [5–9]. However, few studies have investigated its influence on diabetes risk.

Smoking is a well-known risk factor for type 2 diabetes [10]. The association has been attributed to smoking/nicotine-induced insulin resistance [11, 12], but increased systemic inflammation [13] and adverse effects on pancreatic tissue and β-cell function may also contribute to the excess risk [14]. Snus provides similar or even higher nicotine exposure than cigarettes and hence may be a potent promoter of insulin resistance [15].

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Results of studies to date are inconclusive; findings from two small studies based on the Stockholm Diabetes Prevention Programme indicated an increased risk of type 2 diabetes in snus users [16, 17]. By contrast, no association was found in the Swedish MONICA study or in a recent analysis based on two Scandinavian studies in which only never smokers were included, thereby minimizing confounding due to smoking [18, 19]. Despite the latter being the largest study to date including >700 cases, numbers of high consumers were small and therefore risk estimates unstable. There is thus a clear need for additional studies.

To clarify the association between snus use and diabetes risk, we took advantage of a large Swedish pooling project with data from five prospective studies with more than three times more cases than in previous studies. We investigated the risk of type 2 diabetes in relation to the amount and duration of snus use among never-smoking men. This is important given that use of smokeless tobacco is prevalent and increasing in Scandinavia as well as in the USA [20–23]. In addition, such clarification will further our understanding of the health effects of nicotine *per se*, with implications also for users of e-cigarettes [24].

Materials and methods

Study population

We used individual participant data from five Swedish cohort studies conducted between 1991 and 2013: three population-based studies, the Västerbotten Intervention Programme [25], the Stockholm Public Health Cohort [26] and the Malmö Diet and Cancer Study [27]; a study including participants in a national fundraising event, the National March Cohort [28]; and a national twin study, the Screening Across the Lifespan Twin study (SALT) [29]. We restricted the study population to male never smokers, because snus use is rare in women and to minimize confounding from smoking. After exclusion of prevalent cases of diabetes at baseline (n = 2388), our sample for analysis included 54 531 never-smoking men. An overview of the included studies is shown in Table 1. The current project was approved by the Stockholm Regional Ethics Review Board (registration number 2009/971-31/3).

Assessment of tobacco use and potential confounders

Information on tobacco use was collected by questionnaire in all studies, except in SALT in which telephone interviews were used. Individuals were classified as never, former and current users, and current users were categorized according to number of boxes consumed per week and duration of snus use. Information on the amount of snus used was available in all studies (although only for the participants in the 2010 subcohort of the Stockholm Public Health Cohort) except the National March Cohort, and information on duration of use was recorded in all studies except the Malmö Diet and Cancer Study. In addition, the Malmö Diet and Cancer Study did not obtain information on previous use; hence, any former users were included in the reference category of never users. Two of the studies, the Västerbotten Intervention Programme and Stockholm Public Health Cohort, included repeated assessment of tobacco habits and body mass index (BMI) during follow-up. Baseline information on education, alcohol consumption, physical activity and BMI used to control for confounding was primarily obtained by self-report, but the Stockholm Public Health Cohort included registry data on education and anthropometric measurements that were available for calculation of BMI in the Västerbotten Intervention Programme and the Malmö Diet and Cancer Study. Categorization of variables is shown in Table S1.

Identification of diabetes cases

We used all available information collected within each study to identify prevalent diabetes at baseline and incident diabetes during follow-up. Diagnoses of type 2 diabetes were thus identified using several sources and dated according to the first available record. First, registered diagnoses from hospital admissions since 1987 and outpatient specialist care since 2001 were obtained in all studies by record linkage to the National Patient Register, using the unique personal identification number assigned to all Swedish residents. This registry contains information on primary and additional diagnoses coded according to the Swedish version of the International Classification of Disease ninth revision (ICD-9) from 1986 to 1996 (code 250 for diabetes) and ICD-10 thereafter (code E11 for type 2 diabetes and E14 for unspecified diabetes) [30]. Secondly, prescriptions for glucose-lowering drugs were retrieved for all studies from

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Table 1 *Information about the included Swedish cohorts*

	Västerbotten Intervention Programme	Stockholm Public Health Cohort	SALT	Malmö Diet and Cancer Study	National March Cohort	Pooled
Study population	Population-based, Västerbotten County	Population-based, Stockholm County	Twins born in Sweden 1926–1958	Population-based, City of Malmö	Participants in national fundraising event	
No. of participants	22 681	14 519	7498	2944	6889	54 531
Mean age at baseline (years)	45	49	56	59	49	49
Baseline	1992–2013	2002–2010	1998–2002	1991–1996	1997	
End of follow-up	2013	2012	2010	2012	2010	
Mean follow-up (years)	11.6	5.6	9.8	16.3	12.7	10.3
No. of person-years	264 924	86 310	77 348	49 848	72 173	550 603
Current snus users (%)	17	13	13	2	6	13
Additional snus information						
Duration	X	X (for subset)	X		X	
Amount	X	X (for subset)	X	X		
Former users	X	X	X		X	
Identification of diabetes cases	Screening, self-report, national prescription and patient registry	Self-report, national prescription and patient registry	Self-report (baseline), national prescription and patient registry	Self-report and local, national diabetes, prescription and patient registries	Self-report (baseline only), national prescription and patient registries	
No. of incident cases	1033	237	405	461	305	2441
Weight in pooled analysis	0.66	0.11	0.17	0.04	0.03	

SALT, Screening Across the Lifespan Twin study; X, information was available.

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the Prescribed Drug Register [31]. This is a national registry that records all prescriptions since July 2005, according to the Anatomical Therapeutic Chemical (ATC) classification system. ATC group A10 (insulin and oral antidiabetic drugs) was used to identify diabetes. Thirdly, all studies included information on self-reported diabetes at baseline, as well as during follow-up in three studies (Västerbotten Intervention Programme, Stockholm Public Health Cohort and Malmö Diet and Cancer Study). Fourthly, participants from two studies (Västerbotten Intervention Programme and Malmö Diet and Cancer Study) underwent screening with oral glucose tolerance tests at baseline and during follow-up, and individuals with 2-h glucose levels $\geq$12.2 mmol L^{-1} (capillary blood) were classified as having diabetes as stipulated by the World Health Organization. Finally, the Malmö Diet and Cancer Study obtained information from a local diabetes registry and the National Diabetes Register, both of which were used to identify cases [32]. To distinguish between type 2 diabetes and type 1 diabetes, we excluded all cases occurring before 35 years of age and those with a recorded diagnosis of type 1 diabetes according the patient registries.

Statistical analysis

Study-specific hazard ratios (HRs) and their corresponding confidence intervals (CIs) for the association between snus use and type 2 diabetes were assessed by discrete proportional hazard regression and adjusted for age, calendar time, BMI, physical activity, education and alcohol consumption [33, 34]. Age and calendar time in years were combined into a categorical variable to account for both age and the fact that assessment of diabetes changed during follow-up (primarily because of the introduction of the Prescribed Drug Register in 2005).

Person-time was assessed from the date of baseline to the date of censoring or diagnosis based on (i) first recording in the National Prescribed Drug Register, inpatient or outpatient registries, (ii) self-reported diabetes by questionnaire or (iii) screening-detected diabetes, or the end of follow-up, whichever came first. Interaction terms for snus and the timescale variables age and calendar time were nonsignificant ($P > 0.2$), indicating that the assumption of proportional hazards was not violated. Study-specific HRs were calculated and pooled using the inverse variance method [35]. We estimated the fraction of cases attributable to snus use by scoring the fully adjusted model using the original data, but with the exposed set as unexposed.

Results

Characteristics

A total of 54 531 men were included in this study of whom 2441 developed diabetes over 556 777 person-years; this corresponds to an incidence of 4.4 per 1000. Of the incident cases, the first registration was by self-report, through screening and from the prescription, inpatient and outpatient registries for 8%, 3%, 59%, 19% and 10%, respectively. Mean age at baseline was 49 years, and the average follow-up period was 10 years. The prevalence of snus use was 13% in the pooled cohort; the rate was highest in the Västerbotten Intervention Programme (17%) and lowest in the Malmö Diet and Cancer Study (2%) (Table 1). Mean consumption among current users was four boxes of snus per week, and 31% of users consumed >4 boxes per week. Snus use tended to be more prevalent in individuals with high alcohol consumption, high BMI, young age and secondary school education (Table S1). No clear association was seen between snus use and physical activity level. The HRs of diabetes in relation to age, BMI, physical activity, education and alcohol consumption are shown in Table S2.

Snus use and the risk of type 2 diabetes

Overall, current snus users tended to have an increased risk of diabetes (pooled HR 1.15, 95% CI: 1.00–1.32), in contrast to former users (pooled HR 0.86, 95% CI: 0.71–1.05) (Fig. 1). A clear indication of an excess risk was only seen in the Västerbotten Intervention Programme, but CIs in the other studies were wide. Heterogeneity expressed as I^2 was 37%, indicating low–moderate heterogeneity across studies. Analyses of the dose–response relationship indicated that the risk was increased from consumption levels above four boxes per week and at consumption levels of $\geq$7 boxes per week, estimated HR was 1.68 (95% CI: 1.17–2.41) (Fig. 2). Tendencies towards increased risks in the highest consumption group ($\geq$7 boxes per week) were seen in all studies (Table 2). In snus users, each additional box of snus consumed per week yielded an HR of 1.08 (95% CI: 1.01–1.16). There was no clear association between duration of snus use and the risk of type 2 diabetes (Table 2), possibly reflecting a lower absolute risk of diabetes

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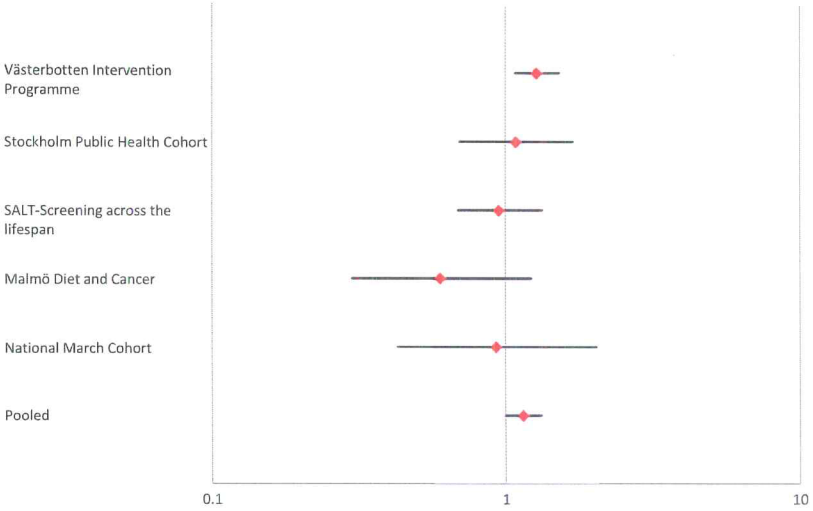


Fig. 1 *Current snus use and risk of type 2 diabetes. Study-specific and pooled hazard ratios and 95% confidence intervals.*

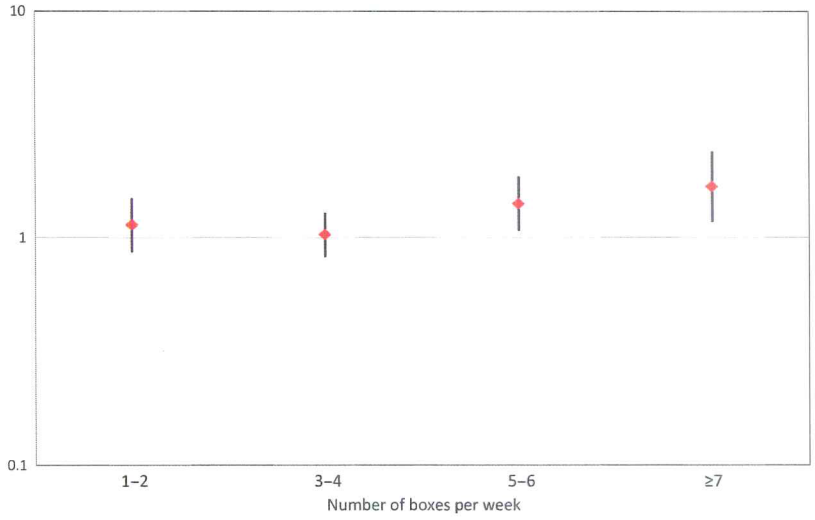


Fig. 2 *Number of boxes of snus consumed per week and risk of type 2 diabetes. Pooled hazard ratios and 95% confidence intervals.*

in younger individuals (who also have shorter duration of disease), leading to higher HRs in the short-duration group. From the calculation of attributable fractions, it appeared that, under the assumption of a causal effect and no measurement error, 13% of all cases of type 2 diabetes among the snus users could be attributed to snus use.

Sensitivity analyses were performed based on data from the Västerbotten Intervention Programme. First, we adjusted the HRs for family history of diabetes, but this had minor influence: HR 1.80, 95% CI: 1.15–2.83 in high (≥7 boxes per week) consumers. We then excluded all individuals in this study who were prescribed insulin during the first year following diabetes diagnosis in an attempt to exclude any remaining individuals with type 1 diabetes. This was performed for the period in which we had access to data from the prescription registry, that is 2006–2013, and again, the HRs were virtually unchanged: HR 1.75, 95% CI: 1.03–2.99 in high consumers.

Discussion

Findings from this large pooling project indicate that a high consumption of snus increases the risk of type 2 diabetes. In fact, our calculation of the

Table 2 *Snus use and risk of type 2 diabetes: study-specific and pooled results*

	Västerbotten Intervention Programme		Stockholm Public Health Cohort[a]		SALT		Malmö Diet and Cancer Study[b]		National March Cohort[c]		Pooled	
	Cases/ person-years	HR (95% CI)	Cases/ person-years	HR (95% CI)	Cases/ person-years	HR (95% CI)	Cases/ person-years	HR (95% CI)	Cases/ person-years	HR (95% CI)	Cases/ person-years	HR (95% CI)
Snus use												
Never	794/200 493	Reference	193/68 502	Reference	349/63 166	Reference	453/48 743	Reference	286/64 751	Reference	2075/445 655	Reference
Current	165/41 029	1.28 (1.08–1.52)	25/11 060	1.09 (0.70–1.69)	43/9469	0.95 (0.69–1.33)	8/1105	0.60 (0.30–1.22)	7/3104	0.93 (0.43–2.03)	248/65 767	1.15 (1.00–1.32)
Former	74/23 402	0.84 (0.66–1.07)	19/6748	1.10 (0.67–1.78)	13/4713	0.66 (0.38–1.16)			12/4318	0.94 (0.51–1.71)	118/39 181	0.86 (0.71–1.05)
Number of boxes per week (current users only)												
1–2	40/10 916	1.12 (0.81–1.54)	0/277	–	11/1792	1.17 (0.64–2.15)	3/265	1.19 (0.37–3.78)			54/12 973	1.14 (0.86–1.50)
3–4	60/16 859	1.17 (0.89–1.52)	3/1153	1.50 (0.45–5.00)	18/5363	0.74 (0.45–1.20)	2/432	0.32 (0.08–1.28)			83/23 807	1.03 (0.82–1.29)
5–6	45/10 007	1.48 (1.09–2.02)	2/520	2.62 (0.62–11.02)	5/1142	1.02 (0.42–2.50)	2/268	0.71 (0.17–2.89)			54/11 937	1.42 (1.07–1.87)
≥7	20/3247	1.79 (1.14–2.80)	1/417	1.50 (0.20–11.28)	9/1129	1.51 (0.76–2.98)	1/58	1.30 (0.18–9.39)			31/4851	1.68 (1.17–2.41)
1–4	100/27 775	1.15 (0.93–1.42)	3/1430	1.15 (0.35–3.81)	29/7155	0.86 (0.58–1.27)	5/697	0.56 (0.23–1.37)			137/37 057	1.08 (0.90–1.29)
≥4	65/13 254	1.56 (1.20–2.03)	3/937	2.09 (0.63–6.93)	14/2271	1.29 (0.74–2.24)	3/326	0.84 (0.27–2.65)			85/16 788	1.43 (1.15–1.79)
Duration of snus use (current users only)												
<30 years	58/23 671	1.40 (1.06–1.84)	2/1547	1.96 (0.45–8.58)	6/2068	0.82 (0.36–1.87)			0/1854	–	66/27 286	1.34 (1.03–1.73)
≥30 years	107/17 358	1.22 (1.00–1.51)	4/1020	1.17 (0.41–3.36)	35/7254	0.97 (0.68–1.40)			6/1137	1.50 (0.65–3.46)	152/26 769	1.17 (0.98–1.39)

[a]Information on number of boxes and years of snus use was only available for a subset of participants who responded to the 2010 questionnaire. The reference group of never smokers consisted of 58 cases per 26 860 person-years. [b]Information on year of snus use was not available. [c]Information on number of boxes consumed was not available. HRs were adjusted for age, calendar year, body mass index, physical activity, education and alcohol consumption.

HR, hazard ratio; CI, confidence interval; SALT, Screening Across the Lifespan Twin study.

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attributable fractions suggests that 13% of cases among snus users were attributed to their snus habit. Our results were consistent with the findings of two small previous studies [16, 17]. By contrast, no association was found in two other studies, including a recent study by us [18, 19]. However, lack of power precluded the possibility of drawing any firm conclusions regarding the risk in high consumers in these previous studies.

We observed elevated risks at consumption levels above four boxes per week and in those consuming ≥7 boxes per week, HR was estimated at 1.68. By comparison, Pan *et al.* [10] reported an HR of 1.57 in heavy smokers (corresponding to one pack of cigarettes per day) in the latest meta-analysis based on 88 observational studies. Hence, smoking and snus use appear to be equally strong risk factors for type 2 diabetes. This is plausible because mechanistic studies also point to nicotine as the possible mediator of such a risk increase. Nicotine can inhibit insulin sensitivity and may stimulate release of cortisol, thus promoting abdominal obesity and subsequently insulin resistance [11, 12, 36]. The nicotine content of one portion of snus is higher than that of one cigarette, but only 10–20% of the nicotine in snus reaches the blood stream [37]. Experimental studies indicate that nicotine is absorbed more rapidly from cigarettes, and after consumption of one cigarette, compared with one portion of snus, the smoker will have a higher peak concentration; however, nicotine levels will be higher in the snus users after 2 h [15]. It has also been shown that regular users of snus and cigarettes tend to have similar blood nicotine levels [38].

A particular strength of this study is the size which enabled restriction of the sample to never smokers, and hence careful control for confounding by smoking, as well as analyses of dose–response relationships. We also adjusted for several other diabetes risk factors including BMI, physical activity, alcohol consumption and education. Information on family history of diabetes was only available in the Västerbotten Intervention Programme, but a sensitivity analysis did not indicate that history of diabetes was a confounder. One concern is that there was no uniform way to identify diabetes patients across all studies. Hence, to optimize case ascertainment, we relied on different sources for each study, including national and local health registries, self-reporting and screening. The incidence of diabetes in the pooled cohort was in line with previous Swedish reports, implying that the assessment method was robust [39]. Furthermore, data from the National Prescribed Drug Register, which has complete information on pharmacologically treated diabetes in Sweden, were available from 2005 in all studies. With regard to the validity of self-reporting, it is noteworthy that when we compared prescription data to self-reports in a subset, we found that 92% of individuals with self-reported diabetes had been prescribed antidiabetic drugs. Undiagnosed diabetes is of concern but, fortunately, the largest and most influential study (Västerbotten Intervention Programme) was also the most detailed as incidence of diabetes could be assessed through screening as well as through self-reporting and registry data. Furthermore, repeated information on both BMI and tobacco use was available during follow-up, allowing us to take into account lifestyle changes after baseline. We used age at onset above 35 years as an indicator of type 2 diabetes and excluded all patients with records of type 1 diabetes in the patient registries. A sensitivity analysis in which we also excluded all cases with insulin prescribed during the first year following diagnosis yielded similar results. Yet, some cases of type 1 diabetes or LADA (latent autoimmune diabetes in adults) may have been misclassified as type 2 diabetes in our main analysis (but this is unlikely to be dependent on snus use), and hence dilute the associations.

The prevalence of snus use varied greatly between studies, which is to be expected as its use has a known north–south gradient in Sweden. Nevertheless, for high consumers (≥7 boxes per week), we consistently found HR values >1 across all studies underlining the robustness of the findings. Self-reported information on tobacco use is a limitation. However, the questionnaire used to elicit information about tobacco use in the Västerbotten Intervention Programme has been validated, and the correlation between self-reported number of boxes of snus used per week was highly correlated with plasma cotinine concentrations ($r = 0.60$, $P = 0.003$) [40]. It is possible that some snus users started smoking during follow-up. Yet, in analyses using updated tobacco exposure employed as a time-varying covariate during follow-up (in the two cohorts in which such data were available), excess risks were still noted in high consumers. Indeed, 91% of participants in the Västerbotten Intervention Programme were stable never smokers at baseline and follow-up, and only 1% of never smokers reported onset of daily smoking. We could

not differentiate between loose and portion-packed snus which have different nicotine yields and hence may affect type 2 diabetes risk differently [38].

Conclusion

Our findings indicate that high consumption of snus increases the risk of type 2 diabetes. This is in contrast to findings for cardiovascular disease as well as cancer [5–9, 41]. The diabetes-promoting effect of cigarettes and snus is hence likely to be mediated by nicotine, whereas the tobacco-related risks of cardiovascular disease and cancer may be due to other components of cigarette smoke. In conclusion, these findings suggest that any health benefit of changing from smoking to snus use does not include reduction in diabetes risk. In addition, this novel finding warrants attention from public health authorities, in the light of the diabetes epidemic and increasing popularity of snus as well as other smoke-free modes of nicotine use such as e-cigarettes [24, 42]. We could not explore the association between snus use and diabetes risk in women, and this is an important topic for future studies.

Author contributions

All authors have made substantial contributions to (i) the conception or design of the study, or the acquisition, analysis or interpretation of the data and (ii) drafting of the manuscript or revising it critically for important intellectual content; all authors have also approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest statement

No conflicts of interest to declare.

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Correspondence: Sofia Carlsson, Institute of Environmental Medicine, Karolinska Institutet, Box 210, S-171 77 Stockholm, Sweden. (fax: 46 8 336981; e-mail: Sofia.carlsson@ki.se).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Numbers (and percentages) of snus users at baseline across categories of age, BMI, alcohol consumption, education and physical activity.

Table S2. The risk of type 2 diabetes in relation to age, BMI, alcohol consumption, education and physical activity.■

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Snus undermines quit attempts but not abstinence: a randomised clinical trial among US smokers

Matthew J Carpenter,[1,2,3] Amy E Wahlquist,[2] Jessica L Burris,[1,4] Kevin M Gray,[1] Elizabeth Garrett-Mayer,[2,3] K Michael Cummings,[1,2,3] Anthony J Alberg[2,3]

[1]Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina (MUSC), Charleston, South Carolina, USA
[2]Department of Public Health Sciences, MUSC, Charleston, South Carolina, USA
[3]Hollings Cancer Center, MUSC, Charleston, South Carolina, USA
[4][Current address] Department of Psychology & Markey Cancer Center, University of Kentucky, Lexington, Kentucky, USA

Correspondence to
Dr Matthew J Carpenter, Department of Psychiatry & Behavioral Sciences, Medical University of South Carolina, 68 President St., MSC 955, Charleston, SC 29425, USA; carpente@musc.edu

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ABSTRACT

Background Observational studies and a few clinical trials suggest that use of low nitrosamine smokeless tobacco (snus) can facilitate smoking cessation. To better understand the real-world impact of snus on smoking behaviour, a large-scale, long-term clinical trial of naturalistic snus use among smokers is needed.

Study design A nationwide clinical trial compared abstinence outcomes among smokers who were randomised to receive free samples of snus versus not. Participants (N=1236) were recruited throughout the US and assessed for 1 year following a 6-week naturalistic sampling period, with high retention throughout. Primary outcomes included self-reported quit attempts, floating abstinence (any 7-day period of non-smoking) and 7-day point-prevalence abstinence at 6 months and 12 months. Secondary outcomes were changes in smoking, motivation and confidence to quit and adverse events. No tobacco industry support was provided.

Results Within snus group, 82% used at least once, and 16% were using regularly at end of sampling period. Compared to control participants, smokers in the snus group were less likely to make any quit attempt (RR=0.83; 95% CI 0.70 to 1.00), and any 24 h quit attempt (RR=0.77; 95% CI 0.63 to 0.95). There were no group differences on any measure of abstinence.

Conclusions Provision of snus in a naturalistic context resulted in minimal uptake, and as a whole, undermined quit attempts and did not increase smoking abstinence. Results do not support the unguided, free provision of snus among smokers not motivated to quit as a means to facilitate quit attempts.

Trial registration number NCT01509586, Results.

INTRODUCTION

The significant increase of alternative tobacco products has given smokers many more options in addition to combustible cigarettes. Low nitrosamine smokeless tobacco, that is, snus, is one such product, widely used in Scandinavian countries. Compared to conventional cigarettes and conventional smokeless tobacco products, snus is associated with reduced harm to individual users.[1–7] For example, in Sweden where snus is widely prominent, rates of lung cancer have dramatically declined[8] without increased risk of other cancers.[9–12]

The population impact of snus depends on if and how it is used among smokers, and particularly whether it is adopted as a substitute for cigarettes. Snus could conceivably help smokers to quit cigarettes, or, alternatively, it could maintain dependence, allowing them to circumvent smoking restrictions, and thus, undermine smoking cessation. Scandinavian studies[13–20] show an association between use of snus and increased quitting of conventional cigarettes. However, most of these studies have been observational, either cross-sectional or cohort studies, and therefore, are limited by self-selection of participants.

The optimal test of this question is a randomised trial, of which there have been four. Two such studies, both from Europe, were cessation trials. Danish smokers wanting to quit were randomised to receive snus or not.[21] Abstinence rates were significantly higher at 7 weeks of follow-up (36% vs 21%; OR=2.1) but did not persist after 6 months (23% vs 21%; OR=1.3). A larger, longer and placebo-controlled study from Serbia found a threefold increase in abstinence at 6 months (9.5% vs 3.7%, adjusted OR/AOR=2.7), and a nearly twofold increase at 1 year (15.8% vs 9.3%, AOR=1.9).[22] Among smokers wanting to stop smoking, this evidence suggests snus might be an effective substitute for cigarettes. However, no randomised studies have investigated the impact on smoking behaviour of providing snus to smokers in a naturalistic setting among smokers not motivated to quit.

There have also been two large-scale clinical trials of snus within the USA. The first was a placebo controlled trial in which 250 smokers wanting to quit were randomised to receive active/placebo snus for 14 weeks, with brief adjunctive behavioural support.[23] Abstinence rates were low, overall, and point-prevalence rates were higher among smokers receiving active vs placebo snus at 6 weeks (18% vs 9%; OR=2.3), 16 weeks (18% vs 8%; OR=2.4), and 28 weeks of follow-up (13% vs 7%; OR=1.9). A more recent study compared effects of snus versus nicotine gum (an active comparator, in contrast with the above studies) among 391 smokers who were 'interested in completely switching'.[24] Abstinence from cigarettes was nearly identical after 3 months of follow-up (27% vs 25%; independently calculated relative risk=1.1). A separate meta-analysis of only the Fagerström (US)[23] and Joksić (Serbian)[22] RCTs suggested a threefold increase in abstinence in favour of snus.[25]

The existing evidence leads to the following inferences: (1) only a small number of randomised trials have tested the impact of snus on smoking behaviour, (2) most studies suggest positive (or sometimes non-significant) effects from snus; no published study has shown negative effects on quitting and (3) all randomised trials, to date, have been abstinence focused, recruiting smokers wanting to quit and providing snus with explicit instructions to do so. To better understand the regulatory issues surrounding snus, a study is

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needed that is both naturalistic (unguided use of snus) and randomised (avoiding biases of self-selection). Additionally, since at any time the majority of smokers are not motivated to quit,[26 27] it is also important to assess how smokers across a broader motivational continuum respond to snus. The present randomised trial was designed to examine the impact of snus use within a naturalistic, non-cessation context. It is the largest and longest trial of snus usage among US smokers.

METHODS

Study overview

Smokers across the USA (N=1236) who did not want to quit were randomised to receive a 6-week supply of snus or not, after which everyone was advised to quit all tobacco products and then followed for 12 months. The primary outcome was incidence and duration of quit attempts. Secondary outcomes were point-prevalence abstinence from cigarette smoking, at 6 months and 12 months, smoking reduction, and associated measures of quitting. No support from the tobacco industry was provided. Given our naturalistic focus, we were granted a waiver of Investigational New Device from the Food and Drug Administration (FDA). All procedures were approved by local (Medical University of South Carolina) IRB review.

Participant recruitment, eligibility, and randomisation

A CONSORT flow of recruitment is shown in figure 1. Participants were recruited via national market research panels (Knowledge Networks (KN), now GfK). KN emailed to potential study participants a link to a brief study description, and interested individuals then completed a brief online screener to determine eligibility (criteria below). A more complete study description was offered to individuals who were eligible and interested in study participation. This description did not explicitly mention any product by name, but rather stated that the study was a test of a 'new, potentially safer tobacco product. While there is no such thing as a safe tobacco product, some evidence suggests that the product we will be testing could be safer than conventional cigarettes'. This recruitment step also emphasised that (1) smokers would be randomised to receive the new tobacco product or not, (2) product use would be entirely self-determined and (3) there would be no requirement to quit. Those who affirmed interest provided name and full contact information, which was forwarded to the central study office. Study personnel then mailed to each individual a packet consisting of (1) two copies of the consent form, (2) a prepaid, preaddressed return envelope and (3) a baseline questionnaire. Many individuals failed to respond to this mailing. Upon receipt of signed consent at the central study office, individuals were contacted for the first study call, formally commencing study participation. Thus, the final sample of enrolled participants consisted of those who were eligible, returned consent, and completed the initial study call (N=1236). Recruitment took place between October 2011 and August 2013. Among those who were eligible for study participation, there were no significant differences between those who did and did not return consent in terms of age, gender, race, or cigarettes smoked per day.

Participants met the following eligibility criteria: (1) age ≥19 years, (2) daily cigarette smoker of ≥10 cigarettes per day, (3) English speaking, (4) live in the contiguous USA, (5) non-use of smokeless tobacco or other reduced exposure products in prior 6 months, (6) not breastfeeding, pregnant, or planning a pregnancy, (7) no cardiovascular trauma (stroke, myocardial infarction, uncontrolled hypertension) in past 6 months, (8) no quit attempt of ≥1 week in past 6 months, (9) no use of cessation pharmacotherapy in past 3 months and (10) unmotivated to quit smoking in next 30 days, operationalised as (1) ≤7 on a 0–10 contemplation ladder[28] and (2) no intention to quit in the next 30 days (asked yes/no).[29]

Participants were allocated to groups using mixed block randomisation (2–8 participants; NQuery Advisor 6.0), stratifying on state-specific cigarette tax (high vs medium vs low), under the notion that uptake of snus and its impact on cessation might vary by state-specific tobacco policies.[30 31] Given our large sample size, we did not stratify on individual-level predictors of quit attempts (eg, dependence, smoking history).

Sampling period and follow-up

During the initial call, participants completed a brief assessment to verify smoking status. Participants were then informed of group status (snus vs control), and briefed on upcoming calls and reimbursement schedule. The sampling period lasted 6 weeks, with an interim call at week 3 (figure 2). The rationale for a 6-week sampling period was to provide (1) participants with sufficient time to sample snus and get adjusted to it and (2) the research team with sufficient time to mail product. At the end of the sampling period (week 6), participants in both groups were given a brief prompt to quit all tobacco products, including snus if still being used. All participants were given a referral to their state quitline. Calls to snus participants were slightly longer in duration during the sampling period as compared with the control group (mean 17.8 vs 13.8 min), but did not meaningfully differ for follow-up calls (mean 9.0 vs 8.3 min for snus, control groups, respectively).

All participants were followed for an additional 12 months, for a total of eight follow-up calls (see figure 2 for study retention). Across all 9888 (8×1236) follow-up calls, 87% were completed (84% snus vs 90% control; $p<0.01$).

Snus group

During the first call, participants randomly assigned to receive snus were given a brief product overview, how it differentiates from traditional smokeless tobacco, and why it might be considered safer than cigarettes. No explicit usage instructions were given, though participants were told they could use snus to cut down and/or quit smoking (ie, completely switch to snus), and/or get through smoking restrictions. A repeated theme was that choice and manner of use was entirely self-determined. In all mailings and phone contacts, participants were reminded that snus should (1) not be shared with anyone, (2) be kept away from children and (3) not be used by anyone pregnant or who could be pregnant.

We considered, but opted against, a menu of snus products from which to choose, and rather chose Camel Snus for several reasons. A menu of options would create unwanted variance and need for larger sample size. Evidence available at the time of study initiation,[32] and since,[33] suggested greatest relief of craving and high favourability for Camel Snus. It was also marketed the most aggressively and was at the time of study initiation the only major smokeless tobacco product with its own dedicated website (camel.com).

Participants were given choice of flavour (Winterchill or Robust), 2.5–2.6 mg nicotine per pouch,[24 34] and were mailed 10 tins (15 pouches per tin; total of 150 pouches) to use over the next 3 weeks, split into two mailings. We knowingly oversupplied smokers with snus so that amount of uptake would not be artificially suppressed. At the interim-sampling phone call (week 3), use of snus was assessed in addition to all other

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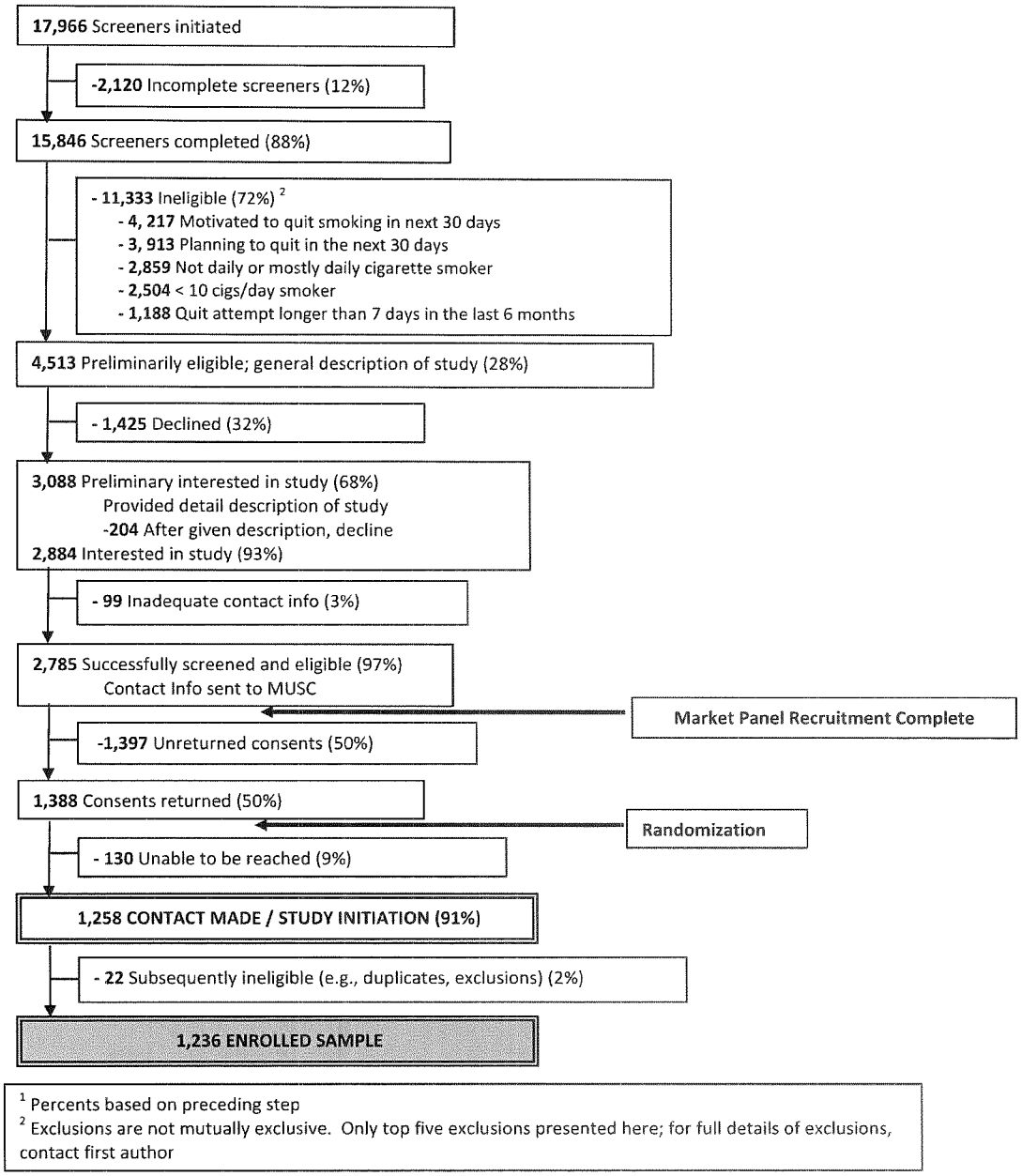


Figure 1 CONSORT recruitment flow. MUSC, Medical University of South Carolina.

outcomes, and again participants were asked if they wished to receive additional product. Another mailing of snus (up to 10 tins) based on preferred flavour was sent, again split across two shipments. Snus was provided free of charge to all participants.

Control group

Participants were matched on all call schedules, including the sampling period during which they were instructed to smoke/reduce/quit cigarettes as they wish. To equate for free product, these participants received extra reimbursement.

Assessments

The primary outcome was the incidence of quit attempts, both any self-defined and any lasting at least 24 h, per Centers for Disease Control definition.[35] We also assessed cessation, defined as 7-day point-prevalence abstinence, at both 6 and 12 months (ie, 32 and 58 weeks), and in all cases report abstinence among the entire sample. In addition, we assessed floating abstinence, that is, any 7-day period of non-smoking at any point during the study. Floating abstinence is a common outcome in cessation-induction studies, allowing for the possibility that successful abstinence may occur at any time.[36 37] The amount of smoking was based on the average number of cigarettes smoked in the past 7 days. Motivation and confidence to quit were assessed via standard (0–10) ladders,[28 38] each asked in reference to quitting within next month, and each with verbal anchors at polar ends only to minimise clustering around labels (0='very definitely no' vs 10='very definitely yes').

Statistical considerations and data analysis

The study was designed to detect a difference in quit attempts between snus and control groups. With a sample size of 1250,

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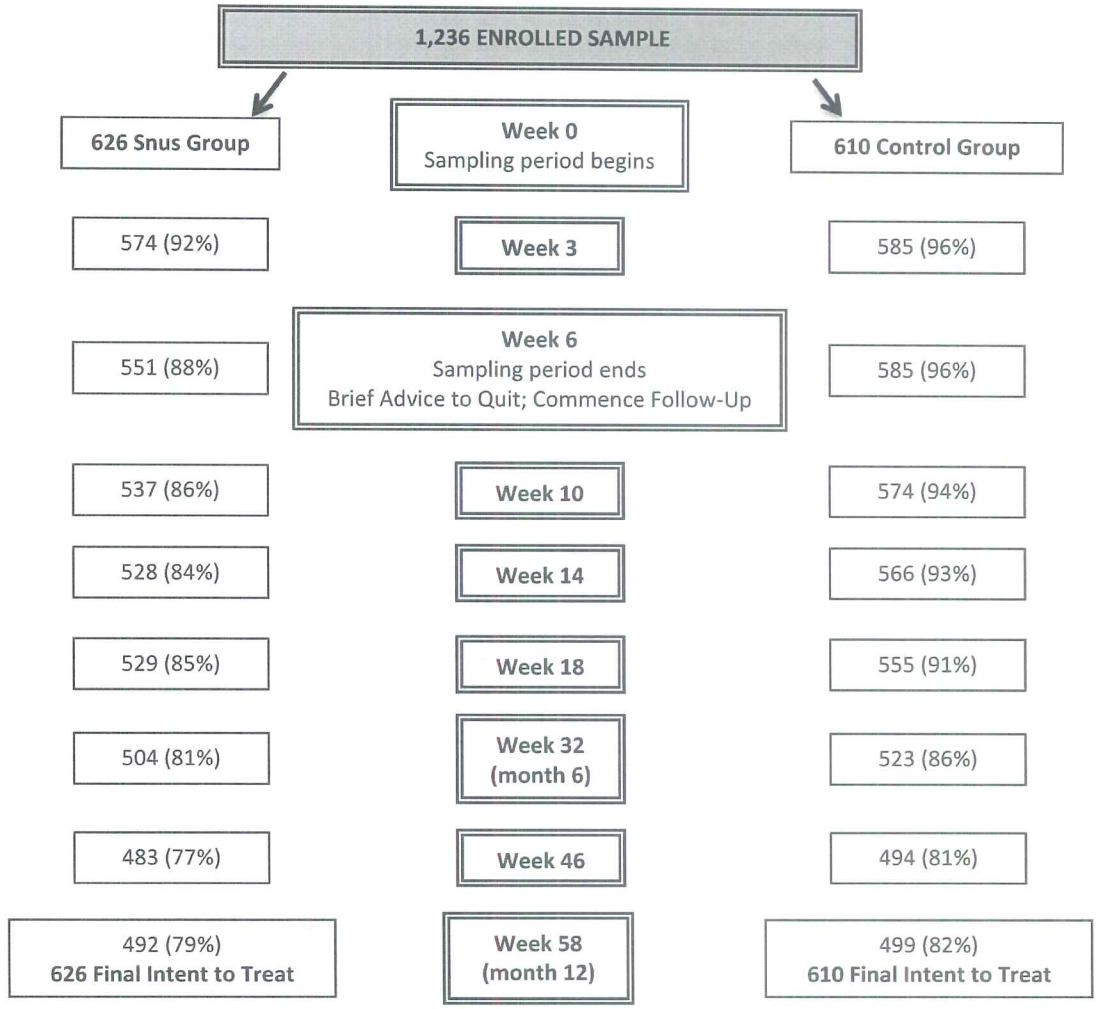


Figure 2 Study retention.

the study was adequately powered (82%) to detect an 8% group difference in the prevalence of quit attempts (eg, 32% vs 40%) with a two-sided α of 0.05. The 32% base rate for quit attempts was based on our prior nationwide study of unmotivated, untreated smokers,[39] conservatively proportioned over a 1-year follow-up. All analyses are based on intent-to-treat, in which participants with missing data were presumed to neither have made a quit attempt nor be abstinent. For the primary outcomes of quit attempts, 24 h quit attempts, 7-day point prevalence abstinence at 6 months and 12 months, and floating abstinence, χ^2 tests were used to compare the rates between groups. Mixed models with repeated measures were used to evaluate outcomes measured multiple times during the study, such as motivation and confidence to quit smoking. Each of these models included group, visit and the group by visit interaction.

RESULTS

Participant characteristics

Participants were generally female, self-identified Caucasian, and middle-aged (table 1). Most were concerned of the health effects of smoking. By design, participants were minimally motivated to quit smoking, and by expectation were minimally confident to quit and had limited quit history in the past year. With the exception of home smoking policies, there were no differences between groups on baseline characteristics.

General snus use

Within the snus group, 96% opted to receive snus at least once during the sampling period, and 82% used it at least once. Forty-two per cent reported use at the end of the sampling period: 165 (26%) using irregularly (1–5 days of preceding week) and 98 (16%) using regularly (6–7 days). Less than half (40%) reported snus use during the 1-year follow-up period, but only 11% reported independent purchase. A small number of participants reported past-week use at 6 and 12-month follow-up (8% and 4%, respectively). Within the control group, ≤1% of participants reported any snus use at any given follow-up week, on their own accord.

Quit attempts and abstinence

Snus participants were significantly less likely than control participants to report a quit attempt of any kind during the course of the study (table 2). During the 6-week sampling period, there were no differences in rates of any quit attempt (6.6% vs 5.3% in snus and control groups, respectively) or in rates of any 24 h quit attempts (3.7% vs 3.0%). Restricting analyses to the follow-up period alone, those in the snus group were

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Table 1 Sample characteristics

	Snus group n=626	Control group n=610	p Value
Age, mean (SD)	48.7 (12.5)	48.7 (12.6)	0.9
Female (%)	70	65	0.1
Race			0.7
Caucasian (%)	89	87	
African-American (%)	9	10	
Other (%)	2	3	
Married or within a couple (%)	47	51	0.2
Full/part employment (%)	42	45	0.3
Education			0.4
HS (%)	32	32	
>HS (%)	64	62	
Very/somewhat concerned of health (%)	74	74	0.9
Age began smoking	16.9 (3.7)	17.0 (4.4)	0.5
Cigarettes per day	20.1 (8.7)	19.9 (8.4)	0.7
Heaviness of smoking index	3.5 (1.2)	3.5 (1.2)	0.9
Quit attempt, past year (%)	9	8	0.6
Live with other smoker	45	47	0.5
Allow smoking indoors at home (%)	55	50	0.01
Heard of low nitrosamine SLT (%)*	76	75	0.6
Prior use of low nitrosamine SLT, lifetime (%)*	7	8	0.4
Heard of Camel snus (%)	70	68	0.7
Prior use of Camel snus, lifetime (%)	5	5	0.4
Intend to quit smoking, next month	1.4 (2.3)	1.3 (2.4)	0.8
Confidence to quit smoking	2.6 (3.1)	2.7 (3.0)	0.4

*Low nitrosamine SLT: Ariva, Revel, Exalt, Stonewall, Camel Snus, Marlboro Snus (exclusive of both e-cigarettes and traditional SLT products).
HS, high school; SLT, smokeless tobacco.

significantly less likely to make any quit attempt (23.2% vs 29.5%; RR=0.78; 95% CI 0.65 to 0.95), and any 24 h quit attempt (18.2% vs 24.4%; RR=0.75; 95% CI 0.60 to 0.93). There were no differences in rates of floating or point-prevalence abstinence, either at 6-month or 12-month follow-up (table 2). Within the snus group alone, rates of cigarette-only versus all tobacco (cigarettes + snus) abstinence were nearly identical: 6% versus 5% at 6 months, and 7% versus 7% at 12 months. Among those who made a quit attempt, the longest duration of abstinence also did not vary between snus (61.1 days; SD=98.4) and control (62.3 days; SD=97.3) participants.

Use of cessation medication at any time during the study was significantly lower among snus participants: 9% vs 13% (RR=0.69; 95% CI 0.50 to 0.96). Use of behavioural treatment for smoking cessation (physician advice, counselling, classes, quitlines) was comparable between groups (25% snus vs 24% control), as was use of any cessation support, either medication or behavioural treatment (28% vs 27%).

Smoking reduction

Participants in both groups reduced the number of cigarettes smoked per day by 23% from baseline to the 1-year follow-up (p<0.0001). There were no between-group differences: 22.4% and 22.9% of snus and control participants, respectively, achieved a 50% reduction in smoking level.

Motivation/confidence to quit

In parallel with the above, there were significant increases over time in both motivation and confidence to quit smoking in the next 30 days (both p<0.0001), but no significant between-group differences and motivation to quit remained low both at the end of the sampling period (mean snus group: 2.0 vs control: 1.9) and at final follow-up (mean snus group: 3.0 vs control: 3.3).

Subgroup comparisons

Sensitivity analyses separately examined incidences of (1) any 24 h quit attempt (figure 3A) and (2) floating abstinence (figure 3B), each as measured across the entire study duration inclusive of the sampling period. Provision of snus significantly undermined quit attempts for participants who were: female; younger than 50 years of age; smoking ≥20 cigarettes per day; participants with tobacco-related health concerns; and who had a history of smokeless tobacco use. There were no differences in rates of floating abstinence within any subgroups tested.

We next compared subgroups of snus participants who (1) never used snus, (2) tried product during sampling period but discontinued use (ie, 'samplers'), (3) reported current infrequent use at end of sampling period, and (4) reported current frequent use at end of sampling period. Compared with control group participants, never and current infrequent users were less likely to make a quit attempt. However, current frequent users of snus at the end of the sampling period were marginally more likely to make a quit attempt as compared with control group participants (RR=1.32; 95% CI 0.99 to 1.83), and were significantly more likely to achieve floating abstinence during the study (RR=1.67; 95% CI 1.15 to 2.43). Within the snus group alone (figure 3C, D), with never users as a referent, participants who become current frequent users at the end of the sampling period were more likely to try (RR=2.24; 95% CI 1.30 to 3.86) and succeed (at any point) in quitting (RR=2.21; 95% CI 1.18 to 4.13).

Table 2 Quit attempts and abstinence

	Number (%) of participants				
	Snus (n=626)	Control (n=610)	Relative risk (95% CI)	Risk difference (95% CI)	p Value
Any quit attempt, ever within study	161 (25.7)	188 (30.8)	0.83 (0.70 to 1.00)	0.05 (0.0008 to 0.10)	0.05
Any 24 h quit attempt, ever within study	124 (19.8)	156 (25.6)	0.77 (0.63 to 0.95)	0.06 (0.01 to 0.10)	0.02
Floating abstinence*	93 (14.9)	99 (16.2)	0.92 (0.71 to 1.19)	0.01 (−0.03 to 0.05)	0.5
Week 32 PPA	39 (6.2)	34 (5.6)	1.12 (0.72 to 1.75)	0.006 (−0.02 to 0.03)	0.6
Week 58 PPA	32 (5.1)	34 (5.6)	0.92 (0.27 to 1.47)	0.005 (−0.02 to 0.03)	0.7

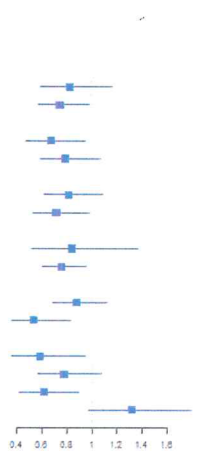
*Floating abstinence: Any 7-day period of non-smoking, ever within study.
PPA, point-prevalence.

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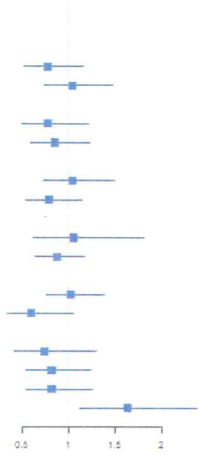
A 24-hr Quit Attempt: Snus vs. Control Group

	Snus #	Group N	Control #	Group N	Relative Risk
Gender					
Male	43	188	58	211	0.83
Female	81	438	98	399	0.75
Age (yrs)					
<50	46	270	60	238	0.68
>=50	60	285	75	282	0.79
CPD (baseline)					
<20	68	327	80	316	0.82
>= 20	56	299	76	293	0.72
Concerned for Health					
No	25	164	29	160	0.84
Yes	99	459	127	450	0.76
Prior SLT Use					
No	99	465	103	424	0.88
Yes	25	161	53	186	0.54
Snus Use					
Never	15	100	156	610	0.59
Sampler	36	180			0.78
Current Infrequent	26	165			0.62
Current Frequent	33	98			1.32



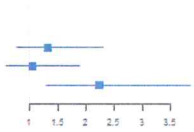
B Floating Abstinence[2]: Snus vs. Control Group[3]

Snus #	Control #	Relative Risk
32	46	0.78
61	53	1.05
32	36	0.78
45	52	0.86
52	48	1.05
41	51	0.79
24	22	1.06
69	77	0.88
77	68	1.03
16	31	0.6
12	99	0.74
24		0.82
22		0.82
26		1.63



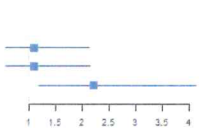
C) 24-hr Quit Attempt: Within Groups of Snus Group

Snus Group Only	Snus Users #	Never Users #	
Sampler	36	15	1.33
Current Infrequent	26		1.05
Current Frequent	33		2.24



D) Floating Abstinence[2]: Within Groups of Snus Group[3]

Snus Users #	Never Users #	
24	12	1.11
22		1.11
26		2.21



Notes:
[1] Snus Group Only analyses compare samplers, current infrequent users, and current frequent users, each, to never users of snus within the snus group (referent); see text for definitions of snus groups
[2] Any 7-day period of non-smoking, ever within study
[3] Subgroup sample sizes (N) are identical as those shown in panels A and C

Figure 3 Subgroup Comparisons for Snus vs. Control Group: (A) Incidence of 24-hr, and (B) Floating Abstinence, and Within Snus Group Alone[1], (C) Incidence of 24-hr, and (D) Floating Abstinence.

Adverse events

During the sampling period alone, 240 snus participants (38%) reported a total of 412 adverse events compared with 187 control participants (31%) who reported a total of 300 adverse events (p=0.005). The between-group self-ratings of symptom severity in the snus versus control group were very similar. There were no snus-related, FDA-defined instances of serious adverse events. The most common side effects reported were nausea (12%), burning in throat or mouth (10%), and heartburn (8%) in the snus group; and headache (16%), nausea (10%), and dry mouth (8%) in the control group.

DISCUSSION

We report herein on 1-year outcomes from a large randomised trial of naturalistic snus use among US smokers, based on smokers not ready to quit, with the notion that assessment of naturalistic use, but under randomised conditions, would best guide policy evaluation of snus. Snus was provided in a manner that optimised the opportunity to observe benefit; that is, removing all barriers to use by providing it at no cost and with home delivery. Our results do not point toward any benefits of snus for quitting, and in contrast with prior studies, suggest that snus may undermine quit attempts, at least among a sample of smokers not ready to quit. We did not find any impact—positive or negative—of snus on abstinence.

The present study contrasts with several prior studies that suggest use of snus may facilitate quitting.[21–25 33 40] Snus may prompt smoking abstinence only when provided to smokers who endorse that intention and who use snus, with accompanying instruction/support to quit. In our study, a small number of snus participants who used the product with regularity showed increased quitting behaviour. On the whole, however, and within all other subgroups tested, our results show that providing snus to smokers who do not wish to quit, devoid of any instruction on how to use it, could be harmful in that it undermines quit attempts, though it has no impact on abstinence.

Within our snus group, there were a small number of participants (n=98; 16%) who, through the sampling period, became frequent snus users. For this subset of participants, outcomes were favourable in terms of inducing quit attempts and abstinence. This subset represents a self-selected sample of participants who were favourable of snus. Separate analyses, presented elsewhere, will differentiate those participants who did versus did not use snus, and will also determine the long-term trajectory of use of snus beyond the sampling period. Nonetheless, snus may offer individual benefits to a small group of smokers who go on to adopt snus.

Though our methods focus on one product alone, we have no reason to suspect that the results for other snus products would not generalise. Of greater concern for generalisability is that our study sample consisted primarily of Caucasian women. While both men and women reported fewer quit attempts within a snus group, this was only significant among women, and this may have influenced our negative results. Additionally, our focus on smokers not wanting to quit may create the impression that it was methodologically biased against snus. We did not believe it ethically appropriate to provide a tobacco product without instructions or support to smokers wanting to quit; anyone with such interest was referred to their state quitline. However, using identical naturalistic sampling methods, we

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have previously shown that provision of nicotine replacement therapy to smokers who do not want to quit, effectively prompts quitting.[37 41] Thus, the results reported herein are specific to snus and are not attributable to the low motivational status of our sample. One study limitation is the lack of biochemical verification of abstinence, though national guidelines suggest no such need within studies of minimal intervention.[42] Few biomarkers exist that differentiate smoked versus smokeless tobacco, and collection of carbon monoxide breathalyser was not possible given a geographically dispersed sample. Finally, though retention rates favoured the control group, the slight differences make it doubtful that this would materially influence the results.

CONCLUSIONS

In a randomised trial of naturalistic snus use among smokers not wanting to quit, provision of snus led to decreased incidence of quit attempts and had no impact on smoking abstinence. Uptake of snus was generally poor, though a minority of smokers did adopt frequent use, with beneficial impact on quitting. Notwithstanding the potential for snus to lower individual health risk, the overall adverse impact of providing snus in an unguided context to smokers on quit attempts provides clinical and regulatory caution against snus as an inducement or aid for smoking cessation, at least among smokers not yet ready to quit.

What this paper adds

What is already known on this subject

- A number of cross-sectional and retrospective surveys, many of which come from Scandinavian settings, suggest an association between use of low nitrosamine smokeless tobacco (ie, snus) and smoking abstinence.
- A small number of randomised clinical trials have examined snus for purposes of quitting, with generally suggestive or positive results; that is, snus promotes abstinence.

What important gaps in knowledge exist on this topic

- The literature lacks a large-scale clinical trial of naturalistic snus use and impact, particularly among smokers not interested in cessation. Such a trial would inform the regulatory debate on snus as a means of harm reduction and/or cessation induction.

What this study adds

- Provision of snus to smokers not yet ready to quit decreased the incidence of quit attempts over a 1-year follow-up period. There was no impact of snus on subsequent abstinence.
- Uptake of snus was generally poor and the negative impact on quit attempts provides caution against wide-scale, unguided use of snus as an aid to cessation.

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Contributors MJC was overall responsible for study conduct, including data analyses and manuscript preparation. AEW was responsible for primary data analyses and edited the manuscript. JLB assisted in study coordination and edited the manuscript. KMG provided medical oversight and edited the manuscript. EG-M provided senior statistical oversight and edited the manuscript. KMC and AJA provide scientific input on study design and edited the manuscript. The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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REVIEW ARTICLE

OPEN ACCESS

A critical review of the literature to conduct a toxicity assessment for oral exposure to methyl salicylate

Tracy Greene, Sarah Rogers, Allison Franzen and Robinan Gentry

Environment and Health Division, Ramboll Environ, Monroe, LA, USA

ABSTRACT

Methyl salicylate is the predominant constituent of oil of wintergreen and is used as a pesticide, a denaturant, an external analgesic, a fragrance ingredient, and a flavoring agent in products such as chewing gum, baked goods, syrups, candy, beverages, ice cream, and tobacco products; and it occurs naturally in some vegetables and berries. Methyl salicylate is of interest to the tobacco industry as oil of wintergreen is used as a flavorant in tobacco products. The purpose of this investigation was to conduct a critical review of the available literature for oral exposure to methyl salicylate, incorporating an analysis of the quality of the studies available and the current understanding of the mode of action. Following a review of all of the available literature, the most appropriate data sets for dose-response modeling were reported by Gulati et al. in which significant changes in reproductive/development endpoints were reported to occur after exposure to 500 mg/kg/d of methyl salicylate in male and female mice. Benchmark dose modeling was performed and the most sensitive endpoint, the number of litters per mating pair, was associated with a BMDL of 220 mg/kg/d. This BMDL was chosen as the point of departure and adjusted by a body weight scaling factor to derive a human equivalent dose. Based on the uncertainty factor analysis, the POD for methyl salicylate was adjusted by a UF of 3 for interspecies uncertainty to derive an allowable daily intake of 11 mg/kg/d.

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Introduction

Methyl salicylate is the predominant constituent of oil of wintergreen (JECFA 2002) and is used as a pesticide, a denaturant, external analgesic, flavoring agent, and fragrance ingredient in a variety of products including chewing gum, baked goods, syrups, candy, beverages, and ice cream (CIR 2003; NFSA 2012). It also occurs naturally in some vegetables and berries. Methyl salicylate is of interest to the tobacco industry as oil of wintergreen is a flavorant in multiple tobacco products, including smokeless tobacco and cigarettes (Chen et al. 2010; Lisko et al. 2014). The goal of this investigation was to conduct a toxicity assessment for the oral consumption of methyl salicylate, considering both non-carcinogenic and carcinogenic endpoints.

Several regulatory agencies have established recommended toxicity levels for oral exposure to methyl salicylate.

CONTACT Tracy Greene tgreene@ramboll.com Ramboll Environ, 3001 Armand, Suite I, Monroe, LA 71201, USA

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The United States Environmental Protection Agency's (USEPA's) Biopesticide and Pollution Prevention Division (USEPA 2005a), Norwegian Food Safety Authority (NFSA 2012), European Food Safety Authority (EFSA 2012), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2002) all recommend an acceptable daily intake for methyl salicylate of 0.5 mg/kg/d.

While regulatory agencies have established recommended levels of methyl salicylate that appear to be safe, none of these assessments considered the quality of the available toxicity studies. If the available toxicity studies for methyl salicylate were not conducted according to certain study quality standards, then this could introduce a level of uncertainty into the toxicity assessment. In addition, the current recommended acceptable daily intakes (ADIs) do not appear to incorporate consideration of the mode of action of methyl salicylate following oral exposure. The purpose of this investigation was to conduct a toxicity assessment for oral exposure to methyl salicylate, incorporating an assessment of the quality of the studies available for methyl salicylate and the current understanding of the mode of action for methyl salicylate.

Methods

This investigation was composed of two main steps: hazard assessment and dose–response assessment. The hazard assessment was initiated with a review of the current regulatory agency information, both in the United States and internationally, to identify studies that have served as the basis for any current regulatory toxicity values for methyl salicylate. In addition, a comprehensive search of the peer-reviewed scientific literature was conducted to identify epidemiological and toxicological studies in which the potential for toxicity following oral exposure to methyl salicylate had been investigated. The hazard assessment included an evaluation of the study quality using a published methodology for assigning study quality criteria, to identify studies considered to be of the highest quality that could be relied upon for the identification of endpoints, both cancer and noncancer, reported following oral exposure to methyl salicylate. Second, a dose–response assessment was conducted on the most appropriate data set, which included identification of points of departure (PODs) including no observed adverse effect levels (NOAELs)/lowest observed adverse effect levels (LOAELs) and benchmark doses or concentrations estimated using Benchmark Dose (BMD) modeling, evaluation of the mode of action of methyl salicylate, determination of human relevance for each endpoint identified, an uncertainty analysis, and the derivation of a toxicity value for the oral consumption of methyl salicylate.

Hazard identification

A hazard assessment includes the identification and review of data pertinent to two questions (1) whether an agent may pose a hazard to humans and (2) under what circumstances an identified hazard may be expressed (USEPA 2005b). The hazard assessment step should include a well-defined literature search strategy, as well as methods for critically reviewing the literature identified. The National Research Council (NRC) (2014) has recommended a systematic approach for hazard assessment that incorporates enhanced transparency and incorporates methods for assessing the quality of the studies identified. As part of the hazard assessment for methyl salicylate, an evaluation of the quality of the studies identified for consideration was also incorporated to ensure that the highest quality data currently available were incorporated into the dose–response assessment for methyl salicylate.

Identification of literature

Prior to conducting a search of the peer reviewed literature, a review of websites for authoritative bodies, both within the United States and internationally, was conducted to identify current recommended toxicity values for methyl salicylate and any published or nonpublished work that may have been relied upon in making decisions regarding potential effects following oral exposure to methyl salicylate.

Based on an initial review of the documents prepared by authoritative bodies, a literature search strategy was developed that included key words associated with common chemical names and synonyms for methyl salicylate identified using the database ChemSpider (2015). These keywords and synonyms were used in combination with the CAS registry number for methyl salicylate and keywords relating to methyl salicylate exposure and relevant toxicity endpoints as well as its mode of action (Table 1). While this assessment focused on oral exposure studies for methyl salicylate, the literature search strategy was also developed to include studies that evaluated the potential for health effects following the inhalation route of exposure. These studies were also considered as they may provide additional information that would potentially address data gaps in the literature. Literature searching was conducted using the US National Library of Medicine's Toxicology Data Network (TOXNET), Medline, and PubMed databases, and searching was limited to the literature published in English. The literature search strings included every possible combination between the categories listed in Table 1 (Compound/Products, Exposure, and Endpoints).

An initial screen of the titles and abstracts was performed using a defined set of inclusion and exclusion criteria that assist in the identification of those studies that could be considered for both hazard identification and dose–response modeling (Table 2). The focus was on studies that provided information on long-term exposure as these types of studies are largely relied upon for the development of acceptable levels of exposure to chemicals of interest (IPCS 1987; USEPA 1993, 1994, 2005b). Based on these criteria, the only *in vivo* studies included for further review were those in which the exposure was to methyl salicylate only, exposure was repeated dose, the route of exposure to methyl salicylate was reported to be oral or inhalation, and the study was conducted in humans or a relevant mammalian species (e.g. rats, mice, dogs, rabbits, guinea pigs, monkeys). The only *in vitro* studies that were included were those in which the exposure was to methyl salicylate only and the study was conducted in cell lines (bacterial, animal, or human) that have been relied

Table 1. Literature search strategy keywords.

Toxicity				
Compound/products	AND	Exposure	AND	Endpoints
Methyl Salicylate OR		Oral OR		Toxicity OR
2 Hydroxy benzoic acid methyl ester OR		inhalation OR		reproductive OR developmental OR
119 36 8 OR		chronic OR repeated OR subchronic		teratogenic OR
Methyl 2 hydroxybenzoate OR				teratogens OR
Oil of wintergreen OR				pregnancy OR
Wintergreen oil				fertility OR
				carcinogenicity OR
				cancer OR
				genotoxicity OR
				mutagenicity
Mechanism of action				
Methyl Salicylate OR	AND	Oral OR	AND	pharmacokinetic OR pharmacodynamic OR metabolism OR
2 Hydroxy benzoic acid methyl ester OR		inhalation OR		mode of action OR
119 36 8 OR		*in vitro*		mechanism OR
Methyl 2 hydroxybenzoate OR				human relevance
Oil of wintergreen OR				
Wintergreen oil				

Table 2. Inclusion and exclusion criteria.

Include/exclude	Criteria
Inclusion	• Exposure was to methyl salicylate only, as opposed to a mixture containing methyl salicylate.
	• Exposure was repeated and not a single acute exposure (with the exception of studies performed *in vitro*).
	• The route of exposure in *in vivo* studies was either via an oral or inhalation pathway.
	• The study was conducted in humans or a relevant mammalian species such as rats, mice, dogs, rabbits, guinea pigs, or monkeys if *in vivo*; and in bacterial, animal, or human cell lines if *in vitro*.
Exclusion	• Exposure was not to methyl salicylate only.
	• Exposure occurred via a single exposure or multiple exposures over a 24 hour period (with the exception of studies performed *in vitro*).
	• The route of exposure for *in vivo* studies was not by the oral or inhalation route.
	• Or for *in vitro* studies, conducted in a cell line that was not bacterial, animal, or human.

upon by authoritative bodies to assess the potential for hazard.

Evaluation of study quality

Toxicity studies that were identified in the literature search and determined to be relevant based on inclusion and exclusion criteria were reviewed and assessed for study quality using criteria reported by Klimisch et al. (1997). The Klimisch et al. (1997) framework provides criteria for scoring studies on a scale of 1–4, with studies scored as a 1 being of the highest quality and studies scored as a 4 of the lowest quality. A detailed description of the criteria applied in the Klimisch et al. (1997) study quality framework is provided in Table 3. The studies receiving the highest score of 1 would be those performed according to and complying with standard study guidelines or methods recommended by regulatory agencies such as the USEPA, the Organization for Economic Co-Operation and Development (OECD), or the European Union (EU) and preferably studies performed according to Good Laboratory Practices (GLP). GLP standards were developed to ensure transparency and consistency when conducting toxicity studies; however, the absence of GLP or the fact that a study was conducted prior to the implementation of GLP does not imply that the study was inadequately conducted. For this investigation, the absence of GLP was not used as a reason for excluding any data for consideration. Studies receiving a score of 2 were those that were not reported to have been conducted according to a published testing guideline and did not report GLP compliance. These studies would also be considered high quality because they would meet the requirements for a well-conducted toxicity study (e.g. appropriate number of animals per dose group; appropriate documentation of test chemical, dose, exposure methods). Studies of lower quality were given a score of 3 or 4. A score of 3 was given to studies for which the methods were not clearly defined or specified in the study, only one dose was administered and dose response could not be determined, or small numbers of animals per group were tested. A score of 4 was given to studies which do not provide sufficient experiment details (e.g. abstracts) or were secondary literature.

The higher quality *in vivo* and *in vitro* toxicological studies (i.e. those receiving quality scores of 1 or 2) were considered to provide the highest quality data. These should be given the most consideration when making conclusions regarding the potential for hazard. In addition, if the highest quality toxicological studies provide quantitative data for use in dose–response modeling, then these would be relied upon for the dose–response assessment. Data from toxicological studies with lower quality (scores of 3 or 4) were used as supporting information in the hazard assessment, but were not considered in the dose–response assessment. Studies that were identified that provided only mechanistic or mode of action data were used as supporting evidence only; therefore, no quality ratings were assigned to these studies.

Dose–response assessment

Utilizing the data identified in the hazard assessment, a dose–response assessment was performed to derive an

Table 3. Criteria from Klimisch et al. (1997).

Quality score	Requirements
1	Studies or data from the literature or reports that are carried out or generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to Good Laboratory Practices [GLP]), or studies in which the test parameters documented are based on a specific (national) testing guideline (preferably performed under GLP), or studies in which all parameters described are closely related/comparable to a guideline method
2	Studies that are not performed according to GLP or specific testing guidelines but are well documented and scientifically acceptable with the following information reported: For *in vivo* animal studies this includes the following criteria: • Data on the test animals including species, sex, and strain; • The purity/composition/origin of the test substance; • The number of animals evaluated; • The scope of the investigation per animal (e.g. clinical chemistry, hematology, organ weights, pathology or histopathology) and description of the methods; • Description of the changes observed; • Control group or historical control data of the laboratory; • Description of the test conditions; • Description of the route and doses of administration; • Dose/concentration relationship is possible. For *in vitro* studies this includes the following criteria: • Description of the test system and test method in details; • Purity/composition/origin of the test substance; • Data on the dose/concentration differentiated according to the toxicity of the test substance on the test system; information on volatility; • Data on secondary effects which may influence a result (solubility, impurities, pH shifts, influence on the osmolarity, etc.); • Appropriate negative/positive controls as integral parts of the test; • References on adequacy of the method should be given or generally known.
3	Studies or data from the literature which do not meet the criteria for a quality score of 2. These include studies for which the methods were not clearly defined or specified in the study, in which only one dose was administered, or small numbers of animals per group were tested. The appropriate number of animals per group was determined based on recommendations from OECD guidelines for comparable study types
4	Studies or data from the literature which do not provide sufficient experiment details and which are only listed in short abstracts or secondary literature (books, reviews, etc.) • Additional criteria for *in vivo* animal studies: • Single exposure; • Inappropriate or irrelevant species tested; • Route of exposure not relevant to humans; • If the animals tested underwent any type of alteration or injury prior to testing.

acceptable level of methyl salicylate that an individual can be exposed to on a daily basis for a lifetime that would not be expected to result in any appreciable cancer or non-cancer health risk (USEPA 1993). Following a review of all the literature identified, a dose–response assessment was conducted utilizing the data from the most appropriate study to identify the most sensitive endpoint that could be relied upon to develop a point of departure (POD). An acceptable level or ADI was then estimated through the application of safety factors (for non-cancer effects) to the POD. For cancer effects, in the absence of an understanding of the mode of action, linear extrapolation from the POD to the dose or concentration associated with a one in a million risk (for cancer effects) is conducted. This approach assumes that the mode of action for the development of the cancer endpoint is a linear process.

Identification of the point of departure and dose–response modeling

The studies with the highest study quality scores were reviewed to determine if there were appropriate quantitative data sets that could be used to estimate a POD. The POD is defined as the estimated dose near the lower end of the observed range, without extrapolation to lower doses (USEPA 2005b). This POD, adjusted for potential uncertainty, could then be used to estimate an acceptable level of oral exposure, such as an oral Reference Dose (RfD) or an ADI. While a POD can be based on a LOAEL or a NOAEL, a POD can also be based on a benchmark dose (BMD) (USEPA 2002, 2005b, 2012). While a NOAEL, LOAEL, or BMD could all serve as the POD, the estimation of a BMD provides a more robust, informative POD, compared with using a NOAEL or LOAEL. BMDs, and their associated statistical confidence intervals (i.e. 95% lower limit on the BMD estimate, or BMDL), account for the shape of the dose-response curve over the continuum of observed exposures, rather than at a single dose level (USEPA 2002, 2012). They also inform the POD with respect to within-group variability of response and reward study designs that utilize a sufficiently large number of subjects and doses, and exposure ranges inclusive of the no response level and the maximally tolerated dose.

BMD modeling was performed on all data sets with adverse effects reported to be biologically significant and statistically significantly increased at any dose when compared to controls. All BMD modeling was performed using BMD Software (BMDS) version 2.6.01 from the USEPA (2016). As part of the BMDS software (USEPA 2016), the BMDS models used were based on the type of results being considered (i.e. continuous, quantal, or nested). Quantal data are presented as a fraction or percent of a population with a condition or effect at a given dose or exposure level and BMDS (2012, 2016) uses probability density models including the Gamma, Logistic, Multistage, Probit, Weibull, and Multistage for quantal data. For continuous data, presented as means and standard deviations or standard errors, BMDS software

(USEPA 2012, 2016) uses continuous models including the polynomial, power, linear, exponential and Hill models. Models used by USEPA (2012, 2016) for use in modeling nested data (i.e. the response is either true or false, or outcomes are measured in the offspring of pregnant animals) include the NLogistic, NCTR, and Rai & Van Ryzin models. Based on recommendations from USEPA (2012), model results were evaluated for goodness of fit and the BMD and BMDL of those models with the lowest Akaike information criterion (AIC) value that had adequate Chi-square *p* values (greater than .1) were considered as PODs. The Chi-square Goodness of Fit Test is "a statistical hypothesis test used to compare observed counts with predicted numbers of independent observations classified into two or more categories" (USEPA 2012). The AIC is defined as "a measure of information loss from a dose–response model that can be used to compare a specified set of models". Among a set of models, the model with the lowest AIC is considered the best" (USEPA 2012). Finally, the lowest BMDL with the most appropriate model fit was chosen as the POD for the estimation of an acceptable daily intake of methyl salicylate (USEPA 2012).

Mode of action and human relevance assessment

The studies identified in the literature search providing mechanistic or mode of action information for methyl salicylate were considered separately in a mode of action assessment. While mode of action data are important in understanding the potential for hazard, a lack of evidence from human studies or the lack of human studies also elevates the importance of mode of action data for understanding the potential for human health effects when the toxicity observed is based on studies conducted in animals (i.e. are the effects reported in animal studies relevant to human health). Once the potential POD was identified for the development of the ADI, the mode of action data relevant to the endpoint associated with the POD were reviewed and considered in order to establish (1) whether the effects reported in animal models (in the absence of human data) are potentially relevant to humans and (2) what uncertainty factors should be applied to the POD.

Adjustments for uncertainty

Traditionally, authoritative bodies have applied factors to adjust the POD that are intended to account for several types of uncertainty. For this assessment, an uncertainty factor analysis was performed in accordance with the methods currently recommended by USEPA (2002). This approach is applied by dividing the POD by factors ranging from 1 to 10 intended to adjust for various types of uncertainty. This includes consideration of five uncertainty factors: interspecies, intraspecies, chronic versus subchronic, LOAEL versus NOAEL, and incomplete database uncertainty factors. Each factor is given a value of 1, 3, or 10 depending on the quality of the information available for each uncertainty category and scientific judgment.

- *Interspecies* (UF_A) – uncertainty in extrapolating from laboratory animal data to humans.
- *Intraspecies* (UF_H) – variation in sensitivity among the members of the human population.
- *Chronic versus subchronic study duration* (UF_S) – uncertainty in extrapolating from data obtained in a study that is of less-than-lifetime exposure (subchronic) to a toxicity value that is defined as a lifetime exposure (chronic).
- *LOAEL versus NOAEL* (UF_L) – uncertainty in using a LOAEL rather than a NOAEL.
- *Incomplete database* (UF_D) – inability of any single study to adequately address all possible adverse outcomes in humans (e.g. incomplete database).

The uncertainty value chosen for each type of uncertainty was based on the quality of the available studies and scientific judgment (USEPA 2002). A factor of 10 is typically used to adjust for each type of uncertainty identified; however, if, based on the available data and scientific judgment, a lower uncertainty value can be justified, values of 3 or even 1 have been applied. In the case of interspecies (UF_A) and intraspecies (UF_H) uncertainty factors, each factor includes two components to account for pharmacokinetic differences (UF = 3) and pharmacodynamic differences (UF =3), for a total default UF value of 10 for each (Kroes et al. 1993; Beck & Clewell 2001; USEPA 2002; Dorne 2004). A reduction in the default UF of 10 should only be used if sufficient data are available to support the conclusion that the data set on which the POD is based is representative of the dose–response data for the most susceptible human subpopulation. In the case of the UF_A, the USEPA (2011a) has recently recommended using an allometric body weight scaling to the 3/4 power approach ($BW^{3/4}$) to adjusted the POD to a human equivalent dose (HED). For this conversion the POD is multiplied by a dosimetric adjustment factor (DAF) calculated as follows:

$$DAF = \left(\frac{BW_A}{BW_H}\right)^{1/4} \quad (1)$$

where BW_A is the body weight of the animal species tested and BW_H is the human body weight. The $^1/_4$ exponent accounts for the application of $BW^{3/4}$ scaling to exposure in units of mg/kg-d ($BW^{3/4}/BW^{1/1} = BW^{1/4}$). Using this approach, a reduced UF_A (default of 3) is applied to the HED. The reduction of the UF_A from a default value of 10 to 3 with the use of $BW^{3/4}$ scaling is based on the qualitative recognition that current scientific knowledge indicates that $BW^{3/4}$ scaling addresses the potential for species differences in both kinetic and dynamic processes, which the UF_A is intended to address (USEPA 2011a).

Derivation of an acceptable daily intake

Based on the POD, an acceptable daily intake was derived. For this investigation, the ADI was considered to be synonymous with the USEPA's oral RfD and is defined as an estimate of the daily oral exposure to the human population that includes adjustments for uncertainty in the data, which is likely to be without an appreciable risk of adverse effects over the course of a lifetime (USEPA 2002). If the POD was based on a non-cancer endpoint or a cancer endpoint associated with a nonlinear mode of action, the ADI was derived

from the POD divided by the appropriate uncertainty factors:

$$\text{ADI (non-cancer)} = \frac{\text{POD} \times \text{DAF}}{\text{UF}_A \times \text{UF}_H \times \text{UF}_S \times \text{UF}_L \times \text{UF}_D} \quad (2)$$

Results

Literature search

The literature search was conducted in two steps. First, a review of websites for authoritative bodies, both within the United States and internationally was conducted. Second, using the literature search strategy presented in Table 1, a search of published literature was conducted. The results of each step are detailed in the following sections.

Documents from authoritative bodies

Documents providing recommendations regarding ADIs for methyl salicylate were identified for four authoritative bodies: NFSA (2012), EFSA (2012), USEPA (2005a), and JECFA (1968, 2002). A review of these documents was conducted to identify studies that had been relied upon in making decisions regarding the potential toxicity of methyl salicylate. Although USEPA has not established a reference dose (RfD) for methyl salicylate, in 2005 the Biopesticide and Pollution Prevention Division of the Office of Pesticide Programs (OPP) (USEPA 2005a) developed a RfD for methyl salicylate as part of the re-registration eligibility document for methyl salicylate of 0.5 mg/kg/d based on the NOEL reported in a 2-year oral study in dogs and a 17-week feeding study in rats of 50 mg/kg/d (Webb & Hansen 1963). Based on the results of this study (discussed in more detail in section Chronic toxicity studies), USEPA (2005a) identified a NOEL for both rats and dogs of 50 mg/kg/d. The documentation indicates that a UF of 10 was applied for interspecies extrapolation, resulting in an RfD of 0.5 mg/kg/d. It appears another UF of 10 must have been applied to result in the recommended RfD and it is likely that this factor was an adjustment for intraspecies uncertainty. However, USEPA (2005a) does not document an adequate adjustment factor to support an RfD of 0.5 mg/kg/d.

JECFA (2002) has also established an ADI for methyl salicylate of 0.5 mg/kg-body weight. According to JECFA (2002), the ADI of 0.5 mg/kg-bodyweight is based on a no observed effect level (NOEL) of 50 mg/kg/d reported in a 2-year dietary study in dogs; however, the citation for the study that serves as the basis for the NOEL is not provided. JECFA concluded that the estimated intake for consumers of 0.1 mg/kg body weight is below the ADI of 0.5 mg/kg body weight and the safety of methyl salicylate as a flavoring agent would not raise concern at the currently estimated level of use. NFSA (2012) relies upon the ADI established by JECFA (2002) and, according to NFSA (2012), the results of Webb and Hansen are relied upon in making recommendations regarding acceptable levels of methyl salicylate in food.

EFSA (2012) also relies upon the results of the study conducted by Webb and Hansen (1963), in which a NOAEL of 50 mg/kg/d was reported based on the results of a 2-year rat dietary study. The EFSA recommendations are for allowable concentrations of methyl salicylate in animal feed and not human exposure. The recommendations were species-specific and ranged from 8 mg methyl salicylate/kg body weight for chickens to 30 mg methyl salicylate/kg body weight for dogs.

Methyl salicylate also has United States Food and Drug Administration (USFDA)-specified uses as an indirect food additive (CFR 2015). No other documents developed by authoritative bodies providing recommendations on acceptable levels of methyl salicylate following oral exposure were identified.

Literature search results

The goal of the literature search was to identify all the peer-reviewed, published scientific literature presenting data in animals or humans related to repeated dose toxicity associated with oral exposure to methyl salicylate. In addition, search terms were included to identify data related to the mode of action of methyl salicylate, as these data would be relevant for drawing conclusions about the shape of the dose-response curve and the potential relevance to human health of endpoints identified in animals. While it was originally thought that searches of the peer reviewed literature could be limited to dates associated with the most recent authoritative reviews to the present, because the available regulatory documents contained only limited data that could be used for quantitative risk assessment, the literature search was not limited to any date range. The literature search conducted based on the key words presented in Table 1 resulted in the identification of 18 toxicity studies for methyl salicylate and 116 mode of action studies. Also, as a result of the review of authoritative review articles, 15 additional toxicity studies and one additional mode of action study were identified. These studies were not identified in the original search because the citations were from an unpublished study or a book chapter. The titles and abstracts (where available) identified from the literature search and a thorough review of documents from authoritative bodies were screened for inclusion based on the criteria presented in Table 2. Following inclusion and exclusion screening of both the literature identified through the literature search and the unpublished studies identified in regulatory documents, a total of 26 toxicity studies and 25 mode of action studies were identified for critical review and consideration in the hazard and dose–response assessments which are presented in Table 4. Of these, three unpublished studies by Abbot and Harrisson (1978), USFDA (1966) and LaWall and Harrisson Research Laboratories Inc. (1964) were not able to be obtained; however, summaries of the studies in CIR (2003) were reviewed. Two of the 26 citations were available as abstracts only (Packman et al. 1961; Harrisson et al. 1963) and were obtained for review.

Study quality assessment

Study quality of the relevant studies identified from the literature searching was assessed using the approaches recommended by Klimisch et al. (1997) (Table 3). Of the 26 toxicity studies noted in Table 4, once obtained and reviewed in

Table 4. Literature identified from authoritative reviews and literature searching.

Toxicity studies

Abbott DD, Harrisson JWE (1978). Methyl salicylate: Studies of osseous changes in the rat, reproduction in the rat and mouse, and liver and kidney effects in the dog. Unpublished communication to the Flavor and Extract Manufacturers Association[a]

Collins TF, Hansen WH, Keeler HV (1971). Effect of methyl salicylate on rat reproduction. Toxicology and Applied Pharmacology 18(4): 755 765

Cosmetic Ingredient Review (2003). Safety assessment of Salicylic Acid, Butyloctyl Salicylate, Calcium Salicylate, C12 15 Alkyl Salicylate, Capryloyl Salicylic Acid, Hexyldodecyl Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Magnesium Salicylate, MEA Salicylate, Ethylhexyl Salicylate, Potassium Salicylate, Methyl Salicylate, Myristyl Salicylate, Sodium Salicylate, TEA Salicylate, and Tridecyl Salicylate. International Journal of Toxicology 22(Suppl 3): 1 108

Cosmetic Ingredient Review (2008). Final report of the safety assessment of Alcohol Denat., including SD Alcohol 3 A, SD Alcohol 30, SD Alcohol 39, SD Alcohol 39 B, SD Alcohol 39 C, SD Alcohol 40, SD Alcohol 40 B, and SD Alcohol 40 C, and the denaturants, Quassin, Brucine Sulfate/Brucine, and Denatonium Benzoate. International Journal of Toxicology 27(Suppl 1): 1 43

Crossland AM (1948). Methyl salicylate poisoning. Canadian Medical Association Journal 58(1): 75 77 (FREE)

Gulati DK, Choudhury H, Chambers R, Sabharwal PS, Lamb JIV (1984). Methyl salicylate: fertility assessment in cd 1 mice when administered by gavage. NTIS (pb 85 164283, ntp 85 022)

Harrisson JWE et al. (1963). Federation Proceedings, Federation of American Societies for Experimental Biology 22: 554.[b]

Hutt AJ, Caldwell J, Smith R (1986). The metabolism of aspirin in man: a population study. Xenobiotica; the fate of foreign compounds in biological systems 16(3): 239 249.

Infurna R, Beyer B, Twitty L, Koehler G, Daughtrey W (1990). Evaluation of the dermal absorption and teratogenic potential of methyl salicylate in a petroleum based grease. Exxon Biomedical Sciences, Inc., East Millstone, New Jersey. Teratology 41: 566

LaWall and Harrisson Research Laboratories, Inc. (1964). Study of Methyl Salicylate on rats. L&H 58188. (Submitted to USFDA in response to an FOI request 1998; 120 pages.)[a]

Morrissey RE, Lamb J, Morris R, Chapin R, Gulati D, Heindel J (1989). Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Developmental and Reproductive Toxicology Group, NIEHS, NTP Program, Research Triangle Park, NC, Analytical Sciences Inc., Durham, North Carolina, Environmental Health Research and Testing, Inc., Lexington, Kentucky; Fundamental and Applied Toxicology 13: 747 777

National Toxicology Program. 1984. Abstract for RACB82104 Methyl salicylate CAS no. 119 36 8. NTIS #RACB82104. Available at: http://ntp.niehs.nih.gov/testing/types/repro/abstracts/racb/index 8.html

Opdyke DL (1978). Acute oral toxicity: rat; acute dermal toxicity: rabbit; and primary dermal irritation:rabbit) for methyl salicylate, fragrance raw materials mono graphs. Food and Cosmetics Toxicology 16: 821 825

Opdyke DL (1979). Monographs on fragrance raw materials. Food and Cosmetics Toxicology 17(4): 357 390

Opdyke DL (1979). Monographs on fragrance raw materials. Food and Cosmetics Toxicology 17(Special Issue V): 695 923

Overman DO, White JA (1983). Comparative teratogenic effects of methyl salicylate applied orally or topically to hamsters. Teratology 28(3): 421 426

Packman E, Abbott D, Wager B, Harrisson J (1961). Chronic and oral toxicity of oil sweet birch (methyl salicylate). Pharmacologist 3: 62[b]

Rainsford K (2004a). History and development of the salicylates. In Aspirin and Related Drugs (K Rainsford, Ed.), Taylor and Francis, New York

Rainsford K (2004b). Pharmacology and biochemistry of salicylates and related drugs. In Aspirin and Related Drugs (K Rainsford, Ed.), pp. 215 366. Taylor and Francis, New York

Rainsford K (2004c). Side effects and toxicology of the salicylates. In aspirin and related drugs (K Rainsford, Ed.), Taylor and Francis, New York

Rudolph AM (1981). Effects of aspirin and acetaminophen in pregnancy and in the newborn. Archives of Internal Medicine 141(3 Spec No): 358 363

Sharpe GL, Larsson KS, Thalme B (1975). Studies on closure of the ductus arteriosus. XII. In utero effect of indomethacin and sodium salicylate in rats and rabbits. Prostaglandins 9(4): 585 596

Ungvary G, Tatrai E, Lorincz M, Barcza G (1983). Combined embryotoxic action of toluene, a widely used industrial chemical, and acetylsalicylic acid (aspirin). Teratology 27(2): 261 269

USFDA (1966). Chronic oral toxicity of Methyl Salicylate using dogs and reproductive and developmental effects oral study of Methyl Salicylate using rats. United States Food and Drug Administration. Studies dated May. (Submitted by FDA in response to an FOI request 1998; 33 pages.)[a]

Warknay J, Takacas E (1959). Experimental production of congenital malformations in rats by salicylate poisoning. American Journal of Pathology 35: 315 331

Webb WK, Hansen WH (1963). Chronic and subacute toxicology and pathology of methyl salicylate in dogs, rats, and rabbits. Toxicology and Applied Pharmacology 5: 576 587

Mode of action studies

Battino M, Ferreiro MS, Armeni T, Politi A, Bompadre S, Massoli A, Bullon P (2005). *In vitro* antioxidant activities of antioxidant enriched toothpastes. Free Radical Research 39(3): 343 350.

Cerletti C, Dell'Elba G, Manarini S, Pecce R, Di Castelnuovo A, Scorpiglione N, Feliziani V, de Gaetano G (2003). Pharmacokinetic and pharmacodynamic differences between two low dosages of aspirin may affect therapeutic outcomes. Clinical Pharmacokinetics 42(12): 1059 1070

Daston GP, Rehnberg BF, Carver B, Rogers E, Kavlock R (1988). Functional teratogens of the rat kidney. I. Colchicine, dinoseb, and methyl salicylate. Fundamental and Applied Toxicology 11(3): 381 400

Davis LE, Westfall BA (1972). Species differences in biotransformation and excretion of salicylate. American Journal of Veterinary Research 33(6): 1253 1262

Davison C, Zimmerman EF, Smith P (1961). On the metabolism and toxicity of methyl salicylate. The Journal of Pharmacology and Experimental Therapeutics 132: 207 211

Dorne JL (2004). Impact of inter individual differences in drug metabolism and pharmacokinetics on safety evaluation. Fundamental and Clinical Pharmacology 18(6): 609 620

Gatti G, Barzaghi N, Attardo Parrinello G, Vitiello B, Perucca E (1989). Pharmacokinetics of salicylic acid following administration of aspirin tablets and three differ ent forms of soluble aspirin in normal subjects. International Journal of Clinical Pharmacology Research 9(6): 385 389

Graham G, Roberts M, Day R, Rainsford K (2004). Pharmacokinetics and Metabolism of the Salicylates. In Aspirin and Related Drugs (K Rainsford, Ed.), Taylor and Francis, New York

Ishidate M, Sofuni T, Yoshikawa D, Hayashi M, Nohmi T, Sawada M, Matsuoka A (1984). Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22: 623 636

Kuboyama, N. and Fujii A. (1992). Mutagenicity of analgesics, their derivatives, and anti inflammatory drugs with S 9 mix of several animal species. The Journal of Nihon University School of Dentistry 34(3): 183 195

Lapczynski A, Jones L, McGinty D, Bhatia S, Letizia C, Api A (2007). Fragrance material review on methyl salicylate. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 45(Suppl 1): S428 452

(*continued*)

Table 4. Continued.

Toxicity studies
Levy G, Weintraub L, Matsuzawa T, Oles S (1966). Absorption, metabolism and excretion of salicylic phenolic glucuronide in rats. Journal of Pharmaceutical Sciences 55(11): 1319 1320
Montgomery PR, Berger LG, Mitenko P, Sitar D (1986). Salicylate metabolism: effects of age and sex in adults. Clinical pharmacology and therapeutics 39(5): 571 576
Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis 8(Suppl 7): 1 119
Murugesh N, Kumar VR, Vembar S, Damodaran C (1981). Studies on erythrocyte membrane. V. Haemolytic effect of methylsalicylate and its possible mechanism. Toxicology Letters 9(3): 225 229
Needs CJ, Brooks PM (1985). Clinical pharmacokinetics of the salicylates. Clinical Pharmacokinetics 10(2): 164 177
Patrono C, Coller B, Dalen J, Fitzgerald G, Fuster V, Gent M, Hirsh J, Roth G (2001). Platelet active drugs: the relationships among dose, effectiveness, and side effects. Chest 119(Suppl 1): 39S 63S
Roberts LJ III, Morrow JD (2001). Analgesic antipyretic and antiinflammatory agents and Drugs Employed in the Treatment of Gout. In Goodman & Gilman's The Pharmacological Basis of Therapeutics (JG Hardman, LE Limbird, Eds.), McGraw Hill, New York, New York
Rotilio D, Joseph D, Hatmi M, Vargaftig B (1984). Structural requirements for preventing the aspirin and the arachidonate induced inactivation of platelet cyclo oxygenase: additional evidence for distinct enzymatic sites. European Journal of Pharmacology 97(3 4): 197 208
Vane JR, Botting RM (2003). The mechanism of action of aspirin. Thrombosis Research 110(5 6): 255 258
Verstraelen S, Nelissen I, Hooyberghs J, Witters H, Schoeters G, Van Cauwenberge P, Van Den Heuvel R (2009a). Gene profiles of a human alveolar epithelial cell line after *in vitro* exposure to respiratory (non)sensitizing chemicals: identification of discriminating genetic markers and pathway analysis. Toxicology Letters 185(1): 16 22
Verstraelen S, Nelissen I, Hooyberghs J, Witters H, Schoeters G, Van Cauwenberge P, Van Den Heuvel R (2009b). Gene profiles of a human bronchial epithelial cell line after *in vitro* exposure to respiratory (non)sensitizing chemicals: identification of discriminating genetic markers and pathway analysis. Toxicology 255(3): 151 159
Vigano G, Garagiola U, Gaspari F (1991). Pharmacokinetic study of a new oral buffered acetylsalicylic acid (ASA) formulation in comparison with plain ASA in healthy volunteers. International Journal of Clinical Pharmacology Research 11(3): 129 135
Wolowich WR, Hadley CM, Kelley M, Walson P, Casavant M (2003). Plasma salicylate from methyl salicylate cream compared to oil of wintergreen. Journal of Toxicology. Clinical Toxicology 41(4): 355 358
Zhang Z, Jia C, Hu Y, Sun L, Jiao J, Zhao L, Zhu D, LI J, Tian Y, Bai H, Li R, Hu J (2012). The estrogenic potential of salicylate esters and their possible risks in foods and cosmetics. Toxicology Letters 209(2): 146 153

[a]This study was identified through a review of the CIR (2003) and was determined to be an unpublished study that is not available publicly.
[b]For this citation, only an abstract was available.

more detail, six were found to be studies other than toxicity studies and were not included in the study quality assessment (Warknay & Takacas 1959; Sharpe et al. 1975; Overman & White 1983; Ungvary et al. 1983; Hutt et al. 1986; Infurna et al. 1990). A list of 20 studies that were assessed for study quality is presented in Table 5, along with the corresponding study quality score and the rationale for the score. Based on the application of study quality scores, no toxicity study identified for methyl salicylate received a study quality score of 1. Two studies (Gulati et al. 1984; Morrissey et al. 1989) received a score of 2 instead of 1 because the studies were not performed according to standard study guidelines recommended by regulatory agencies (e.g. USEPA or OECD). The study reported by Webb and Hansen (1963) also received a study quality score of 2 because the study was conducted prior to the establishment of Good Laboratory Practices (GLP) and the study was not performed according to standard study guidelines recommended by regulatory agencies (e.g. USEPA or OECD). Gulati et al. (1984), Morrissey et al. (1989) and Webb and Hansen (1963) were retained for further critical review for consideration in the dose-response assessment.

One study, Collins et al. (1971), received a score of 3 and was not considered further for quantitative analysis because the study was conducted prior to the establishment of GLP, the study was not performed according to standard study guidelines recommended by regulatory agencies, and the purity of the test substance was not reported. The remaining sixteen studies received a score of 4 and were not considered further for quantitative analysis (Table 5). These were mainly studies that were cited in authoritative reviews and were not publicly available. The information provided in the authoritative review was limited. It also included citations for which only abstracts for the studies were available, or the studies were review articles and did not provide any original data.

The following sections provide a summary of the studies that were considered for the dose–response assessment based on study quality scores of 1 or 2 and the inclusion of quantitative data that could be used for dose–response modeling. No studies received a study quality score of 1; however, two studies received study quality scores of 2. These include one chronic toxicity study conducted in rats (Webb & Hansen 1963) and one reproductive/developmental study conducted in two separate laboratories (Gulati et al. 1984; NTP 1984; Morrissey et al. 1989). When reviewing these studies, only those effects considered to be treatment related and biologically significant; and reported to be statistically significantly different from the control were considered as potential effects associated with methyl salicylate exposure and these were the only effects considered for evaluation in the dose–response assessment.

Chronic toxicity studies. Webb and Hansen (1963) conducted a chronic dietary study in which Osborne–Mendel rats were administered diets for 2 years containing 0, 0.1, 0.5, 1.0, or 2.0% methyl salicylate (Table 6). Each study group consisted of 25 male and 25 female rats, with the exception of the 2.0% group, which included 24 males and 26 females. Rats were fed *ad libitum* and weighed weekly. Hematologic examinations were performed at 3, 11, 17, and 22 months. Weights of the heart, liver, kidneys, spleen, and testes were

Table 5. Results of the study quality analysis.

Study	Study quality score	Rationale for study quality score
Gulati et al. (1984)	2	Study was performed according to GLP. The study did not specify that it was performed according to standard study guidelines recommended by regulatory agencies (e.g. USEPA or OECD); however, the methods are well documented and scientifically acceptable.
Morrissey et al. (1989)	2	Study was performed according to GLP. The study did not specify that it was performed according to standard study guidelines recommended by regulatory agencies (e.g. USEPA or OECD); however, the methods are well documented and scientifically acceptable.
Webb and Hansen (1963)	2	The study was performed prior to GLP. The study was not performed according to standard study guidelines recommended by regulatory agencies (e.g. USEPA or OECD); however, the methods are well documented and scientifically acceptable.
Collins et al. (1971)	3	The study was performed prior to GLP and was not performed according to standard study guidelines recommended by regulatory agencies (e.g. USEPA or OECD). The purity of the test substance was not specified.
Packman et al. (1961)	4	Only an abstract was available. Limited study details were available.
Harrisson et al. (1963)	4	This citation could not be obtained in the publically available literature. Information considered was based on the summary reported in NFSA (2012).
USFDA (1966)[a]	4	This is an unpublished study and could not be obtained. Information considered was based on the summary reported in CIR (2003). Due to lack of appropriate protocol and dose response information presented in CIR (2003) the study was not considered for this assessment.
LaWall and Harrisson Research Laboratories, Inc. (1964)[a]	4	This is an unpublished study and could not be obtained. Information considered was based on the summary reported in CIR (2003). Due to lack of appropriate protocol and dose response information presented in CIR (2003) the study was not considered for this assessment.
Abbot and Harrisson (1978)[a]	4	This is an unpublished study and could not be obtained. Information considered was based on the summary reported in CIR (2003). Due to lack of appropriate protocol and dose response information presented in CIR (2003) the study was not considered for this assessment.
Rudolph (1981)	4	This is a review article and not a toxicity study.
Crossland (1948)	4	This is a review article and not a toxicity study.
Opdyke (1979a)	4	This is a review article and not a toxicity study.
Opdyke (1979b)	4	This is a review article and not a toxicity study.
Rainsford (2004a)	4	This is a review article and not a toxicity study.
Rainsford (2004b)	4	This is a review article and not a toxicity study.
Rainsford (2004c)	4	This is a review article and not a toxicity study.
Opdyke (1978)	4	This is a review article and not a toxicity study.
CIR (2003)	4	This is a review article and not a toxicity study.
CIR (2008)	4	This is a review article and not a toxicity study.

[a]This study was identified through a review of the CIR (2003) and was determined to be an unpublished study that is not available publicly.

recorded at study termination. Gross pathological and histopathological examinations of the thyroid, parathyroid, lung, heart, stomach, pancreas, spleen, liver, kidney, adrenal gland, lymph node, small intestine, bone marrow smear, leg bone, leg muscle, urinary bladder, testis and prostate (males), and ovary and uterus (females) were performed following necropsy. In animals administered the highest concentration, 50% died by week eight of the experiment and all were dead by week 49. A statistically significant decrease in body weight was reported for rats administered diets containing 1% or 2% methyl salicylate (through week 49 when all animals in the high concentration group were reported to have died). Absolute organ weights were not statistically significantly different from control animals; however, significant changes in organ weights relative to body weight were reported. Statistically significant increases in testis weight relative to body weight were reported in male rats administered diets containing 1.0% methyl salicylate. In addition, statistically significant increases in heart and kidney weight relative to body weight were reported in females administered diets containing 1.0% methyl salicylate, compared with controls. The authors reported that the incidence of an induced lesion in the leg bone of rats administered diets containing 2.0% methyl salicylate was the most prominent histopathological change. However, a histopathological examination of the leg bone was only conducted in a subset of animals ($n = 10–11$) and the results were reported as an increase of cancellous bone in the metaphysis of bones examined per dose group with no indication of how many bones per rat were examined and no information reported for the control group for this effect. Therefore, the data reported by the study authors appear to support the presence of a dose-related increase in the incidence of cancellous bone and the authors state that the increased incidence in the high dose group was very apparent compared with the controls; however, the data reported in the study were insufficient to determine if the incidence of these lesions was statistically significantly increased compared to control animals. The histopathological changes reported in the bone consisted of an increased amount of cancellous bone (i.e. trabecular bone or spongy bone) in the metaphysis. A subset of the bones of rats in the 0.5 and 1.0% groups were also examined and the same lesions in the bone were reported at a lesser degree of severity in two of 11 bones in the 1.0% dose group and one of 11 bones in the 0.5% dose group. In addition to histopathological changes noted in the bone, the authors reported

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Table 6. Summary or studies considered for eva uation in the QRA

Study	Species	Route of exposure	Number of anima s/sex/group	Administered dose	Duration	NOAEL (mg/kg/d)	Endpoint	Study qua ity score	Rationa e for study qua ity score
Chronic toxicity studies									
Webb and Hansen (1963)	Rats	diet	25	0, 0.1, 0.5, 1, 2% (0, 50, 250, 500, 1000 mg/kg/d)[a]	2 years	500	Cance ous bone in the metaphysis	2	The study was performed prior to GLP. The study was not performed accord- ing to any specific pub ished testing guide ines; however, the study proto- co was we documented and scientif- ica y acceptab e
	Dogs	capsu e	2	0, 50, 150, 350 mg/kg/d	2 years	350	No adverse effects reported	2	The study was performed prior to GLP. The study was not performed accord- ing to any specific pub ished testing guide ines; however, the study proto- co was we documented and scientif- ica y acceptab e.
Reproductive/developmental studies									
Morrissey et (a . 1989), Gu ati et a . (1984), and NTP (1984)	Mice	Gavage	40 (contro); 20 (treated)	0, 25, 50, 100 mg/kg/d (NTP 1984) 0, 100, 250, 500 mg/ kg/d (Gu ati et a . 1984)	7 d (premating); 100 d (cohabited)	100	Decreased number of itters, number of pups per itter, proportion of pups born a ive and decreased mean pup weight	2	Study was performed according to GLP. Testing guide ine is a pub ished guide ine but not OECD. Testing guide ine is we documented and sci- entifica y acceptab e.

[a]Concentrations were converted from percent methy sa icy ate in the diet to doses of mg/kg/d assuming a food factor of 0.05 for rats or 0.13 for mice (USEPA 1985). Food consumption data was not reported in the study.

similar types and numbers of tumors at all dose levels except the 2.0% dose group. These included mammary tumors and benign and malignant pituitary tumors; however, the authors did not report incidence data nor did they conclude that any of the tumors were treatment related. The authors also noted that pituitary tumors were reported in 22 females (occurrence by dose level was not specified) and only one male rat (dose level not specified).

Webb and Hansen (1963) also performed a chronic study in beagle dogs (two/sex/dose) in which the animals were orally administered capsules containing 0, 50, 150, or 350 mg/kg/d of methyl salicylate 6 d a week for 2 years. The dogs were weighed weekly and hematological examinations were performed at 2 weeks, 1 month, 3 months, 6 months, 1 year, and 2 years after the start of the experiment. One female dog in the 350 mg/kg/d group died of infectious canine hepatitis after 33 d of exposure and her replacement died of canine distemper after 19 weeks of exposure. At the time, the replacement was added to the high-dose group, a sibling was also added to the control group increasing the total number of dogs to 18, with three females and two males in the control and 350 mg/kg/d dose groups and two females and two males in the 50 and 150 mg/kg/d dose groups. No significant hematologic effects were noted at any of the examination time points. Necropsies were performed on all dogs dying or killed during the experiment or upon termination, and all major organs were weighed. In the surviving dogs in the high-dose group, microscopic examinations were conducted on the brain, pituitary, salivary gland, thyroid, parathyroid, thymus, lymph nodes, lung, heart, stomach, spleen, pancreas, kidney, adrenal, liver, gallbladder, small intestine, large intestine, urinary bladder, rib, skeletal muscle, testis and prostate or ovary, and uterus. Body weights of the dogs administered 150 or 350 mg/kg/d were reported to be decreased (statistical significance not specified) at the termination of the experiment; and enlarged livers were reported in dogs administered 150 or 350 mg/kg/d (statistical significance not specified). Although changes in body weight are reported, it is unclear if these changes were over the duration of the study or observed at terminal sacrifice. For all 18 dogs, including the two that died earlier, bone marrow smears, fat content of the liver and kidney, and adrenal glands were examined and no significant changes were reported. Necropsies were conducted on dogs that died prior to the end of the experiment and on dogs that were killed at termination. Results of histopathological examinations were only reported in the three dogs (two male and one surviving female) in the high-dose group. Microscopic examinations in these three dogs included examination of the brain, pituitary, salivary, spleen, pancreas, kidney, adrenal, liver, gallbladder, small intestine, large intestine, urinary bladder, rib, skeletal muscle, and testis and prostate or ovary and uterus. No significant histopathological effects were reported.

Reproductive/developmental toxicity studies. Morrissey et al. (1989) reported the methods and results from the Reproductive Assessment by Continuous Breeding (RACB) study, which includes 48 continuous breeding studies performed for various chemicals including methyl salicylate

Table 7. Reproductive performance of fertile pairs during continuous breeding (task 2) methyl salicylate (Gulati et al. 1984).

	Treatment group (methyl salicylate concentration)			
Reproductive parameter[a]	Control	100 mg/kg	250 mg/kg	500 mg/kg
Litters per pair	4.92 ± 0.044 (38)[b]	4.82 ± 0.128 (17)	4.78 ± 0.129 (18)	4.50 ± 0.258 (16)*
Proportion of pups born alive	0.97 ± 0.010 (38)	0.98 ± 0.010 (17)	0.96 ± 0.017 (18)	0.91 ± 0.033 (16)*
Live pups per litter				
Male	5.73 ± 0.209 (38)	5.41 ± 0.249 (17)	4.75 ± 0.384 (18)	4.32 ± 0.364 (16)**
Female	5.55 ± 0.206 (38)	5.05 ± 0.228 (17)	4.83 ± 0.313 (18)	3.46 ± 0.351 (16)**
Combined	11.29 ± 0.364 (38)	10.47 ± 0.348 (17)	9.58 ± 0.648 (18)	7.78 ± 0.635 (16)**
Live pup weight (g)				
Male	1.65 ± 0.014 (38)	1.64 ± 0.020 (17)	1.64 ± 0.018 (18)	1.60 ± 0.023 (16)
Female	1.60 ± 0.014 (38)	1.59 ± 0.021 (17)	1.57 ± 0.020 (18)*	1.53 ± 0.016 (16)*
Combined	1.62 ± 0.013 (38)	1.62 ± 0.021 (17)	1.60 ± 0.017 (18)	1.57 ± 0.021 (16)*
Adjusted live pup weight (g)[c]				
Male	1.67 ± 0.014 (38)	1.65 ± 0.019 (17)	1.63 ± 0.019 (18)	1.57 ± 0.021 (16)**
Female	1.61 ± 0.013 (38)	1.59 ± 0.019 (17)	1.56 ± 0.018 (18)*	1.51 ± 0.021 (16)**
Combined	1.64 ± 0.013 (38)	1.62 ± 0.018 (17)	1.59 ± 0.018 (18)*	1.54 ± 0.020 (16)**

[a]Mean ± SE
[b]Number of pairs providing data indicated in ().
[c]Means adjusted for total number of live and dead pups per litter by analysis of covariance.
*Significantly different $p < .05$ from control group.
**Significantly different $p < .01$ from control group.

(Table 6). A review of Morrissey et al. (1989) suggested that the information reported specifically for methyl salicylate was from studies conducted in two separate laboratories: Environmental Health Research Testing (Lab A) and Research Triangle Institute (Lab B). The methods and results from Lab A have been published in a detailed technical report (Gulati et al. 1984), while a summary of the methods and results for Lab B are provided in Morrissey et al. (1989) and in an abstract reported by NTP (1984) (Table 6). The study protocol for each lab consisted of four tasks: Task 1 – dose range finding study, Task 2 – continuous breeding phase, Task 3 – crossover mating phase, and Task 4 – second-generation phase.

In the study conducted at Lab A (Gulati et al. 1984), groups of male and female mice were administered methyl salicylate by gavage at doses of 0, 100, 250, or 500 mg/kg/d, while animals in Lab B (Morrissey et al. 1989; NTP 1984) were administered doses of 0, 25, 50, or 100 mg/kg/d by gavage. In Task 1, eight mice per sex per dose group were dosed for 14 days, and clinical signs, mortality, body weight gain, and food and water consumption were reported. In task 2 (continuous breeding phase), dose groups included 20 mice per sex per dose group with 40 mice per sex in the control group. During task 2, male and female mice were housed separately and dosed for 7 d before mating. The mice were then randomly paired for mating and housed together while receiving daily exposures for 98 d. Body weight, proportions of males, number or litters per pair, and number of live and dead pups were recorded within 12 h of birth in the first generation (F_1) newborn pups, then the litters were discarded.

In task 3, the crossover mating phase, F_0, mice continued to this phase only if any significant adverse effects on fertility were observed in the F_1 pups. In order to determine if the effects seen were sex dependent, F_0 mice in the high-dose group were mated with control mice in different combinations. Control males were mated with control females, control males were mated with high-dose females, and high-dose males were mated with control females. Litters were observed for litter size and sex, pup weight was measured and F_0 mice were necropsied. Endpoints measured in F_0 necropsied female mice included selected organ weights (brain, kidneys/adrenals, liver, ovary/oviducts, pituitary, reproductive tract, and uterus) and histology. In F_0 males, selected organ weights (brain, kidneys/adrenals, and liver), histology, percentage motile sperm, epididymal sperm concentration and percentage of abnormal sperm were measured.

In task 4, the second-generation phase, mice were exposed to methyl salicylate for a 7 d premating period and then were randomly grouped into mating pairs and cohabitated for 98 d with continuous treatment. The F_1 mice born within the next 21 d were exposed throughout lactation until weaning, and body weight, proportion of males, number of litters per pair, number of live, and dead pups were assessed. F_1 mice were continually dosed after weaning and then mated, at about 74 d, from the same treatment group (20 mice/group/sex). Litter size, sex, and pup weight were recorded for second-generation (F_2) mice.

The results from the dose-setting study (task 1) were not reported. For task 2, no statistically significant changes in body weights or fertility indices were reported following exposure to any dose of methyl salicylate (Gulati et al. 1984). A statistically significant decrease in the mean number of litters, the average number of pups per litter, the proportion of pups born alive, and mean live pup weights were reported to occur after exposure to 500 mg/kg/d of methyl salicylate (Gulati et al. 1984) (Table 7). A statistically significant decrease in live pup weights was reported to occur in females and in the adjusted live pup weight (adjusted for total number of live and dead pups per litter) in females and combined total pups (male and females) after exposure to 250 or 500 mg/kg/d of methyl salicylate (Gulati et al. 1984). For Lab B, NTP (1984) and Morrissey et al. (1989) reported no significant effects on body weight or reproductive indices in the F_1 or F_2 generation at any of the doses tested.

Summary of the available data. In summary, based on the highest quality studies identified, a chronic 2-year toxicity study conducted in rats and dogs (Webb & Hansen 1963) and a RACB study conducted in mice (Gulati et al. 1984; Morrissey et al. 1989) was available to assess the potential hazard from

chronic oral exposure to methyl salicylate. While only minimal effects (changes in body weight) were noted in rats and dogs following two years of oral (capsule) exposure to methyl salicylate (Webb & Hansen 1963); statistically significant reproductive/developmental effects were reported by Morrissey et al. (1989) and Gulati et al. (1984) in a RACB study in mice, in which oral exposure occurred during gestational development, considered a sensitive window of exposure. These effects included significant decreases in the mean number of litters, the average number of pups per litter, the proportion of pups born alive and mean live pup weights reported to occur after oral exposure to methyl salicylate (Gulati et al. 1984; Morrissey et al. 1989).

While the studies or abstracts that received quality scores of 3 and 4 were not considered for the quantitative assessment, they were reviewed and considered qualitatively. The only study receiving a study quality score of 3 was a reproductive toxicity study conducted in rats fed methyl salicylate in the diet at concentrations of 0, 500, 1500, 3000, or 5000 ppm (Collins et al. 1971). The study received a quality score of three because it was performed prior to GLP, no testing guidelines were followed, and the purity of the test substance was not specified in the study report. However, the results reported by Collins et al. (1971) in the second generation rats are comparable the results reported in Morrissey et al. (1989) and Gulati et al. (1984). Collins et al. (1971) reported no treatment related effects in the parental group. A significant decrease in the fertility index was reported in the 5000 ppm groups in the second and third generations. Significant decreases in mean litter size, mean number of live born pups per mated female, and mean number of offspring that survived to day 4 per mated female were reduced at 3000 ppm and 5000 ppm in the second generation only. However, these same effects were not noted in the first and third generations at any concentration tested.

Fifteen studies received study quality scores of 4. Of the 15, 10 were considered review articles and did not report original study data, and were, therefore, not considered either qualitatively or quantitatively. One of the 15 receiving a score of 4 was only available in an abstract form (Packman et al. 1961). Packman et al. (1961) exposed groups of rats to methyl salicylate in the diet at concentrations of 0, 700, or 2100 ppm for 2 years. The authors reported no treatment-related effects on growth, survival, or food consumption, no changes in blood or urine parameters and no increase in treatment related lesions following pathological evaluation.

Four of the 15 studies receiving a quality score of 4 (Harrisson et al. 1963; LaWall & Harrisson Research Laboratories, Inc. 1964; USFDA 1966; Abbot and Harrisson 1978) were unpublished studies that were not publically available; however, summaries were presented in the CIR (2003) and NFSA (2012). Abbot and Harrisson (1978) and LaWall and Harrisson Research Laboratories, Inc. (1964) exposed rats to various concentrations of methyl salicylate in the diet for durations ranging from 10 to 30 weeks. Abbot and Harrisson (1978) reported increased bone density at 1.13% and 2% methyl salicylate and bone lesions at 2% methyl salicylate in the diet for 10 to 12 weeks, while LaWall and Harrisson Research Laboratories, Inc. (1964) reported increased bone density in rats feds diets containing 11 250 and 20 000 ppm methyl salicylate for 30 weeks. A short summary of Harrisson et al. (1963) can be found in the NFSA (2012) and indicates increased bone density was reported in rats fed diets containing methyl salicylate for 10–12 weeks.

LaWall and Harrisson Research Laboratories, Inc. (1964) and USFDA (1966) also performed studies in dogs administered oral capsules of methyl salicylate at doses ranging from 50 to 800 mg/kg/d for 6–7.5 months. Survival was decreased in dogs dosed at 500 and 800 mg/kg/d and some increases in liver and kidney weight were noted at 150 mg/kg/d and higher; however, the pathologist reported no lesions or deleterious alterations at any dose tested. While the summaries of these unpublished studies presented in CIR (2003) indicate that the results support those reported by Webb and Hansen (1963) of increased incidence of cancellous bone in rats with an absence of the same results reported in dogs, the information reported in the CIR (2003) was not adequate to perform a quantitative assessment of these results.

Dose–response assessment

Identification of study and endpoints for dose–response modeling

The ADI reported by JECFA (1968, 2002) of 0.5 mg/kg/d is based on a NOEL of 50 mg/kg/d reported in a 2-year study in dogs. While JECFA (2002) does not cite the study reference that is the basis of the ADI, it is assumed that the data that serve as the basis for the ADI are that reported in the only 2-year dog study identified in the literature and performed by Webb and Hansen (1963). Results reported by Webb and Hansen (1963) for both rats and dogs indicated that the only adverse effect was the increased incidence of cancellous bone reported in a subset of rats fed 2% methyl salicylate in the diet with no statistically significant adverse effects reported in dogs. An increased incidence of cancellous bone in the metaphysis was reported in a subset of rats ($n = 10$ or 11) and the incidence appeared to be dose related with an incidence rate of 1/11 bones, 2/11 bones, and 9/9 bones in the low-, mid-, and high-dose groups. However, no information concerning the incidence of cancellous bone in the control group was given in the study. While the authors state that the increased incidence in the high-dose group was very apparent compared with the controls, the data reported in the study were insufficient to determine if the incidence of these lesions was statistically significantly increased compared with control animals. In addition, it is unclear why all animals from a treatment group were not examined and whether or not each bone examined was from separate animals. In the absence of the incidence for the control group, it is not possible to conduct dose–response modeling on the incidence of cancellous bone. Therefore, this data set from Webb and Hansen (1963) was not considered for the development of a POD. The biological impact of the increased amount of cancellous bone (also referred to as spongy bone or trabecular bone) in the metaphysis of rats is also unclear. No data were identified to clarify the biological significance of this bone

Table 8. Litter data for individual mating pairs (Gulati et al. 1984).

	Number of live pups in each litter					Minimum number of dead pups in each litter					Total pups dead and alive[a]				
Litter number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Control	12	15	11	11	10	0	0	0	0	0	12	15	11	11	10
	12	9	9	10	12	0	0	0	0	0	12	9	9	10	12
	8	11	4	14	15	0	1	0	0	1	8	12	4	14	16
	11	12	10	13	12	0	0	0	0	0	11	12	10	13	12
	11	13	11	11	11	0	0	0	1	0	11	13	11	12	11
	15	15	17	16	13	0	0	0	1	0	15	15	17	17	13
	11	12	12	11	9	0	0	1	1	0	11	12	13	12	9
	13	14	12	5	0	0	0	0	3	6	13	14	12	8	6
	11	12	10	13	12	0	0	0	0	0	11	12	10	13	12
	10	14	15	13	6	0	0	0	0	0	10	14	15	13	6
	10	9	15	16	0	1	0	0	0	3	11	9	15	16	3
	6	4	8	5	8	0	0	0	0	3	6	4	8	5	11
	9	12	6	8	14	0	0	0	0	0	9	12	6	8	14
	10	11	12	11	7	0	0	0	0	0	10	11	12	11	7
	12	13	13	11	7	0	0	0	0	0	12	13	13	11	7
	9	14	14	15	12	0	0	0	0	0	9	14	14	15	12
	7	9	2	10	11	0	0	3	0	0	7	9	5	10	11
	11	15	8	0	0	0	0	0	9	6	11	15	8	9	6
	9	12	11	10	13	0	0	0	0	0	9	12	11	10	13
	11	10	11	10	6	0	0	0	0	0	11	10	11	10	6
	12	14	13	14	8	0	1	0	0	0	12	15	13	14	8
	15	17	16	17	16	0	0	0	0	1	15	17	16	17	17
	11	14	11	13		0	0	0	0		11	14	11	13	
	9	9	13	12	13	0	0	0	0	0	9	9	13	12	13
	10	12	8	11		0	0	0	0		10	12	8	11	
	12	14	11	13	12	0	1	0	0	0	12	15	11	13	12
	9	12	12	10	10	0	0	0	0	0	9	12	12	10	10
	10	14	16	15	13	0	2	0	0	0	10	16	16	15	13
	11	12	0	0	12	0	2	2	3	0	11	14	2	3	12
	14	15	7	12	10	0	0	0	0	0	14	15	7	12	10
	10	11	11	9	11	0	0	0	0	0	10	11	11	9	11
	10	14	12	15	15	1	0	0	0	0	11	14	12	15	15
	13	13	14	16	13	0	0	0	0	0	13	13	14	16	13
	13	10	13	11	10	0	0	0	0	0	13	10	13	11	10
	10	12	12	12	14	0	0	0	0	0	10	12	12	12	14
	13	11	11	12	11	0	0	0	0	0	13	11	11	12	11
	15	16	19	16	15	0	0	0	0	0	15	16	19	16	15
	17	10	15	13		0	1	0	0		17	11	15	13	
MEAN	11.1	12.3	11.2	11.4	10.3	0.1	0.2	0.2	0.5	0.6	11.2	12.5	11.4	11.9	10.9
SE	0.4	0.4	0.63	0.6	0.7	0	0.1	0.1	0.3	0.3	0.4	0.5	0.73	0.9	1
0.1 g/kg	15	10	4			0	0	0			15	10	4		
	14	12	5	11	2	0	0	0	0	3	14	12	5	11	5
	11	12	11	9	8	0	0	0	0	0	11	12	11	9	8
	9	10	10	10	12	0	0	0	0	0	9	10	10	10	12
	10	12	11	8	10	0	0	0	0	0	10	12	11	8	10
	11	12	12	10	11	0	0	0	0	0	11	12	12	10	11
	10	13	11	10	10	0	0	0	0	0	10	13	11	10	10
	10	12	16	13	9	1	1	0	0	0	11	13	16	13	9
	6	13	10	10	13	0	0	0	0	0	6	13	10	10	13
	11	13	13	9	11	0	0	0	0	0	11	13	13	9	11
	14	12	12	11	10	0	0	0	0	1	14	12	12	11	11
	14	3	7	12	12	0	0	0	0	0	14	3	7	12	12
	11	14	13	14	12	0	0	0	0	0	11	14	13	14	12
	13	10	11	12	9	1	0	0	0	0	14	10	11	12	9
	11	11	10	11		1	0	0	0		12	11	10	11	0
	11	12	8	10		0	0	1	0		11	12	9	10	0
	7	15	11	13	10	1	0	0	0	0	8	15	11	13	10
	10	6	9	0	7	0	2	2	1	1	10	8	11	1	8
Mean	11	11.2	2.9	10.2	9.7	0.2	0.2	0.2	0.1	0.3	11.2	11.4	3.1	10.3	10
SE	0.6	0.7	0.7	0.7	0.7	0.1	0.1	0.1	0.1	0.2	0.7	0.8	0.8	0.8	0.9
0.25 g/kg	9	9	6	6	8	2	0	0	1	0	11	9	6	7	8
	10	7	11	10	7	0	0	0	0	0	10	7	11	10	7
	12	10	9	7	10	0	0	0	0	0	12	10	9	7	10
	11	14	13	10	9	0	0	0	0	0	11	14	13	10	9
	11	11	13	12		0	0	0	0		11	11	13	12	0
	14	12	15	12	11	0	0	0	0	0	14	12	15	12	11
	9	3	11	1	0	0	0	0	1	2	9	3	11	2	2
	11	11	11	11	10	1	0	0	0	0	12	11	11	11	10
	11	2	5			0	0	1			11	2	6	0	0

(*continued*)

Table 8. Continued.

	Number of live pups in each litter					Minimum number of dead pups in each litter					Total pups dead and alive[a]				
Litter number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	10					0					10	0	0	0	0
	11	14	13	13	14	0	0	0	0	0	11	14	13	13	14
	6	7	7	5	9	0	0	0	2	0	6	7	7	7	9
	11	14	11	10	10	0	0	0	0	0	11	14	11	10	10
	11	15	10	14	11	0	0	0	0	0	11	15	10	14	11
	10	12	7	2	8	0	0	3	0	0	10	12	10	2	8
	10	15				0	0				10	15	0	0	0
	10	13	0	2		0	0	7	3		10	13	7	5	0
	11	11	13	15	12	0	0	0	0	0	11	11	13	15	12
	14	14	9	12	11	0	0	0	0	0	14	14	9	12	11
	6	8	7	12		0	1	0	0		6	9	7	12	0
Mean	10.4	10.6	9.5	9.1	9.3	0.2	0.1	0.6	0.4	0.1	10.6	10.7	10.1	9.5	9.4
SE	0.4	0.9	0.9	1.1	0.9	0.1	0.1	0.4	0.2	0.1	0.5	1	1.3	1.3	1
0.5 g/kg	8	9	12	7		0	0	0	0		8	9	12	7	
	13	6	6	11	1	0	1	0	0	0	13	7	6	11	1
	10	12	13	14	13	0	0	1	0	0	10	12	14	14	13
	10	5	8	11		0	0	0	0		10	5	8	11	0
	10	11	12	11	9	0	2	0	0	0	10	13	12	11	9
	7	0	8	0		0	4	0	4		7	4	8	4	0
	2	9	11	10		2	2	1	0		4	11	12	10	0
	10	8	9	11	8	0	0	0	0	0	10	8	9	11	8
	9	11	11			0	0	0			9	11	11	0	0
	6	6	5	7	6	1	1	0	1	1	7	7	5	8	7
	10					0					10				
	2	12	15	17	13	0	0	0	0	0	2	12	15	17	13
	8	8	11	10	0	1	0	0	0	2	9	8	11	10	2
	10	9	11	9	8	0	0	0	0	0	10	9	11	9	8
	3	8	11	0	0	8	0	0	7	2	11	8	11	7	2
	9	4	7	3	4	0	1	0	0	1	9	5	7	3	5
	10	6	12	0		0	2	0	5	0	10	8	12	5	0
	5					0					5				
	13	12	7	7	0	0	0	0	0	2	13	12	7	7	2
Mean	8.2	8	9.9	8	5.6	0.6	0.8	0.1	1.1	0.7	8.8	8.8	10	9.1	6.3
SE	0.8	0.8	0.7	1.3	1.5	0.4	0.3	0.1	0.6	0.3	1.2	1.1	0.8	1.9	1.8

[a]The total number of pups was not reported, so it was assumed the total pups equaled sum of the live and dead pups.
indicates no litter data available.

lesion and determine the historical incidence of cancellous bone in rats.

The results from the RACB reproductive/developmental study for methyl salicylate (Gulati et al. 1984; Morrissey et al. 1989) were reviewed to determine if the data could be relied upon to quantify the potential toxicity of methyl salicylate (Table 6). The authors reported no statistically significant changes in body weights or reproductive indices in the F_1 or F_2 generation mice exposed to doses of 25, 50, or 100 mg/kg/d, compared with controls (NTP 1984). Gulati et al. (1984) reported a statistically significant decrease in the number of litters per pair, the number of live pups per litter, and the proportion of pups born alive following administration of doses of 500 mg/kg/d (Table 7). A statistically significant decrease in live pup weight and adjusted live pup weight (adjusted for the total number of live and dead pups per litter by analysis of variance) was also reported following administration of doses of 250 or 500 mg/kg/d (Table 7). Following a review of the studies identified that had the appropriate criteria, data, and study quality scores (Webb & Hansen 1963; Gulati et al. 1984; Morrissey et al. 1989), it was determined that the reproductive/developmental endpoints reported by Gulati et al. (1984) were the only results for which sufficient data were available to be used for dose-response modeling. Webb and Hansen (1963) reported no significant effects in rats following chronic exposure; and the effects reported in dogs following oral exposure were not sufficiently reported for use in dose-response modeling.

Dose–response modeling and identification of point of departure (POD)

Based on the effects reported in Gulati et al. (1984) and NTP (1984), a POD using the NOAEL of 100 mg/kg/d could be assumed based on statistically significant decreases in the number of litters, number of pups per litter, proportion of pups born alive, or mean pup weight reported in animals administered 250 mg/kg/d. Following review of the data reported by Gulati et al. (1984), it was determined that the endpoints for which the data necessary for BMD modeling were available included average number of litters per pair (Table 7), live pup weight adjusted for the average litter size (Table 7), and the proportion of pups born alive per fertile pair (Tables 7 and 8). Because many of the endpoints from developmental studies are reported with litter as the statistical unit, data from these types of studies are often best modeled using nested models (USEPA 2012). Nested models account for any intra-litter correlation, or the tendency of

Table 9. BMD results from Gulati et al. (1984).

					(Continuous 1 STD change, extra risk) (g/kg/d)	
Endpoint	Model	AIC	Chi square or likelihood ratio goodness of fit *p* value	Scaled residual at observed dose closest to BMD	BMD	BMDL
Average pup weight	Exponential2	362.11	.664	0.081	0.41	0.29
	Exponential3	360.13	.373	0.007	0.41	0.29[a]
	Exponential4	362.11	.664	0.081	0.41	0.24
	Exponential5	358.13	N/A	0.007	0.41	0.29
	Hill	358.13	N/A	0.006	0.42	0.23
	Linear	362.12	.667	0.065	0.41	0.30
	Polynomial	360.14	.374	0.001	0.42	0.30
	Power	360.13	.373	0.007	0.42	0.30
Litters per mating pair	Exponential2	44.09	N/A	0.212	0.42	0.21
	Exponential3	44.09	N/A	0.212	0.42	0.21
	Exponential4	43.44	N/A	0.506	0.42	0.13
	Exponential5	43.44	N/A	0.506	0.42	0.13
	Hill	43.73	.104	0.547	0.43	
	Linear	43.96	.110	0.222	0.43	0.22[a]
	Polynomial	43.06	.068	0.435	0.41	0.15
	Power	43.96	.940	0.222	0.43	0.22[a]

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: lower bound on BMD.
[a]Lowest BMDL with the best model fit.
N/A: not available. A *p* value could not be determined by BMDS. Model output indicates that other models should be considered because model fit cannot be confirmed. Benchmark computation failed.

littermates to respond more similar to one another relative to the other litters in a dose group. If this correlation (which may vary with dose) is not estimated, variance estimates, and hence the confidence limits on benchmark responses and doses, will generally be miss-specified. In addition, these models often include provision for a litter-specific covariate, such as the litter size, which may be correlated with the outcome of interest (but not necessarily with the treatment) and may help clarify the response pattern (USEPA 2012).

The average litter size data (Table 7) were modeled in BMDS (USEPA 2016) as continuous data with a benchmark response (BMR) of one standard deviation below the control mean to determine a BMD and a 95% lower bound confidence limit (BMDL). For continuous data, USEPA (2012) recommends a BMR be defined based on the level of change in the endpoint at which the effect is considered to become biologically significant. However, in the absence of the level of response to consider adverse, a change in the mean equal to one control standard deviation from the control mean is recommended (USEPA 2012). Because the variance was not homogeneous across the dose groups, a non-homogeneous variance model was used. A nested model was not appropriate to use to model this particular endpoint because the nested model shows the risk of an effect nested within the litter; however, a change in litter size is the effect for this endpoint. Therefore, continuous models including the polynomial, power, linear, exponential, and Hill models were used (Table 9). Model results were then evaluated for goodness of fit and the BMD and BMDL of those models with the lowest Akaike information criterion (AIC) value that had an adequate Chi-square *p* value (greater than .1) were considered in identifying a POD for use in estimating an ADI. The linear and power models, which produced the same fit, were chosen as the best fit models for the litter per pair endpoint and produced a BMD of 428 mg/kg/d (0.428 g/kg/d) and a BMDL of 220 mg/kg/d (0.22 g/kg/d). The modeling results are summarized in Table 9 and graphs of the BMDL output from both the linear and power models are presented in Figure 1(a) and (b).

The live pup weights (that were not adjusted for litter size) were not modeled (Table 7), because these weights do not account for the significant change noted in the average litter size in treated animals compared with untreated controls. The more appropriate data to model were the live pup weight adjusted for litter size (Table 7). Although it would be most appropriate to model this endpoint with a nested model, the available models in BMDS require specific information. First, the individual pup weights would be necessary and Gulati et al. (1984) do not provide this information. In addition, the only nested models available in BMDS require dichotomized data, which would require a transformation of the continuous body weights for the pups to a dichotomized form. Because of this, the adjusted pup weight data (Table 7) were modeled using the continuous models (not nested), provided in BMDS. The variance for the adjusted pup weight was homogeneous across dose groups so the homogenous variance option for continuous data was used for the BMDS models, with a BMR of one standard deviation below the control mean used to determine a BMD and a BMDL. The linear model was chosen as the model providing the best fit to the adjusted average pup weights, with an estimated BMD of 412 mg/kg/d (0.412 g/kg/d) and a BMDL of 297 mg/kg/d (0.297 g/kg/d). The modeling results are summarized in Table 9.

In addition to requiring dichotomous data, the nested models in BMDS only allow for an event in which the incidence is increasing. For the endpoint of proportion of pups born alive per fertile pair, the results are expressed as a decreasing proportion born alive with increasing concentration of methyl salicylate (Table 7) (Gulati et al. 1984). This required some transformation of the individual data to convert the result to the number of dead pups in each

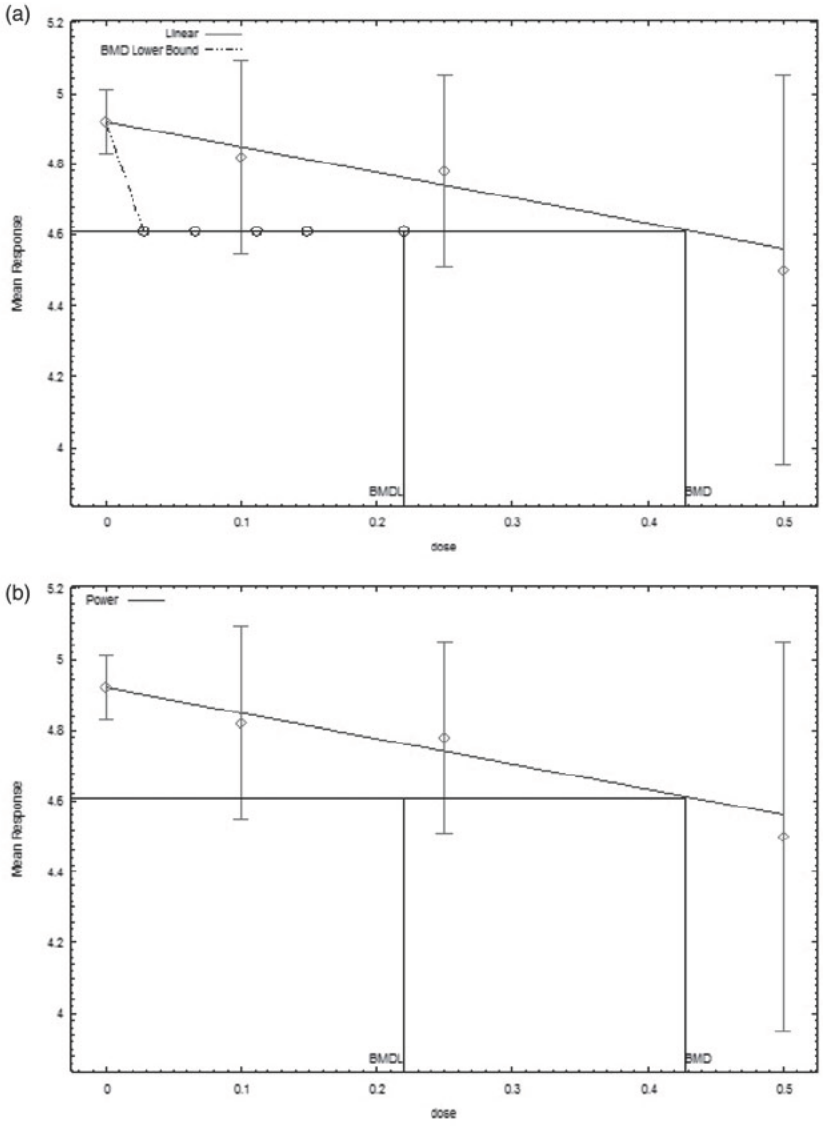


Figure 1. (a) Graph of benchmark modeling output for number of litters per mating pair reported in Gulati et al. (1984). Linear model with BMR of 1 standard deviation for the BMD and 0.95 lower confidence limit for the BMDL. (b) Graph of benchmark modeling output for number of litters per mating pair reported in Gulati et al. (1984). Power Model with BMR of 1 standard deviation for the BMD and 0.95 lower.

litter (Table 8). The result would be an endpoint where adverse changes would be associated with an increase in incidence with increasing dose, rather than a decrease in incidence with increasing dose. The individual data were available for this endpoint (Table 8) and were used in the BMD modeling. Because the BMD and BMDL for these nested models are computed in terms of a fixed litter size, both the mean litter size of the control group and the mean litter size over all dose groups were used. In addition, the models were fit both using the litter as a covariate and without the additional covariate. The NLogistic model using litter size as a covariate with the control group average litter size used to determine the BMD and BMDL provided the best fit model to the data, with a BMD of 2710 mg/kg/d (2.71 g/kg/d) and a BMDL of 770 mg/kg/d (0.77 g/kg/d). If the average litter size over all dose groups was used to determine the BMD and BMDL, the results were slightly different with a BMD of 1670 mg/kg/d (1.67 g/kg/d) and a BMDL of 600 mg/kg/d (0.60 g/kg/d). All modeling for this endpoint was conducted with a 95% confidence level with a BMR of 5% as recommended by Allen et al. (1994), which concluded that benchmark doses developed from nested models with BMRs at the 5% level of risk were similar to no observed adverse effect levels determined by statistical tests of trend from a large dataset of reproductive/developmental studies. USEPA (2012) also notes "most reproductive and developmental studies with nested study designs support a BMR of 5%". The modeling results are presented in Table 10.

A BMDL based on the best model fit for each of the datasets modeled was determined. For decreases in the numbers of litters per mating pair, the best model fit was produced by both the linear and power models and produced a BMD of 428 mg/kg/d (0.428 g/kg/d) and a BMDL of 220 mg/kg/d (0.22 g/kg/d). Modeling results for the adjusted average pup weights indicated the exponential 3 model was the model providing the best fit with an estimated BMD of 415 mg/kg/d (0.415 g/kg/d) and a BMDL of 293 mg/kg/d (0.293 g/kg/d). Finally, for the number of dead pups per individual breeding

Table 10. ndividua breeding pair BMD mode ing resu ts for Gu ati et a . (1984).

Endpoint	Mode	Covariate	A C	Chi-Square P-va ue (bootstrap)	Average sca ed residua for dose group nearest the BMD	Average ABS(sca ed residua) for dose group nearest the BMD	Mean itter specific covariate type for BMD and BMDL	BMD 5 (g/kg/d)	BMDL 5 (g/kg/d)
No. dead pups/ individua breeding pair	NLogistic	Litter size	843.58	.782	−0.180	0.283	Contro mean =11.5	2.71	0.77[a]
	NCTR	Litter size	864.47	.935	−0.366	0.366		0.49	0.49
	Rai_and_Van_Ryzin	Litter size	836.38	–	–	–		–	–
	NLogistic	Not used	879.54	.287	−0.444	0.444		0.41	0.30
	NCTR	Not used	879.72	.303	−0.444	0.444		0.48	0.48
	Rai_and_Van_Ryzin	Not used	879.72	.321	−0.444	0.444		0.48	0.48
	NLogistic	Litter size	843.58	.763	0.279	0.760	Overa mean =10.6	1.67	0.60
	NCTR	Litter size	864.47	.920	−0.118	0.610		0.48	0.48
	Rai_and_Van_Ryzin	Litter size	836.38	–	–	–		–	–
	NLogistic	Not used	879.54	.309	−0.190	0.606		0.41	0.30
	NCTR	Not used	879.72	.322	−0.189	0.606		0.48	0.48
	Rai_and_Van_Ryzin	Not used	879.72	.315	−0.189	0.606		0.48	0.48

A C, Akaike information criterion; BMD: benchmark dose; BMDL, ower bound on BMD. –: Benchmark computation fai ed.
[a]Lowest BMDL with the best mode fit.

pair, the NLogistic model provided the best fit and a BMD of 2710 mg/kg/d (2.71 g/kg/d) and a BMDL of 770 mg/kg/d (0.77 g/kg/d). Overall, the most sensitive endpoint determined from BMDS modeling was the number of litters per mating pair with a BMDL of 0.22 g/kg/d or 220 mg/kg/d. This BMDL will serve as the POD in the derivation of an allowable daily intake for methyl salicylate.

Mode of action and human relevance assessment

As the endpoints of interest for dose–response modeling were all reproductive/developmental endpoints, consideration of the available mode of action information associated with these types of effects following oral exposure to methyl salicylate was evaluated. The results from studies that focus on the mode of action of methyl salicylate were used to determine (1) whether the reproductive effects reported in animal models are relevant to humans and (2) what uncertainty factors should be applied to the POD estimated from Gulati et al. (1984) in order to estimate an acceptable daily intake. The most conservative BMDL or POD of 220 mg/kg/d was derived based on a significant decrease in the number of litters per pair, in mice orally exposed to methyl salicylate during mating. No human data related to oral toxicity of methyl salicylate were identified in the literature. In addition, a review of the available literature indicated limited data are available related to the mode of action of methyl salicylate following oral exposure.

Once absorbed, the majority of methyl salicylate is converted to salicylic acid (Opdyke 1978). Salicylic acid is also the major metabolite of acetylsalicylic acid or aspirin for which mode of action data are readily available (Hutt et al. 1986). Therefore, similarities in the mode of action for methyl salicylate and acetylsalicylic acid can be assumed. Davison et al. (1961) conducted a study to compare the toxicity and metabolism of methyl salicylate and salicylic acid. Davison et al. (1961) concluded that the results indicated the toxicity and metabolism of both methyl salicylate and salicylic acid are comparable across multiple species (rats, dogs, and monkeys).

The mode of action for salicylic acid, the metabolite of both methyl salicylate and acetylsalicylic acid, has been well studied and is known to involve the inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activity, which in turn leads to a decrease in the synthesis of prostaglandins and thromboxane (Patrono et al. 2001; Roberts & Morrow 2001; Vane & Botting 2003). The inhibition of COX-2 leads to the therapeutic effects associated with the use of salicylates as antipyretic, analgesic, and anti-inflammatory agents, and it can also lead to unwanted side effects due to decreased prostaglandin formation (Roberts & Morrow 2001). Prostaglandins play an important role in reproductive functions (Rudolph 1981). The inhibition of prostaglandins may interfere with uterine contractility, platelet function, and fetal vascular structure (Rudolph 1981). Offspring of women that ingested salicylates for long periods during pregnancy have been reported to have significantly reduced body weights (Roberts & Morrow 2001). In pregnant women administered aspirin during the third trimester, increased perinatal

mortality, anemia, antepartum and postpartum hemorrhage, prolonged gestation, and complicated deliveries were reported (Roberts & Morrow 2001).

Similar reproductive effects to those reported following oral methyl salicylate exposure in animals have also been reported following oral exposure to acetylsalicylic acid. These effects include decreased litter size and fetal weights in rats orally exposed to salicylic acid during gestation (CIR 2003). Therefore, based on similar reproductive effects noted in animals and similar metabolism of methyl salicylate; along with the reported reproductive effects in humans following exposure to acetylsalicylic acid during pregnancy, it was assumed that the reproductive effects reported in rats following methyl salicylate exposure are relevant to humans. This is supported by the mode of action of salicylates, which involves the inhibition of prostaglandin synthesis which has been reported to interfere with uterine contractility, platelet function, and fetal vascular structure (Rudolph 1981).

Adjustments for uncertainty

Based on the study characteristics and the mode of action information available in animals following oral exposure to methyl salicylate, an uncertainty factor analysis was performed in accordance with the methods currently recommended by USEPA (2002). As noted previously, a factor of 1–10 is commonly used to adjust for each type of uncertainty considered based on the available data and scientific judgment.

Interspecies uncertainty factor (UF_A). There are very limited data available to characterize the pharmacokinetic and/or pharmacodynamic differences between animals and humans following exposure to methyl salicylate. No data were identified to compare the pharmacokinetics of methyl salicylate in humans and mice, which is the species upon which the POD is based. Based on the data reported in Davison et al. (1961), there appear to be some slight differences in the metabolism of methyl salicylate between humans and animals. Methyl salicylate administered orally to dogs was nearly completely hydrolyzed to salicylic acid within 1 h of administration (Davison et al. 1961). However, hydrolysis was slower in humans when compared with animals with 21% total salicylate measured in the plasma 90 min following oral administration of methyl salicylate compared with the negligible amounts noted in dogs at 60 min post-administration. Therefore, based on recommendations by USEPA (2011a) an animal to human body weight scaling factor was calculated and applied to the POD to derive a HED. The DAF of 0.15 was estimated using the study specific mean maternal body weight at parturition reported by Gulati et al. (1984) of 0.042 kg divided by the mean body weight for a pregnant female of 75 kg provided in the USEPA Exposure Factors Handbook (USEPA 2011b) and raised to the ¼ power as shown in Equation (1). Because body weight scaling was applied, the default UF_A of 3 as recommended by USEPA (2011a) was used.

Intraspecies uncertainty factor (UF_H). When considering the pharmacokinetic variability component of the UF_H, the only available data specifically for methyl salicylate is provided by Davison et al. (1961). In this study, six volunteers were administered a single dose of methyl salicylate orally at an average dose of 7 mg/kg. Plasma levels of methyl salicylate, free salicylate, and total salicylate were measured at 15 and 90 min post-administration. The available results suggested limited variability in plasma concentrations among the volunteers. In addition, Davison et al. (1961) also administered a single dose of acetylsalicylic acid to the same six volunteers and measured total salicylate in the plasma at 15 and 90 min post-exposure. Results indicated variability in total salicylate plasma concentrations in humans following exposure to methyl salicylate did not differ markedly from total salicylate plasma concentrations following administration of acetylsalicylic acid.

While limited pharmacokinetic data were available in humans exposed to methyl salicylate that could be used to characterize variability in human pharmacokinetics; based on the similarities in metabolism and mode of action between methyl salicylate and acetylsalicylic acid pharmacokinetic data available for acetylsalicylic acid were used as surrogate data to characterize the potential variability associated with the pharmacokinetics of methyl salicylate in humans. In a review of the pharmacokinetic data in humans for salicylates as a class of compounds, Needs and Brooks (1985) concluded there were no significant differences in the pharmacokinetics of salicylates in the elderly or in children compared with young adults (Needs & Brooks 1985), indicating that there was limited human variability across age groups. In addition, human variability was limited when measured area under the curve (AUC) and peak serum concentrations in volunteers administered acetylsalicylic acid were compared across several studies (Gatti et al. 1989; Cerletti et al. 2003; Lucker et al. 2003).

When considering interspecies and intraspecies variability, the default factor of 10 for each is comprised of two components, pharmacokinetic differences and pharmacodynamics differences, each having a default value of 3 (Kroes et al. 1993; Beck & Clewell 2001; USEPA 2002; Dorne 2004). However, in cases where there are sufficient pharmacokinetic and/or pharmacodynamic data available, a reduction in the default UF_A or UF_H to 3 or 1 could be justified. Therefore, because there are sufficient data available to suggest limited human pharmacokinetic variability for methyl salicylate and the pharmacokinetic component of the UF_H was decreased from 3 to 1.

No data were identified that could be used to characterize human variability in the pharmacodynamics of methyl salicylate. However, the endpoint relied upon is from a sensitive subpopulation. The UF_A incorporates a factor to account for the potential that humans are more sensitive than animals. The available pharmacokinetic data also suggest limited variability across subpopulations. Therefore, the pharmacodynamic component of the UF_H was decreased from 3 to 1. The total UF_H would be 1, which is composed of a factor of 1 for pharmacokinetics and 1 for pharmacodynamics.

Chronic versus subchronic uncertainty (UF_S). The POD is based on a reproductive endpoint reported in a well-

conducted continuous breeding reproductive study (Gulati et al. 1984). While the exposure duration in reproductive studies is less than chronic, the duration or exposure and the timing of exposure in these types of studies are designed to occur during critical windows of reproductive and development physiology. Therefore, no uncertainty factors were applied to the POD to account for less than chronic exposure and the UF_S was 1.

LOAEL versus NOAEL uncertainty (UF_L). Because a BMDL is being recommended as the POD, which is considered a NOAEL equivalent, no uncertainty factor adjustment will be made. The UF_L was 1.

Incomplete database uncertainty (UF_D). The UF_D is intended to account for any underestimates of an acceptable daily intake due to incomplete characterization of the chemical's toxicity. In the case of methyl salicylate, the database characterizing toxicity associated with oral exposure includes chronic/carcinogenicity studies in multiple species, as well as, reproductive/developmental studies conducted in mice. Therefore, the data are considered complete and no adjustment for database insufficiency will be made to the POD. The UF_D will be 1.

Based on the uncertainty factor analysis, the POD for methyl salicylate will be adjusted by a total UF of 3 (3 for UF_A, 1 for UF_H, and 1 for UF_S, UF_L, and UF_D).

Estimation of an acceptable daily intake

An ADI is an estimate that includes adjustments for uncertainty in the data, of the daily oral exposure to the human population that is likely to be without an appreciable risk of adverse effects over the course of a lifetime. An ADI is derived from a POD, which is the dose–response point equivalent to the beginning of a low-dose extrapolation and includes body weight scaling adjustment (DAF) and an adjustment for uncertainty using UFs. An oral ADI of 11 mg/kg/d was derived using the BMDL estimated from data reported in Gulati et al. (1984) of 220 mg/kg/d based on significant decreases in the number of litters per pair in rats orally exposed to methyl salicylate during mating (Table 7). The BMDL was converted to a HED by multiplying by the DAF ($BW^{3/4}$ scaling factor) and adjusted by the uncertainty factors determined in the uncertainty factor analysis of 3 for UF_A, 1 for UF_H, and 1 for UF_S, UF_L, and UF_D. Equations (3) and (4) were used to calculate the ADI:

$$\mathrm{ADI} = \frac{\mathrm{BMDL(mg/kg/day)} \times \mathrm{DAF}}{\mathrm{UF_A} \times \mathrm{UF_H} \times \mathrm{UF_S} \times \mathrm{UF_L} \times \mathrm{UF_D}} \quad (3)$$

$$\mathrm{ADI} = \frac{22(\mathrm{mg/kg/day}) \times 0.15}{3 \times 1 \times 1 \times 1 \times 1} = 11(\mathrm{mg/kg/day}) \quad (4)$$

Discussion/conclusions

Several authoritative agencies have recommended an ADI for ingestion of methyl salicylate, which is used as a denaturant, external analgesic, flavoring agent, and fragrance ingredient in a variety of products including chewing gum, baked goods, syrups, candy, beverages and ice cream (CIR 2003; NFSA 2012). JECFA (1968, 2002) recommended an ADI of 0.5 mg/kg-body weight for methyl salicylate based on a NOEL of 50 mg/kg/d reported in a 2-year study in dogs; however, the specific study was not cited (JECFA 1968, 2002). JECFA (2002) also reports an estimated daily per capita intake for methyl salicylate of 0.7 mg/kg-body weight. In addition, NFSA (2012) relies upon the JECFA (2002) ADI of 0.5 mg/kg/d in making recommendations regarding acceptable levels of methyl salicylate in food. In making recommendations regarding concentrations of methyl salicylate in animal feed, EFSA (2012) relies upon a study conducted by Webb and Hansen (1963), which appears to be the same study relied upon by JECFA (2002) for recommending an ADI. Methyl salicylate also has USFDA-specified uses as an indirect food additive (CIR 2003).

Based on a review of the available published literature and summaries of unpublished literature identified through review documents for methyl salicylate, while chronic toxicity studies were available, no studies were identified that reported statistically significant increases in tumors following oral exposure to methyl salicylate. The studies relied upon by JECFA (2002), NFSA (2012), and EFSA (2012) were identified for review of non-cancer endpoints. However, studies were also identified that evaluate the potential reproductive/developmental toxicity of methyl salicylate following oral exposure (Gulati et al. 1984; NTP 1984; Morrissey et al. 1989; CIR 2003) which were available at the time the ADIs from EFSA, JECFA and NFSA were recommended but do not appear to have been considered by authoritative agencies when developing ADI's for methyl salicylate.

When considering the currently recommended ADI's for methyl salicylate, several points should be considered that could increase uncertainty in these ADI's. Based on current recommendations from the NRC (2014) in reviewing the processes applied by the EPA in estimating acceptable intake of chemicals that approaches be developed to identify studies in a systematic, defined manner and that study quality be considered. The literature search strategy used to identify the studies and the criteria used to assess study quality should be well documented. This ensures that all the available toxicological literature have been considered and the highest quality data currently available were incorporated into the dose-response assessment. A literature search strategy, the results of the literature search, and an assessment of study quality based on criteria recommended by Klimisch et al. (1997) were all incorporated into this investigation. The study quality analysis was conducted and a transparent set of study quality criteria were used to determine the highest quality studies receiving scores of 1 or 2, which were considered for evaluation for the dose response assessment. In order to receive a quality score of 1 or 2, the study must have been conducted according to a published testing guideline and preferably according to GLP. Studies receiving a score of 2 were those that were not reported to be conducted according to published guidelines; however, met the reporting requirements outlined by Klimisch et al. (1997) (Table 3). For this investigation, no studies received a score of 1. Studies receiving a score of 2 included the chronic toxicity

study relied upon by EFSA (2012) (and presumably JECFA (2002) and NFSA (2012)) conducted in rats and dogs (Webb and Hansen 1963); and one reproductive/developmental study conducted in two laboratories (Gulati et al. 1984; NTP 1984; Morrissey et al. 1989).

A second consideration that should be addressed when comparing the current recommended ADIs to the ADI proposed in this assessment is the ADI recommended by JECFA (2002), NFSA (2012) and EFSA (2012) is based on a NOEL or no observed effect level, for changes in body weights, an endpoint that is not typically considered adverse in the absence of accompanying histopathological changes when it is the only significant effect reported in a study (USEPA 1988). In addition, while a POD can be based on a NOEL, a POD based on the estimation of a BMD provides a more robust, informative POD because BMDs account for the shape of the dose-response curve over the continuum of observed exposures, rather than at a single dose level.

Webb and Hansen (1963) reported no significant effects in rats following chronic exposure; and the studies conducted in dogs following oral exposure do not provide adequate detail to allow for the use in a dose response assessment. However, based on the available data provided by Gulati et al. (1984), BMD modeling could be conducted for selected reproductive/developmental endpoints. These reproductive effects of methyl salicylate do not appear to have been considered for the ADIs recommended by JECFA (2002), NFSA (2012) and EFSA (2012), even though reproductive/developmental effects are typically considered more sensitive endpoints because they occur in sensitive subpopulations and the studies by Morrissey et al. (1989) and Gulati et al. (1984) were published and available at the time the JECFA, EFSA, and NFSA documents were published. This could possibly be because the ADI developed by JECFA of 0.5 mg/kg/d was higher than the expected daily consumption rate of 0.1 mg/kg/d. This could also be because a NOEL approach was used for the estimation of the POD for the ADIs and the NOAEL for reproductive effects would have been higher at 100 mg/kg/d reported by Gulati et al. (1984) compared with the NOEL for body weight changes in dogs of 50 mg/kg/d. The reproductive data may also not have been considered because many of the endpoints from developmental studies are reported as nested data with litter as the statistical unit. These data can be more difficult to model and are best modeled using nested models (USEPA 2012), which account for intra-litter correlation.

Benchmark modeling, using either continuous or nested models where appropriate, was conducted on datasets associated with decreased average pup weight, adjusted average pup weights, and the number of dead pups per individual breeding pair (Gulati et al. 1984). BMDLs for each dataset were chosen based on the best model fit and ranged from a BMDL of 220 mg/kg/d for decreases in average pup weight to a BMDL of 770 mg/kg/d for the number of dead pups per individual breeding pair. Overall, a BMDL of 220 mg/kg/d associated with the most sensitive endpoint (i.e. the number of litters per mating pair) was chosen as the BMDL to serve as the POD in the derivation of an ADI for methyl salicylate.

Due to a lack of relevant epidemiological studies in humans following oral exposure to methyl salicylate, a review of the mode of action was conducted to determine if the available data suggested that the most sensitive endpoint identified in animals (i.e. reproductive toxicity) was relevant to humans. Limited mode of action data were available for methyl salicylate; however, both methyl salicylate and acetylsalicylic acid are metabolized to the same active metabolite (i.e. salicylic acid) and there are data to characterize the mode of action for acetylsalicylic acid (Opdyke 1978; Hutt et al. 1986). The mode of action for salicylic acid involves the inhibition of COX-1 and COX-2 activity, leading to an inhibition in the synthesis of prostaglandins (Patrono et al. 2001; Roberts & Morrow 2001; Vane & Botting 2003). Prostaglandins play an important role in reproductive functions, and their inhibition has been linked to changes in uterine contractility, platelet function, and fetal vascular structure (Rudolph 1981). Therefore, based on the available mode of action data for acetylsalicylic acid and the fact that similar reproductive toxicity has been reported in both humans and animals following exposure to acetylsalicylic acid (Roberts & Morrow 2001; CIR 2003), the reproductive endpoints reported in mice following oral exposure to methyl salicylate were considered relevant to humans.

An uncertainty factor analysis for methyl salicylate resulted in a total recommended uncertainty factor of 3. This was based on uncertainty factors of 1 representing uncertainty associated with study length (UF_S), use of LAOEL/NOAEL (UF_L) and database insufficiencies (UF_D); and 3 for uncertainty associated with intraspecies (UF_A) and 1 for uncertainty associated with interspecies (UF_H). The factor of 3 for UF_A is the default UF_A recommended by USEPA (2011a) used in combination with a body weight scaling adjustment to the POD. There are sufficient data to suggest limited human variability in the pharmacokinetics associated with methyl salicylate (Davison et al. 1961) and salicylates as a class of compounds (Needs & Brooks 1985; Gatti et al. 1989; Cerletti et al. 2003; Lucker et al. 2003). There are limited pharmacodynamic data for methyl salicylate; however, the available pharmacokinetic data suggest limited variability across subpopulations and the endpoint relied upon for the POD is from a sensitive subpopulation. Therefore, the pharmacodynamic component of the UF_H was decreased from 3 to 1.

In conclusion, while the only ADIs recommended by authoritative bodies were based on NOELs for body weight changes which were the lowest doses tested from chronic studies conducted in dogs and rats, through a critical review of the available peer-reviewed literature, a reproductive/developmental study was identified that could be used to derive a BMDL. BMD analysis of the data reported by Gulati et al. (1984) indicated the lowest BMDL associated with the best model fit was 220 mg/kg/d. Therefore, the BMDL of 220 mg/kg/d was considered as a POD and adjusted by a DAF of 0.15 based on study specific animal body weight reported by Gulati et al. (1984) and a total uncertainty factor of 3 to derive an ADI for methyl salicylate of 11 mg/kg/d.

In comparison with the ADI recommended by JECFA (2002), which is assumed to be based on the body weight changes and bone effects in rats at 50 mg/kg/d reported by Webb and Hansen (1963), the POD of 220 mg/kg/d based on the data reported by Gulati et al. (1984) is greater than four

times higher. A NOEL or no observed effect level is a level at which no effects are noted; however, those effects are not necessarily toxicologically significant or considered to be precursors to adverse effects (Lewis et al. 2002). A NOAEL or no observed adverse effect level is a level at which there are no statistically significant or biologically significant adverse effects (Lewis et al. 2002; USEPA 2002). Because JECFA (2002) defines the POD as a NOEL with no other information as to the endpoint or even the study on which the POD is based, it must be assumed that the POD is based on the results of Webb and Hansen (1963) and, based on the definition of a NOEL, the effects that serve as the basis of the POD were not considered adverse. The POD proposed in this investigation of 220 mg/kg/d is based on the most sensitive, biologically significant adverse effect reported in both of the studies reviewed and considered for use in estimating the ADI. The four-fold difference in the POD used by JECFA and the POD recommended in this assessment is likely due to two reasons: (1) the POD used by JECFA (2002) is likely based on effects that are not considered biologically significant or adverse that are occurring at lower doses than the adverse reproductive effects reported in Gulati et al. 1984), and (2) the POD recommended in this assessment is based on the results of benchmark modeling that provides a more robust, informative POD compared with the NOAEL that accounts for the shape of the dose–response curve over the continuum of observed exposures, rather than at a single dose level (USEPA 2002, 2012). The differences in the PODs resulted in the recommended ADI in this investigation of 11 mg/kg/d being 22 times higher than the current ADI recommended by JECFA (2002) of 0.5 mg/kg-body weight.

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Declaration of interest

The conclusions of this assessment are those of the authors. The employ ment affiliation of the authors is as shown on the cover page. All authors are employed by Ramboll Environ, a global environmental consultancy. This project was supported by funding provided by Altria Client Services (ALCS), LLC, which provides professional services and support to the Altria Group and its operating companies. Altria Group consists of a fam ily of companies that are leading manufacturers and producers of tobacco products and wines. Methyl salicylate is used in some of these tobacco products as an additive.

ALCS had no role in study design, data collection and analysis, deci sion to publish, or preparation of any portion of the manuscript. ALCS were given the opportunity to review a draft manuscript while it was under review and consideration by the journal; however, no comments or suggested revisions were provided by the funders. The analyses, opin ions, and conclusions presented in this manuscript are exclusively those of the authors and do not necessarily represent the view of ALCS. The authors' scientific conclusions and professional judgments were not sub ject to the funders' control.

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An investigation on the use of snus and its association with respiratory and sleep-related symptoms: a cross-sectional population study

Arna Ýr Gudnadóttir,[1,2] Inga Sif Ólafsdóttir,[2,3] Roelinde Middelveld,[4] Linda Ekerljung,[5] Bertil Forsberg,[6] Karl Franklin,[7] Eva Lindberg,[1] Christer Janson[1]

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For numbered affiliations see end of article.

Correspondence to
Dr Christer Janson;
christer.janson@medsci.uu.se

ABSTRACT

Introduction Studies of the health effects of moist oral tobacco, snus, have produced inconsistent results. The main objective of this study is to examine the health effects of snus use on asthma, respiratory symptoms and sleep-related problems, a field that has not been investigated before.

Methods and material This cross-sectional study was based on a postal questionnaire completed by 26 697 (59.3%) participants aged 16 to 75 years and living in Sweden. The questionnaire included questions on tobacco use, asthma, respiratory symptoms and sleeping problems. The association of snus use with asthma, respiratory symptoms and sleep-related symptoms was mainly tested in never-smokers (n=16 082).

Results The current use of snus in never-smokers was associated with an increased risk of asthma (OR 1.51 (95% CI 1.28 to 1.77)), asthmatic symptoms, chronic bronchitis and chronic rhinosinusitis. This association was not present among ex-snus users. Snoring was independently related to both the former and current use of snus ((OR 1.37 (95% CI 1.12 to 1.68)) and (OR 1.59 (95% CI 1.34 to 1.89), respectively)). A higher risk of difficulty inducing sleep was seen among snus users.

Conclusion Snus use was associated with a higher prevalence of asthma, respiratory symptoms and snoring. Healthcare professionals should be aware of these possible adverse effects of snus use.

Strengths and limitations of this study

- This is one of the first studies to investigate the association between the use of snus and respiratory and sleep-related symptoms.
- The population is large which enables us to investigate subgroups such as never-smokers.
- Data are self-reported and some subjects may therefore be misclassified.
- The study is cross-sectional which makes it difficult to distinguish between causes and outcomes.

INTRODUCTION

Snus is a smokeless, moist tobacco product consisting mainly of tobacco, salt, water, humectants and flavouring.[1] The tobacco in snus contains a number of harmful substances, including nicotine and tobacco-specific nitrosamines.[2] In Sweden, where it is a very popular tobacco alternative, with 18% men and 4% women being current users, snus is regulated under food legislation.[3 4] The highest proportion of snus users is found among men aged 40 years.[5] In the mid-1990s, the prevalence of snus use among Swedish men surpassed the prevalence of smoking. The proportion of female snus users is rising, but it has still not reached the prevalence for women smokers.[5] Compared with smoking, it has been suggested that the addiction to snus use is stronger, due to a lower cessation rate[6] and reports of greater experience of nicotine dependence,[7] however, other data suggest that snus cessation may be less difficult than cigarette cessation.[8] Some authors have suggested that snus is as a good alternative for smoking cessation due to the beneficial health effects compared with cigarettes.[9]

Studies aiming to identify the risk of health effects as a result of snus use have not reported consistent results. A significant increase in pancreatic cancer has been observed,[10 11] but reports regarding the association between snus use and oral and pharyngeal cancer are inconclusive.[10–12] Snus use appears to increase the risk of short-term case fatality after suffering from acute myocardial infarction[13] and stroke.[14] An increased risk of heart failure among snus users has been reported, with a particularly high risk of non-ischaemic heart failure among elderly men.[15]

There is only sparse evidence regarding the potential effect of snus on respiratory health and sleep. An association between snus and asthma has been reported in one study[16] and an elevated risk of insufficient sleep was found among smokeless tobacco users in

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another.[17] The main objective of this paper was to investigate the health effects of snus use on asthma, respiratory symptoms and sleep-related problems in a large general population sample.

METHODS

Study design and participants

This cross-sectional study is based on observations from a postal questionnaire sent to 45 000 randomly selected subjects as part of the Global Allergy and Asthma European Network (GA^{2}LEN) survey in 2008.[18] In Sweden, 26 697 (59.3%) participants responded. The subjects were aged 16 to 75 years and lived in four Swedish cities (Uppsala, Stockholm, Umeå and Gothenburg).[16]

Ethical approval was granted by the Regional Ethical Review Board in Uppsala, Sweden.

GA^{2}LEN questionnaire

The questionnaire included questions on respiratory symptoms, asthma and smoking. The questions also covered gender, age, weight and height. Body mass index (BMI) was calculated using the values of weight and height. In Sweden, the questionnaire also included questions on the use of snus and sleep-related symptoms. Listed below are definitions relevant to this paper.

Snus *users* were defined as those giving a positive answer to both the questions 'Have you ever used snus every day for at least 6 months?' and 'Do you currently use snus?'.

Smokers were defined as those giving a positive answer to the questions 'Have you ever smoked at least one cigarette a day for at least 1 year?' and 'Have you smoked at all during the last month?'. Based on the answers to questions on snus use and smoking, the participants were divided into four groups: tobacco free, snus users, smokers and dual users.

Based on answers to the questions on snus use and smoking, the subjects were further divided into never-snus, ex-snus and current snus users, as well as into never-smokers, ex-smokers and current smokers.

Asthma was defined as a positive answer to either of the questions 'Have you had an asthma attack during the last 12 months?' or 'Are you currently taking any asthma medication including inhalers, sprays or tablets?'. Childhood asthma was defined as reporting having had an attack of asthma before the age of 13 years.

Questions regarding a*sthmatic symptoms* during the last 12 months included: (1) wheezing in the chest; (2) wheezing together with breathlessness; (3) wheezing without having a cold; (4) waking up with tightness in the chest; (5) waking up with shortness of breath and (6) waking up with a coughing attack.

Chronic bronchitis was defined as a positive answer to the question: 'Are you used to having a cough almost every day with sputum production that lasts for at least 3 months every year during the winter?'.

Allergic rhinitis was defined as a positive answer to the question 'Have you had hay fever or a runny nose because of other allergies during the last 12 months?'.

Chronic rhinosinusitis was defined as suggested by the European Position Paper on Rhinosinusitis and Nasal Polyps (EP^{3}OS) criteria 2007.[19] It was considered to be present if participants stated that the following symptoms had been present for >12 weeks during the last 12 months: (1) nasal blockage, as well as one of the subsequent symptoms: (2) facial pain or pressure, (3) discoloured nasal discharge or (4) reduction or loss of smell. The disease was also considered to be present if both symptoms (2) and (3) were reported.

Sleep-related problems examined in this study were (1) snoring that is loud and interrupting, (2) difficulty inducing sleep (DIS), as in having a hard time falling asleep at night, (3) difficulty maintaining sleep (DMS), as in repeatedly waking up during the night, (4) being sleepy during the day (EDS) and (5) early morning awakening (EMA), as in waking up too early and having a hard time falling asleep again.[20] Each group included subjects who claimed they had the problem at least three to five times a week. The use of hypnotics was defined as a positive answer to the question 'Do you take medication for sleeping problems?'.

Educational level was divided into three categories. (1) College was defined as having attended college/university for >2½ years. (2) High school was defined as having attended high school or vocational school for >2 years. (3) Elementary school was defined as any education below the level of high school.

Activity level was divided into three categories depending on hours spent on intensive exercising per week. (1) Physically inactive was defined as zero hours a week. (2) Moderately physically active was defined as 0.5 to 3 hours a week. (3) Vigorously physically active was defined as 4 to 7 hours a week.

Data analysis

For statistical analyses, Stata V.12 was used. When comparing the characteristics of the study population, univariate analyses using the χ^2 test were used. Multivariate logistic regression models were used to study independent associations between various symptoms and different groups of tobacco use after adjusting for potential confounders: gender, age, BMI, centre, educational level and physical activity. Subanalyses were performed in never-smokers and in never-smokers with reported asthma. Analyses of possible heterogeneity between the centres were performed using random effects meta-analysis. A p value of <0.05 was regarded as statistically significant.

RESULTS

The frequency of snus use among men was highest in the 25–35 age group. The number of snus-using women was highest in the 45–55 age group, with a steep decrease

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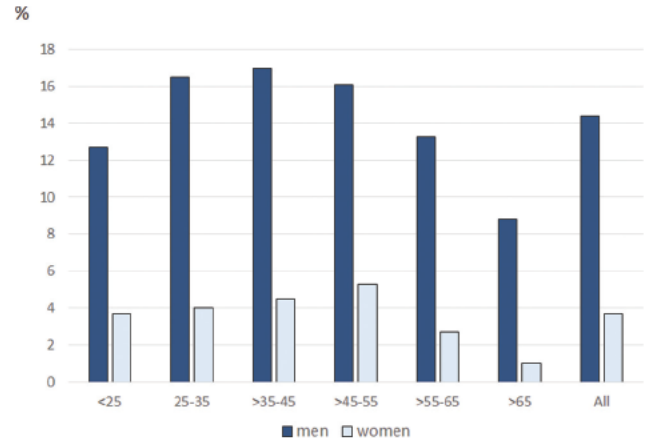


Figure 1 Proportion of men and women using snus among age groups.

thereafter. In overall terms, 18.0% of men and 4.7% of women in the study population used snus (figure 1).

In the whole population, snus use and dual use were highest in the 25–35 age group, whereas smoking was most prevalent at 55–65 years of age. The group of snus users had a higher BMI than the other groups. They were also more likely to be ex-smokers than persons in the tobacco-free group. Educational level and physical activity level were higher among snus users compared with smokers and dual users but lower compared with those who were tobacco free (table 1).

Tobacco use and symptoms

In table 2, we examined the association between tobacco use and symptoms after adjusting for likely confounders. Having asthma was independently related to using snus but not to smoking or the dual use of snus and cigarettes. Although the strongest associations with respiratory symptoms were found among smokers and dual users, snus users were more likely to suffer from wheezing and night-time chest tightness, as well as chronic bronchitis, allergic rhinitis and chronic rhinosinusitis, compared with the tobacco-free group. Snoring, DIS, EDS and the use of hypnotics were associated with all three groups of tobacco use. Snus users had a decreased risk of DMS (table 2). The corresponding unadjusted associations were fairly similar to the adjusted estimates (see online supplementary Table S1).

Table 1 Characteristics of the study population (%)

		Tobacco users			
	Tobacco free (n=20 699)	Snus (n=2265)	Smokers (n=3136)	Smokers and snus (n=597)	p Value
Women	57.7	23.5	62.9	25.0	<0.001
Age (years)					<0.001
16–25	15.4	12.8	12.4	15.2	
25–35	21.4	23.2	17.4	26.7	
35–45	17.6	21.5	16.7	16.8	
45–55	15.4	19.7	20.0	21.5	
55–65	17.7	16.5	23.2	14.9	
>65	12.4	7.2	10.1	6.7	
Body mass index					<0.001
<20	8.6	4.7	9.9	5.8	
20–25	51.9	46.6	48.9	48.1	
25–30	30.1	36.2	30.9	35.3	
>30	9.5	12.4	10.3	10.9	
Ex-smokers	26.8	48.1	—-	——	<0.001
Educational level					<0.001
Elementary school	15.0	12.5	23.6	18.6	
High school	31.6	42.9	40.7	45.8	
College	53.5	44.7	35.7	35.6	
Activity level					<0.001
Physically inactive	18.1	19.3	32.8	27.8	
Moderately physically active	62.8	62.0	55.1	58.7	
Vigorously physically active	19.0	18.7	12.1	13.5	

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Snus use in never-smokers

In never-smokers, there was an association between snus use and asthma, all the asthmatic symptoms, chronic bronchitis and chronic rhinosinusitis. Sleeping problems with an increased risk among snus users were snoring and DIS. Also, among never-smokers, snus users had a decreased risk of DMS (table 3). The associations with asthma and nocturnal breathlessness became statistically non-significant when excluding subjects who have had attacks of asthma before the age of 13 years (n=1466) (adjusted OR (95% CI) 1.24 (0.89 to 1.71) and 1.32 (0.97 to 1.77), respectively). All the other association above remained statistically significant.

No significant heterogeneity between the centres was found between snus use and the health outcomes except for bronchitis where the association was particularly strong in Stockholm (adjusted OR (95% CI) 2.35 (1.52 to 3.62)), whereas no significant association was found in Uppsala (OR 0.97 (0.60 to 1.57), $p_{heterogeneity}=0.04$). The pooled estimates of the meta-analyses were very similar to those found in table 3 (data not shown). When snus users among asthma patients who had never smoked were examined, the only symptom with a significantly elevated risk was snoring (OR 2.68 (1.58 to 4.55)).

History of snus use in never-smokers

Current snus use was an independent risk factor for having asthma, asthmatic symptoms, chronic bronchitis and chronic rhinosinusitis, while being an ex-snus user was not (table 4). Snoring was independently related to both the former and the current use of snus. A higher risk of DIS was seen among current snus users. Current snus users had a decreased risk of DMS, whereas ex-snus use was an independent risk factor for the problem. Being an ex-snus user was also an independent risk factor for EMA (table 4).

DISCUSSION

Our results reveal a previously unknown association between snus use and negative health effects on the respiratory tract. An increased risk of asthma, asthmatic and other respiratory symptoms was observed among snus users. An association between snus use and sleep-related problems was mixed with an increased risk of snoring and DIS but a decreased risk of DMS.

In the present study, 18% of men and 4.7% of women were using snus either exclusively or in combination with smoking. This is similar to the prevalence of snus use reported in a Swedish official statistics.[4] According to our findings, snus use is proportionally higher in younger

Table 2 The independent association between tobacco use and respiratory health and sleep-related symptoms (*adjusted* OR (95% CI))

	Tobacco users		
	Snus users (n=2265)	Smokers (n=3136)	Smokers and snus users (n=597)
Asthma	**1.51 (1.28 to 1.77)**	0.96 (0.82 to 1.13)	0.93 (0.65 to 1.33)
Asthmatic symptoms			
Wheezing	**1.50 (1.33 to 1.69)**	**2.89 (2.64 to 3.17)**	**2.09 (1.71 to 2.55)**
Wheezing and breathlessness	**1.42 (1.23 to 1.65)**	**2.11 (1.89 to 2.37)**	**1.46 (1.12 to 1.90)**
Wheezing without having a cold	**1.50 (1.30 to 1.73)**	**2.67 (2.40 to 2.98)**	**2.17 (1.73 to 2.73)**
Night-time chest tightness	**1.21 (1.05 to 1.40)**	**1.57 (1.40 to 1.75)**	**1.43 (1.12 to 1.82)**
Night-time attacks of breathlessness	1.02 (0.83 to 1.24)	**1.44 (1.24 to 1.67)**	**1.58 (1.16 to 2.13)**
Night-time coughing	1.10 (0.99 to 1.23)	**1.79 (1.64 to 1.94)**	**1.79 (1.49 to 2.15)**
Chronic bronchitis	**1.19 (1.03 to 1.37)**	**2.39 (2.16 to 2.65)**	**1.85 (1.48 to 2.31)**
Allergic rhinitis	**1.17 (1.05 to 1.30)**	1.01 (0.91 to 1.11)	0.92 (0.75 to 1.13)
Chronic rhinosinusitis	**1.28 (1.09 to 1.50)**	**1.78 (1.57 to 2.02)**	**1.78 (1.38 to 2.29)**
Sleeping problems			
Snoring	**1.41 (1.25 to 1.58)**	**1.78 (1.60 to 1.97)**	**2.16 (1.77 to 2.63)**
DIS	**1.76 (1.56 to 1.99)**	**1.98 (1.79 to 2.19)**	**2.95 (2.43 to 3.58)**
DMS	**0.74 (0.66 to 0.83)**	0.97 (0.88 to 1.06)	0.91 (0.75 to 1.12)
EDS	**1.18 (1.07 to 1.31)**	**1.29 (1.19 to 1.41)**	**1.38 (1.16 to 1.65)**
EMA	0.87 (0.76 to 1.00)	**1.14 (1.03 to 1.27)**	0.91 (0.70 to 1.17)
Use of hypnotics	**1.33 (1.07 to 1.65)**	**2.08 (1.81 to 2.39)**	**2.77 (2.05 to 3.74)**

Associations with a p value of <0.05 are marked as bold.
Adjusted for gender, age, BMI, centre, educational level and physical activity.
BMI, body mass index; DIS, difficulty initiating sleep; DMS, difficulty maintaining sleep; EDS, excessive daytime sleepiness; EMA, early morning awakening at least three to five nights/week.

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Table 3 Association between snus and respiratory health and sleep-related symptoms in never-smokers (%) and *adjusted* OR

	Never smoked			
	Tobacco free (n=14 914)	Snus users (n=1168)	p Value	OR (95% CI)
Asthma	6.9	10.1	<0.001	**1.49 (1.20 to 1.85)**
Asthmatic symptoms				
Wheezing	12.9	18.8	<0.001	**1.56 (1.32 to 1.84)**
Wheezing and breathlessness	8.2	10.9	0.002	**1.38 (1.12 to 1.69)**
Wheezing without having a cold	8.0	11.8	<0.001	**1.48 (1.21 to 1.80)**
Night-time chest tightness	9.4	12.0	0.004	**1.41 (1.16 to 1.71)**
Night-time attacks of breathlessness	4.8	6.1	0.045	**1.39 (1.07 to 1.82)**
Night-time coughing	23.1	23.1	0.987	**1.27 (1.09 to 1.47)**
Chronic bronchitis	9.0	12.5	<0.001	**1.47 (1.21 to 1.78)**
Allergic rhinitis	24.7	28.0	0.012	1.14 (0.99 to 1.31)
Chronic rhinosinusitis	7.1	9.3	0.005	**1.37 (1.11 to 1.70)**
Sleeping problems				
Snoring	11.7	19.0	<0.001	**1.53 (1.29 to 1.82)**
DIS	11.1	16.5	<0.001	**1.71 (1.44 to 2.03)**
DMS	25.3	15.8	<0.001	**0.71 (0.59 to 0.84)**
EDS	28.7	29.8	0.433	1.08 (0.94 to 1.24)
EMA	12.7	9.0	<0.001	0.83 (0.67 to 1.04)
Use of hypnotics	4.0	3.1	0.143	1.24 (0.85 to 1.80)

Associations with a p value of <0.05 are marked as bold.
Adjusted for gender, age, BMI, centre, educational level and physical activity.
BMI, body mass index; DIS, difficulty inducing sleep; DMS, difficulty maintaining sleep; EDS, excessive daytime sleepiness; EMA, early morning awakening at least three to five nights/week.

age groups and among men than women, indicating an earlier initiation.

When compared with the tobacco-free group, a significant risk of asthma was observed among snus users but not among smokers and dual users. There is a possibility of reverse causation. Smoking is known to cause negative effects on asthma,[21] the switch from smoking to snus use among asthmatic patients could possibly explain some of the associations between snus use and asthma. However, the fact that snus users who had never smoked also had an elevated risk of asthma and asthmatic symptoms excludes this possibility. This difference between snus users and smokers also raised concerns about whether asthmatic patients could be more prone to initiating snus use than cigarette smoking. Because the association with asthma and asthmatic symptoms was only present among current snus users but not ex-users of snus, this seems an unlikely explanation. We also found an association between snus use and respiratory symptoms when excluding participants who had childhood asthma. These findings suggest that snus causes an alteration in the lower respiratory tract, resulting in an increased likelihood of suffering from asthma and asthmatic symptoms. As asthmatic patients are a growing challenge among health professionals today,[22] these results deserve attention and further investigation. Similarly, to asthma and asthmatic symptoms, the risk of chronic bronchitis and chronic rhinosinusitis was only elevated among current snus users but not ex-snus users.

Current snus use was associated with snoring and, as the association remained present among ex-snus users, this suggests a partly irreversible effect from snus use on factors leading to snoring. Snoring is caused by high-frequency oscillation of the soft palate, pharyngeal wall, epiglottis and tongue during sleep, due to the limited flow of air through the upper airways.[23] The causes of limited airflow among patients who snore are diverse.[24 25] There are data indicating that, once snoring occurs, it causes progressive irreversible local neurogenic lesions, caused by the trauma of snoring.[26] In former studies, hypnotics[27] and alcohol consumption[28] have been associated with snoring. Adjustment for the use of hypnotics did not have any impact on the risk (results not shown), excluding it as an interfering factor in this study. However, we were not able to adjust for alcohol consumption, as the GA^{2}LEN questionnaire did not include any questions about alcohol. It could thus serve as a confounder in our results. Obesity, gender, age and cigarette smoking are also possible confounders,[27] all of which were adjusted for in our analyses.

In the group of snus users who had never smoked, being an asthma patient elevated the risk of snoring from 1.53 to 2.68. This implies that being an asthmatic patient

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Table 4 Association between a history of snus use and respiratory health and sleep-related symptoms among never-smokers (*adjusted* OR (95% CI)

	Ex-snus users (n=832)	Snus users (n=1169)
Asthma	1.06 (0.79 to 1.40)	1.50 (1.21 to 1.86)
Asthmatic symptoms		
Wheezing	1.10 (0.89 to 1.36)	1.56 (1.32 to 1.84)
Wheezing and breathlessness	1.00 (0.76 to 1.31)	1.37 (1.12 to 1.69)
Wheezing without having a cold	1.24 (0.97 to 1.59)	1.51 (1.23 to 1.84)
Night-time chest tightness	1.01 (0.78 to 1.30)	1.41 (1.16 to 1.71)
Night-time attacks of breathlessness	1.27 (0.92 to 1.76)	1.42 (1.09 to 1.86)
Night-time coughing	1.14 (0.96 to 1.37)	1.29 (1.11 to 1.50)
Chronic bronchitis	0.91 (0.70 to 1.19)	1.45 (1.20 to 1.76)
Allergic rhinitis	0.95 (0.80 to 1.12)	1.13 (0.98 to 1.30)
Chronic rhinosinusitis	0.95 (0.71 to 1.28)	1.36 (1.10 to 1.70)
Sleeping problems		
Snoring	1.37 (1.12 to 1.68)	1.59 (1.34 to 1.89)
DIS	0.81 (0.62 to 1.05)	1.68 (1.41 to 2.00)
DMS	1.20 (1.01 to 1.42)	0.72 (0.60 to 0.85)
EDS	1.00 (0.85 to 1.18)	1.08 (0.94 to 1.24)
EMA	1.28 (1.03 to 1.59)	0.85 (0.69 to 1.06)
Use of hypnotics	1.32 (0.87 to 2.01)	1.26 (0.87 to 1.84)

Adjusted for gender, age, BMI, centre, educational level and physical activity.
BMI, body mass index; DIS, difficulty initiating sleep; DMS, difficulty maintaining sleep; EDS, excessive daytime sleepiness; EMA, early morning awakening at least three to five nights/week.

increases the sensitivity to possible effects of snus use contributing to snoring. Deterioration in health-related quality of life in asthmatic patients suffering from snoring has been reported.[29] The possible prevention of snoring by reducing snus initiation among those suffering from asthma should therefore be prioritised.

The risk of DMS was found to be decreased among current snus users but increased among ex-snus users. Ex-snus users also ran an increased risk of EMA. These results suggest that snus use improves sleep and that a cessation leads to exacerbation. In spite of this, current snus users had an increased risk of DIS, but ex-snus users did not, which indicates mixed effects on the quality of sleep.

The biological explanation for the association between snus use and health outcomes in the present study is unknown. Gastro-oesophageal reflux is associated with smoking,[30] respiratory symptoms and snoring,[31] but the association between reflux and snus use is less clear.[32] Snus use was in one study found to be associated with an increased transfer factor for nitric oxide and a decrease in alveolar nitric oxide concentration indicating that snus may have an effect also on the lower airways.[33]

The main strength of this study is the use of a large database from a random general population sample. The response rate was lowest in Gothenburg (54.9%) and highest in Uppsala (59.1%).[20] This response rate is somewhat lower than that previous epidemiological studies of the Swedish population have achieved,[34 35] which is a limitation. In addition, the fact that this is a cross-sectional study makes it difficult to distinguish between causes and outcomes. A longitudinal study would be beneficial in order to indicate whether causation is present between snus use and these symptoms or only a connection. The fact that participants reported their history of snus use was, however, a great advantage, which helped us to draw conclusions about associations. It would have been beneficial to include questions on the amount of snus used. We also lack data on alcohol consumption, which is a limitation as there are studies showing an association between use of snus and a higher use of alcohol.[36] It is also possible that some of the associations between snus use and snoring are related to higher alcohol use in participants using snus.[37] Another limitation is the fact that the answers to the questionnaire are self-reported which might lead to underestimation or overestimation in some categories, especially those demanding more than a yes or no answer.[16] It is, however, unlikely that the degree of underestimation or overestimation would differ between groups of tobacco use. We did not adjust for passive smoking, but we know from previous studies that the prevalence of passive smoking is very low in Sweden (<10%)[38] so we do not think that this has influenced our results.

To the best of the authors' knowledge, this field of effects has not been examined before. Further investigations will be needed to draw conclusions on possible reasons for limited airflow in the upper airways, an inflamed/mucus-secreting

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respiratory tract and a mixed impact on the quality of sleep among snus users. Moreover, these results should be supported by further studies. Our results are important when considering tobacco control policies and can provide input to the discussion of whether snus is a good alternative for smoking cessation.

Author affiliations
[1]Deparment of Medical Sciences: Respiratory, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden
[2]Faculty of Medicine, University of Iceland, Reykjavik, Iceland
[3]Department of Respiratory Medicine and Sleep, Landspitali University Hospital, Reykjavik, Iceland
[4]The Centre for Allergy Research and the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[5]Department of Internal Medicine and Clinical Nutrition, Göteborgs Universitet, Goteborg, Sweden
[6]Department of Public Health and ClinicalMedicine, Occupational and Environmental Medicine, Umeå University, Umeå, Sweden
[7]Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden

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From the Department of Women's and Children's Health
Karolinska Institutet, Stockholm, Sweden

PRENATAL NICOTINE EXPOSURE AND EFFECTS ON THE HEALTH OF THE NEWBORN

Anna Gunnerbeck



Karolinska Institutet

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Prenatal nicotine exposure and effects on the health of the newborn
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Anna Gunnerbeck

Principal Supervisor:
Associate Professor Ronny Wickström
Karolinska Institutet
Department of Women´s and Children´s Health
Division of Neuropediatrics

Co-supervisor(s):
Associate Professor Anna-Karin Edstedt Bonamy
Karolinska Institutet
Department of Women's and Children's Health
Department of Medicine
Division of Clinical Epidemiology Unit

Professor Sven Cnattingius
Karolinska Institutet
Department of Medicine
Division of Clinical Epidemiology Unit

Opponent:
Professor Jorge Tolosa
Oregon Health and Science University
Department of OB/GYN
Division of Maternal Fetal Medicine

Examination Board:
Associate Professor Caroline Nilsson
Karolinska Institutet
Department of Clinical Research, SÖS

Associate Professor Karin Pettersson
Karolinska Institutet
Department of Clinical Science, Intervention and Technology

Associate Professor Fredrik Ahlsson
Uppsala University
Department of Women´s and Children´s Health
Division of Neonatology

Den mätta dagen, den är aldrig störst.
Den bästa dagen är en dag av törst.

Nog finns det mål och mening i vår färd –
men det är vägen som är mödan värd.

Karin Boye (1900-1940). Från *I rörelse/On the Move* (Ur Härdarna, 1927).

The day of plenty, never is the greatest.
The best day is a day of craving thirst.

Yes, there is a meaning in our journey –
but 'tis the pathway, which is worth our while.

The excerpt from the poem by Karin Boye is published with kind permission from Mats Boye and from Hans Corell, who made the translation.

To my family

ABSTRACT

Maternal smoking is one of the most important preventable risk factors in pregnancy. Whether it is nicotine or combustion products, or possibly both, that cause the adverse effects is not clear. The common substance in snuff and cigarette smoke is nicotine.The main objective was to study whether it is safe to use snuff in pregnancy, and if cessation of tobacco use influences the risk of adverse effects on the health of the newborn.

Methods: Studies I-III were all based on data from the Swedish Medical Birth Register. Information on tobacco use three months before pregnancy and in early pregnancy was obtained from the register and categorized as nonuser, smoker 1-9 cigarettes/day, smoker≥10 cigarettes/day or snuff user. Most epidemiological studies on tobacco use are based on self-reported information. Snuff use in pregnancy has not been validated previously. In study IV, self-reported snuff use in a cohort of pregnant women was validated by the use of a biomarker of nicotine exposure, cotinine.

Results: In study I, the association between smoking or snuff use in pregnancy and risk of neonatal apnea was investigated. Maternal snuff use was associated with an almost twofold risk of neonatal apnea. In contrast, the increased risk of apnea in smokers was not significant after adjustment for gestational age, indicating different mechanisms for nicotine and tobacco smoke.

Study II investigated the association between tobacco use and risk of oral cleft malformations. Both maternal smoking and snuff use were associated with increased risk of oral cleft malformations. However, infants of women who had stopped smoking or using snuff before the antenatal booking were not at an increased risk of oral cleft malformations compared to that of nonusers.

In study III, the association between maternal smoking, snuff use and preterm birth was studied. Maternal smoking was associated with extremely (<28 weeks), very (28-<32 weeks) and moderately (32-<37 weeks) preterm birth. Snuff use in pregnancy was associated with extremely preterm birth and moderately preterm birth, whereas the association with very preterm birth was of borderline significance. Importantly, with cessation of tobacco use in early pregnancy, there was no increased risk.

In Study IV, self-reported snuff use was validated by measuring cotinine in maternal urine and meconium of the newborn. Self-reported use of snuff was found valid in late pregnancy. However, there was a large proportion of misclassification of snuff use in the Medical Birth Register in late pregnancy.

Conclusion: Snuff use and smoking in pregnancy are associated with increased risk of adverse effects such as neonatal apnea, oral cleft malformation and extremely preterm birth. Importantly, infants of women who stopped using tobacco were not at an increased risk. This thesis indicates that no forms of nicotine or tobacco are to be regarded as safe to use in pregnancy.

SVENSK SAMMANFATTNING

Globalt är rökning en av de främsta förebyggbara riskfaktorerna under graviditet. Rökning under graviditet är associerad med en ökad risk för placentablödning, prematur födsel, intrauterin dödföddhet, tillväxthämning hos fostret och ökad morbiditet och mortalitet i nyföddhetsperioden. Rökning under graviditet ökar också risken för plötslig spädbarnsdöd. Huruvida det är nikotin eller förbränningsprodukter i cigarettrök som orsakar de skadliga effekter som ses hos barn till rökare är inte helt klarlagt. Det är dock en viktig fråga, eftersom det i Sverige finns en utbredd användning av snus. Till skillnad från cigaretter innehåller snus inga förbränningsprodukter men däremot nikotin.

Syftet med denna avhandling var att undersöka effekterna på fostret av prenatal nikotinexponering i form av snus för att bedöma huruvida snus är säkert att använda under gravididitet, samt om snusstopp i tidig graviditet påverkar eventuella risker.

Studie I visade att rökning och snusanvändning ökade risken för andningsuppehåll (apné) i nyföddhetsperioden. Den ökade risken för neonatal apné hos barn till rökare förklarades av den ökade risken för prematur födsel. Hos barn till snusare kvarstod den fördubblade risken också efter justering för prematuritet.

Studie II visade att både rökning och snusanvändning ökade risken för läpp-käk-gomspalt (LKG) missbildning. Kvinnor som slutat röka eller snusa i tidig graviditet, före första besöket hos mödravården, hade ingen ökad risk för att föda ett barn med LKG-missbildning.

Studie III visade att både rökning och snusanvändning under graviditet ökade risken för prematur födsel. Rökning ökade risken för extremt, mycket och måttligt för tidig födsel, medan snusanvändning i graviditet var associerad med ökad risk för extremt prematur födsel och måttligt prematur födsel. Hos varken rökare och snusare sågs någon ökad risk för prematur födsel vid rök- eller snusstopp i mycket tidig graviditet.

Studie IV visade att självrapporterad snusanvändning under graviditet är valid i Sverige. Dock påvisades en omfattande missklassificering av kvinnor i sen graviditet i Medicinska födelseregistret. Kvinnor med kotininnivåer som indikerade tobaksanvändning samt som rapporterat snusanvändning under graviditet i studiens frågeformulär var registrerade som icke-tobaksanvändare i MFR.

Sammanfattningsvis var både rökning och snusanvändning under graviditet associerade med en ökad risk för andningsuppehåll i nyföddhetsperioden, LKG-missbildning samt extremt för tidig födsel. Ett viktigt folkhälsobudskap är att vid rök- och snusstopp i tidig graviditet ses inga förhöjda risker. Samtliga studier i denna avhandling pekar på att snus liksom rökning är förenade med allvarliga skadeverkningar för fostret och den nyföddes hälsa. Cigaretter, snus och andra nikotinprodukter bör inte användas under graviditet.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following original articles and manuscripts. The papers will be referred to in the text by their Roman numerals (I-IV).

I. **Gunnerbeck A**, Wikström A-K, Edstedt Bonamy A-K, Wickström R, Cnattingius S
Relationship of maternal snuff use and cigarette smoking with neonatal apnea
Pediatrics 2011; Vol 128, 503-509

II. **Gunnerbeck A,** Edstedt Bonamy A-K, Wikström A-K, Granath F, Wickström R, Cnattingius S
Maternal snuff use and smoking and risk of oral cleft malformations
- a population based cohort study
Plos One 2014; Vol 9, 1-8

III. Dahlin S**, Gunnerbeck A,** Wikström A-K, Cnattingius S, Edstedt Bonamy A-K
Maternal tobacco use and extremely premature birth
-a population based cohort study
BJOG 2016; Vol 123, 1938-1946

IV. **Gunnerbeck A,** Raaschaou P, Cnattingius S, Edstedt Bonamy A-K, Wickström R
Snuff use during pregnancy and biomarkers of exposure
- a validation study
Submitted

LIST OF ABBREVIATIONS

BMI	body mass index
CI	confidence interval
CL/P	cleft lip with or without cleft palate
CP	isolated cleft palate
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
DNA	deoxyribonucleic acid
FSH	follicle stimulating hormone
HPG	hypothalamic-pituitary-gonadal
HPLC-MS/MS	high-performance liquid chromatography tandem mass spectrometry
ICD-10	international classification of diseases
IVF	in vitro fertilization
LOQ	lowest limit of quantification
MBR	the Medical Birth Register
nAChR	nicotine-acetylcholine receptor
NPV	negative predictive value
NRT	nicotine replacement therapy
OR	odds ratio
PAH	polycyclic aldehydes
PIN	personal identification number
PPV	positive predictive value
PPROM	preterm premature rupture of membranes
ROC	receiver operator characteristics
SAB	spontaneous abortion
SGA	small for gestational age
SHS	second hand smoking
SIDS	sudden infant death syndrome
ST	smokeless tobacco
TSNA	tobacco specific nitrosamines

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1 INTRODUCTION

Smoking is one of the most important avoidable risk factors in pregnancy worldwide. Maternal smoking is causally related to preterm birth and fetal growth restriction and associated with adverse pregnancy complications, like ectopic pregnancy, placenta abruption, stillbirth, preterm birth and intrauterine growth restriction (IUGR).[1] Smoking in pregnancy is also associated with detrimental effects on the health of the newborn, such as an increased risk of sudden infant death syndrome (SIDS). Furthermore, maternal smoking is associated with long-term effects such as asthma and respiratory disease in childhood and morbidity caused by prematurity and intrauterine growth restriction.[2, 3]

The prevalence of smoking during pregnancy ranges from 5-20% in the Unites States and the European countries.[2, 4, 5] In Sweden, the prevalence of smoking during pregnancy is only 5%.[6] In Sweden, smokeless tobacco in the form of moist oral snuff, or snus, is widely used, also among women. Snuff use is most common among young women in reproductive age and the prevalence of snuff use in pregnancy is 1.3%.[6, 7]

Cigarette smoke contains nicotine and numerous combustion products. Despite the common perception that nicotine is responsible for many of the adverse effects of smoking in pregnancy, there is an ongoing discussion of whether it is safe to use nicotine-replacement therapy, NRT, in pregnancy.[8-10] Randomized controlled trials on NRT have been difficult to interpret from because of lack of compliance.[11]

Swedish snuff contains nicotine but no combustion products.[12, 13] Thus, studying snuff use in pregnancy offers an opportunity to differentiate between effects caused by combustion products in cigarette smoke and those caused by nicotine in snuff.

At the end of the 20th century, snuff use among women aged 16-24 almost tripled.[14] International researchers and policy makers, including the tobacco companies, proclaimed Swedish Snuff, or snus, as a harm reduction product in the struggle against smoke-related morbidity and mortality in high income countries.[13, 15-17] However, there was very little knowledge about the effects of using snuff in pregnancy.

Even though it is probably better for the health of the pregnant woman to switch from cigarettes to snuff, it does not necessarily mean that it is also better for the health of the newborn. The main aim of this thesis was to investigate if nicotine, in the form of snuff, is safe to use in pregnancy.

Figure 1. Cigarette advertising



Source: From the collection of Stanford Research Into the Impact of Tobacco Advertising (tobacco.stanford.edu).

2 BACKGROUND

"I say, if you can´t send money, send tobacco."
US President George Washington´s request to help finance the American Civil War (1776)

The earliest cultivation of the tobacco plant is believed to have occurred in America in around 6000 B.C. Christopher Columbus discovered the plant in 1492 and brought it to Europe, and from Europe it spread to the European colonies in Africa and Asia. It was originally believed to have healing properties and was used in medicine.[5]

Throughout history, the mode of nicotine use has changed from mainly chewing tobacco or inhaling dry tobacco powder as snuff to cigar and cigarette smoking.

World War I (1914-1918) effectively spread the habit of smoking, and with the rise of the manufactured cigarette in the 20th century the use of cigarettes exploded. Tobacco companies marketed cigarettes for women, and smoking rates among female teenagers tripled in the years 1925-1935. During World War II (1939-1945), cigarette sales increased to all time high levels.[2]

In the 50´s and 60´s, smoking was associated with success and fame. Manufactured cigarettes were very common among both men and women in the Unites States (US) and Europe.[2, 18] At the same time the first major reports on hazardous effects of smoking were published. In 1950, Richard Doll stated that smoking was causally related to lung cancer,[19] and in1964 the Surgeon General's report on "Smoking and Health" showed hazardous effects of smoking on health.[2]

2.1 THE PREVALENCE OF SMOKING

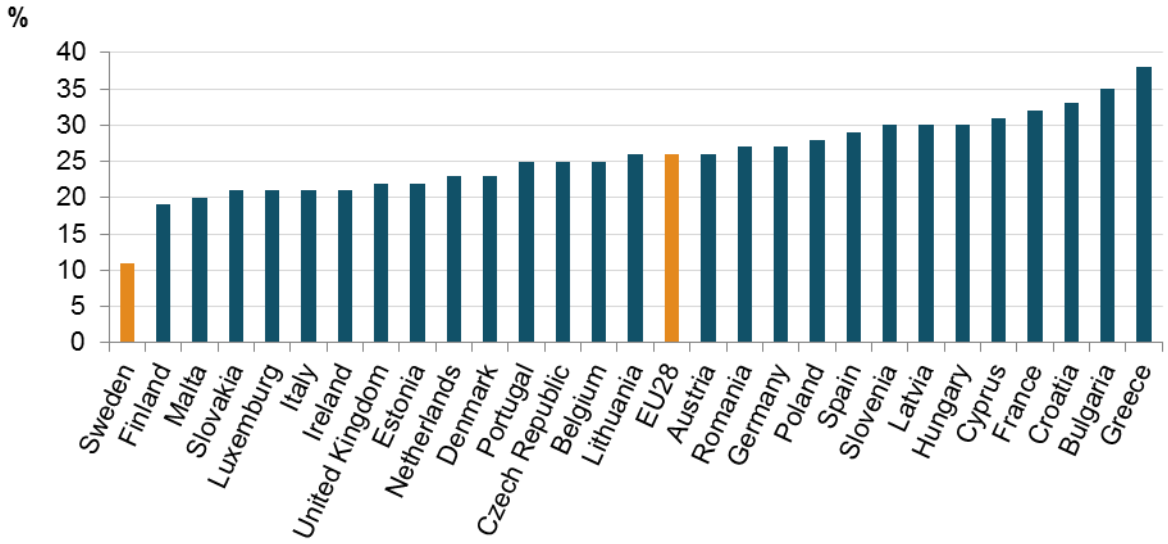
"…the current lower level of tobacco use among women in the world…does not reflect health awareness, but rather social traditions and women's low economic resources."
Dr. Gro Harlem Brundtland, Director General, WHO, 1998

Globally, approximately 967 million men and women smoke daily.[20] The top five high consumption countries of cigarettes in the world are China, Japan, the US, Russia and Indonesia.[5] China is the largest tobacco consuming nation in the world, and one third of all smoking men in the world live in China. According to the World Health Organization (WHO) Global Adult Survey data, 2010, the prevalence of smoking among men in China was 53%.[5, 20, 21]

In the last 30 years, the estimated prevalence of smoking in the world has decreased, but because of population growth the total number of smokers has increased.[20] There has been a shift in smoking prevalence from high-income countries, where smoking is declining, to low-income countries and developing countries where the smoking prevalence is continuously high. In some countries, such as Africa and Eastern Europe, the prevalence is actually rising.[20, 21]

In the US, the prevalence of cigarette smoking has declined from 42% in 1965 to 18% in 2012.[2] The decline of smoking is seen in both men and women and in all age-groups. In the US, there are today more former smokers than smokers and it is estimated that more than 50% of ever smokers have quit smoking.[2]

Figure 2. The prevalence of smoking in Europe, 201



Source 'Special Eurobarometer 429', European Commission, 2015

The average smoking prevalence in Europe is approximately 26%, with great diversity between countries.[4] Smoking is more common in southern and eastern Europe with the highest prevalence in Greece (40%), Bulgaria (36%) and Latvia (36%), in comparison to Sweden, where the smoking prevalence is around 10%.[4, 7] In the United Kingdom (UK), Sweden and many other European countries, smoking has decreased considerably among both men and women since the 1970's and 1980's.

2.2 DEFINITION AND PREVALENCE OF SMOKELESS TOBACCO

Smokeless tobacco (ST) is an umbrella term for a wide range of tobacco products that are not smoked. When used orally, ST is often used in the form of oral moist snuff, such as Swedish snus, and as chewing tobacco. It can also be sucked, applied to the gums as a paste, dissolved in the mouth, gargled or inserted in betel quid. ST can also be inhaled as a powder, called dry snuff.[22]

Over 300 million people around the world use smokeless tobacco. The vast majority, 89%, live in South Asia, especially India and Bangladesh, where the variety of products is large. In these countries, the most commonly used ST products are betel quid with tobacco, khanini and products applied to teeth and gum, like gul and mishri. The prevalence of ST use in India and Bangladesh is 25.9 % and 27.2%, respectively.[23]

In Europe and North America, the most common ST products are chewing tobacco and oral moist snuff. In the US, 7.1% of males use oral moist snuff. [23] In Scandinavia, and in Sweden in particular, oral moist snuff, or Swedish snus, is most widely used, with a prevalence of 20% among males and 3-4% among females.[7]

The diversity of ST products, combined with differences in characteristics between ST users in different regions of the world, makes health implications of ST use difficult to generalize. In this thesis, snuff use will be defined as the use of Swedish snus. Other forms of ST will not be discussed further.

Figure 3. Smokeless tobacco products

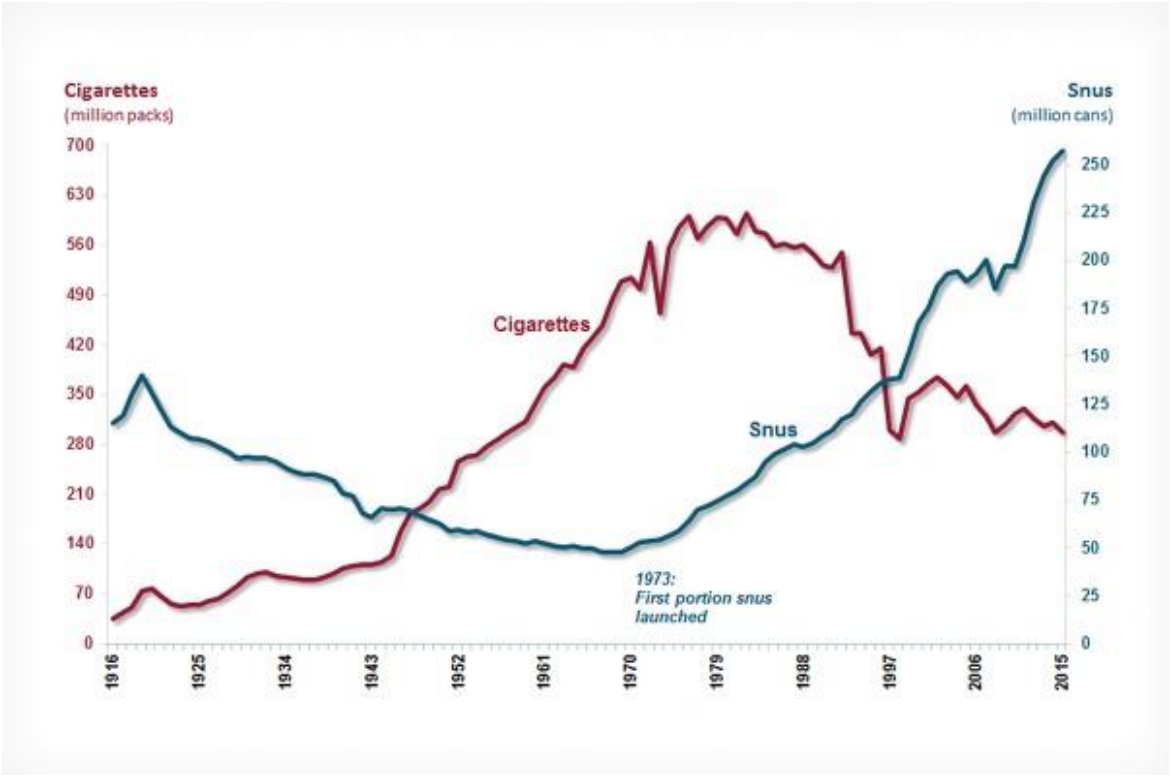
Source: Smokeless tobacco and public health: A global perspective, National Cancer Institute Centers for Disease Control and Prevention U.S. Department of Health and Human Services

2.3 SMOKING AND SNUFF USE IN SWEDEN

"EG? Inte utan min prilla!"
"The EU? Not without my pouch of snuff." (Bumper sticker attributed to Anders Andersö, "Tecknar-Anders", in the campaign for Swedish snuff 1994)

At the beginning of the19th century, snuff, in the form of oral moist snuff, became increasingly common and was used mainly by working class men in Sweden. In 1915, AB Svenska Tobaksmonopolet, today known as Swedish Match, was founded, and after World War I the use of snuff use peaked in Sweden with a consumption of 1.2 kg/per capita.[18] After World War II, though, cigarette smoking grew popular and was associated with fame and high social class. As a consequence, snuff use in Sweden declined substantially. In the 1960's, with 50% of all Swedish men being smokers, it was even discussed to stop the production of snuff altogether in Sweden. However, with increasing knowledge of the hazardous effects of smoking, the number of smokers has declined since the 1970's, and as a consequence the use of snuff, which is regarded as less harmful, has increased. In 1973, portions of snuff were launched as a popular alternative to loose snuff.[18, 24]

Figure 4. Tobacco consumption, cigarettes and snuff from 1916-2015.



Source: Published with permission from Swedish Match

Smoking has continued to decline in both sexes, but Sweden is one of the few countries in the world where more women than men smoke. The prevalence of smoking among women was 11% and among men 9% in 2016.[7] In contrast, most snuff users are men. Approximately, 20% of Swedish men and 3-4% of Swedish women use snuff. It is primarily young women in fertile age, aged 16-29 , who use snuff daily (4%) and sporadically (3%).[7]

Important dates for the tobacco policy in Sweden

Tobacco Act 2016 (SFS 2016:353)

- EU countries are required to have spacious health warnings with text and images on cigarette packs, on more than 65% of the size of the package.
- The text on tobacco products is not allowed to propose that the product is a healthier product in comparison to other tobacco products.
- No information about proportions of nicotine, tar and carbon monoxide is allowed.
- Information on taste or smell additives is not allowed on tobacco products, with the exception of snus.

EU membership 1995

- At the time of Sweden's entry into the EU 1995, tobacco products for oral use, except smoking or chewing tobacco, were been banned. As Swedish snus is neither smoked nor chewed it is prohibited for sale in EU. However, Sweden was granted a permanent exemption from the sales ban on snus.

Tobacco Act 1993 (1993:581)

- Ban of commercial advertising of tobacco products.
- Ban of smoking in public places, with the exception of outdoor seating at cafés and restaurants.

Source: Photo by Henrik Dahl, 2017

2.4 CHARACTERISTICS OF TOBACCO USERS

"It is important to know as much as possible about teenage smoking patterns and attitudes. Today´s teenager is tomorrow´s potential regular customer, and the overwhelming majority of smokers first begin to smoke while still in their teens. The smoking patterns of teenagers are particularly important to Philip Morris." Philip Morris Companies Inc. 1981

Tobacco use is correlated with social inequalities. Globally, high-income countries have lower prevalence of smoking than developing countries.[20] Both smoking and use of ST is more common in rural than in urban areas. Use of tobacco is correlated with lower education, unemployment, poverty, consumption of alcohol and substance use, as well as with psychiatric illness.[2, 4, 23, 25] More than 80% of smokers begin before 18 years of age, and smokers who started smoking early are less likely to quit.[1]

Worldwide, more men than women use tobacco. However, the gender differences in tobacco use vary between countries and cultural contexts. In many of the countries with the highest prevalence of smoking, like China, Eastern Europe, Russia and Asia, men smoke 10 times more than women.[5] In contrast, in high income industrialized countries, the gap between women and men is declining. According to the WHO report on the global tobacco epidemic 2015, 19% of females in Europe smoked tobacco, in comparison to 2-3% in Africa, Southeast Asia and the Eastern Mediterranean and Western Pacific Regions.[21] Sweden and Norway are exceptions with higher smoking prevalence among women than men.[7]

Also the use of smokeless tobacco shows gender differences. The majority of ST users worldwide are men, but in some countries, as for example India (18.4%), Bangladesh (26.4%), and South Africa (10.9), the prevalence among women is very high.[23]

2.5 SUBSTITUENTS OF TOBACCO

Smoked tobacco contains more than 4000 different chemicals, including nicotine, carbon monoxide, CO, tobacco specific nitrosamines (TSNA), polycyclic aldehydes (PAH), nitrate and aromatic amines. It also contains heavy metals like cadmium, arsenic, lead, nickel, chrome, mercury and polonium.[3] The concentration of heavy metals in tobacco is much dependent on the concentration in the soil where tobacco is grown.[23] The levels of TSNA are especially frequent in fire-cured tobacco, but also dependent on the level of nitrate producing bacteria. PAH and formaldehydes are carcinogenic and formed when tobacco is burned and are always present in tobacco smoke.[3]



Figure 5. Nicotiana tabacum

In addition to nicotine, smokeless tobacco also contains numerous toxic substances found in tobacco smoke, including TSNA and PAH. The le differ among products, depending on which type of nicotine plant that is used, the soil where it grows, the production and storage process and the presence of nitrate producing bacteria. In addition, the tobacco is sometimes flavored or used together with other products which also affect the levels of nicotine content and carcinogens.[23]

Swedish snuff, or snus, contains powered tobacco, water, sodium carbonate, sodium chloride, moisturizer and flavoring.[13] Snus differs from other forms of smokeless tobacco, containing considerably lower levels of toxic substances than smoked tobacco and any other ST product.

Since 2000, Swedish Match has its own quality standard, Gothiatek,[26] which controls every step of the production process, including packaging and storage of snuff.[12] The tobacco is air-dried and pasteurized, which decreases the level of TSNA. Gothiatek controls that levels of nitrate, TSNA, NMDA (volatile nitrosamine), PAH and heavy metals (cadmium, lead, arsenic, nickel and chromium) in Swedish snuff are below WHO recommendations for smokeless tobacco.[27] Swedish snuff is also regulated by the Swedish National Food Agency, and production and additive standards are therefore the same as for those for food products.[12, 28, 29] American oral moist snuff is different from Swedish snuff regarding toxicity and level of harmful substances. Because of different production processes, the levels of TSNA, PAH, formaldehydes and metals are considerably higher in American snuff. Mean TSNA in Swedish snuff is 1.1ug/g[29], in comparison to that of American moist snuff with TSNA levels ranging from 4.87ug/g to 90.02ug/g tobacco.[30]

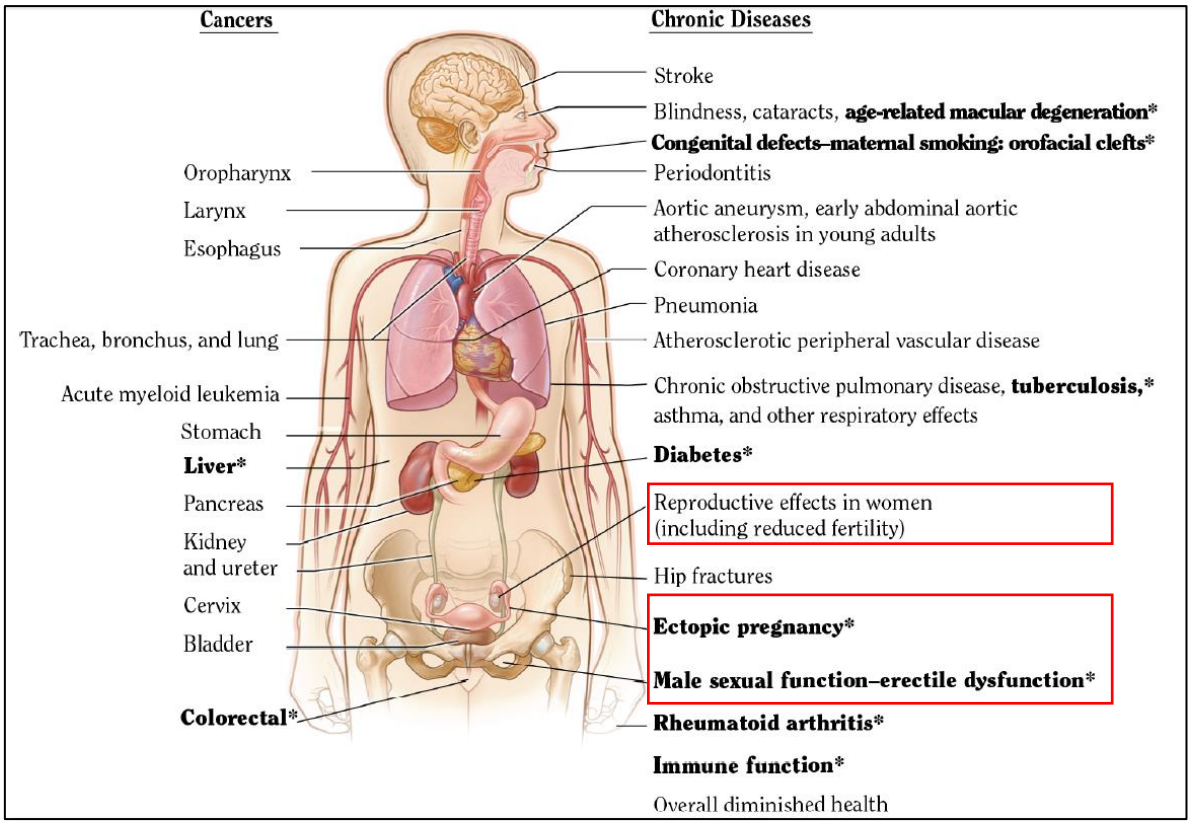
2.6 HEALTH EFFECTS OF SMOKING

"Smoking is a custom loathsome to the eye, hateful to the nose, harmful to the brain, dangerous to the lungs, and in the black, stinking fume thereof nearest resembling the horrible Stygian smoke of the pit that is bottomless." James I of England, A counterblast to tobacco, 1604

Smoking is predicted to account for 1 billion deaths in the 21th century. [31] Europe and North and South America have the highest proportion of smoke related mortality (25 and 17%, respectively) because of their long history of smoking.[31]

There is a causal relationship between smoking and diminished overall health as well as an increased risk of all-cause mortality in men and women.[2] Tobacco smoke has adverse effects on almost all organs. Smoking is a risk factor for non-communicable diseases like cancer, respiratory problems, cardiovascular disease, periodontitis, cataract and reproductive problems. Smoking also increases the risk of attracting communicable diseases like pneumonia and tuberculosis.[2]

Figure 6. Health effects related to smoking



Source: Adapted from USDHHS 2004, 2006, 2012, 2014
Note: Each condition presented in bold text and followed by an asterisk (*) is a new disease that has been causally linked to smoking in the USDHHS 2014 report.

Approximately 70% of all lung cancer is attributable to tobacco smoking. Tobacco smoke is also associated with oropharyngeal cancer, hepatocellular cancer, cancer in esophagus, pancreas, kidney, bladder, colorectal cancer and cervix cancer. Furthermore, the results of cancer treatment is poorer in smokers with cancer.[2]

Both active smoking and exposure to second-hand smoking increase the risk of adverse cardiovascular effects like atherosclerosis, acute myocardial infarct (MI), stroke, aortic aneurysm and sudden cardiovascular death.[2]

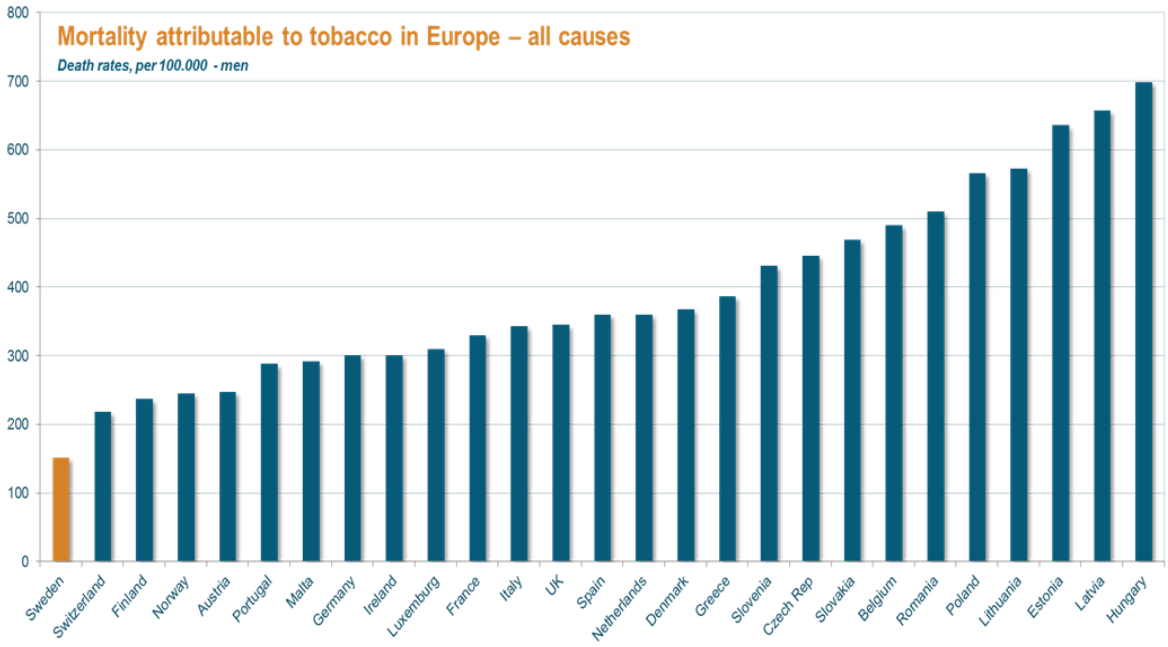
Combustion products in tobacco smoke increase the risk of developing chronic obstructive pulmonary disease, COPD, and more than 42% of all COPD is attributable to smoking. There is also an increasing risk of pneumonia and exacerbation of bronchitis in smokers with COPD. In addition, smoking is also associated with poor asthma control and exacerbation of asthma.[2]

2.7 HEALTH EFFECTS OF SWEDISH SNUFF

"The Swedish Experience." Swedish Match

Sweden has the lowest smoke–related mortality and morbidity in Europe, although the total level of tobacco consumption is comparable to the average consumption in Europe.[4] This is explained by the extensive use of snuff among men in Sweden.

Figure 7. The proportion of mortality attributable to tobacco in European males



Source: Swedish match. Adapted from WHO Mortality Attributable to tobacco, 2012

Internationally, "the Swedish Experience" is debated by researchers, politicians and public health experts, discussing if snuff is to be looked upon as a harm reduction product[32] in the struggle against the smoking related health problems encountered worldwide or as yet another tobacco product causing addiction[32, 33] and contributing to poorer health.[13, 16, 34, 35]

A few studies have found associations between snuff use and increased risk of pancreatic [36, 37] and esophageal cancer,[38] but the risk is significantly lower than with smoking.[39] Compared with non-tobacco users, snuff users generally have a slightly poorer cancer outcome, but if that is attributable to Swedish snuff or to confounding factors is not clear.[40]

Oral mucosal lesions from snuff use have not been associated with cancer.[41] In a review, Lee et al. argue that the evidence for snuff and risk of cancer is weak.[41]

There is little evidence that snuff use is associated with increased risk of acute myocardial infarct or stroke.[41] No association has been found between snuff use and hypertension.[41] Likewise, safety studies of nicotine replacement therapy have not been associated with increased cardiovascular risk.[3] However, there is growing evidence that snuff use, like smoking, is associated with increased insulin resistance and with increased risk of type 2-diabetes, and that the risk increases with increasing snuff use.[42, 43]

2.8 TOBACCO USE AND REPRODUCTIVE HEALTH

Smoking is associated with a poorer reproductive health in both men and women. The Practice Committee of the American Society has stated that 13% of infertility in the US may be attributable to smoking.[44] Studies of snuff use and fertility are lacking.

Smoking is associated with erectile dysfunction,[2] decreased production of testosterone in the Leydig cells[2] and an impaired spermatogenesis with DNA damage.[3] In females, smoking is associated with a shortened menstrual cycle, an earlier menopause and subfertility.[2, 3, 44] Smoking is also associated with hormonal disturbances, such as elevated levels of follicle stimulating hormone (FSH) and decreased progesterone levels.[2, 3]

Prenatal exposure to smoke has been associated with impaired fertility in adulthood in both men and women.[3, 45]

There is evidence in animal studies that nicotine is involved in the mechanisms behind reproductive dysfunction.[46] Nicotine seems to affect the hypothalamic-pituitary-gonadal (HPG) axis, controlling the hormonal balance and the sexual development and function.[3]

2.9 TOBACCO USE AND NICOTINE EXPOSURE IN PREGNANCY

”Smoking behavoiur of women differs from that of men….more highly motivated to smoke….they find it harder to stop smoking…. women are more neurotic than men… there may be a case for launching a female oriented cigarette with relatively high deliveries of nicotine…” 1976 research report, British American Tobacco

Maternal smoking is still one of the most important preventable risk factors for maternal and neonatal health globally. It is associated with adverse fetal and neonatal outcomes with increased risk of neonatal morbidity and mortality but also with long-term effects of the health of the child.[1]

The common substance in tobacco smoke and snuff is nicotine, and effects on the health caused by nicotine should theoretically be similar between smokers and snuff users. However, in the debate about Swedish snuff as a means of smoking cessation and a possibility to reduce the smoke related morbidity and mortality, the adverse effects of nicotine and use of snuff in pregnancy are rarely discussed.

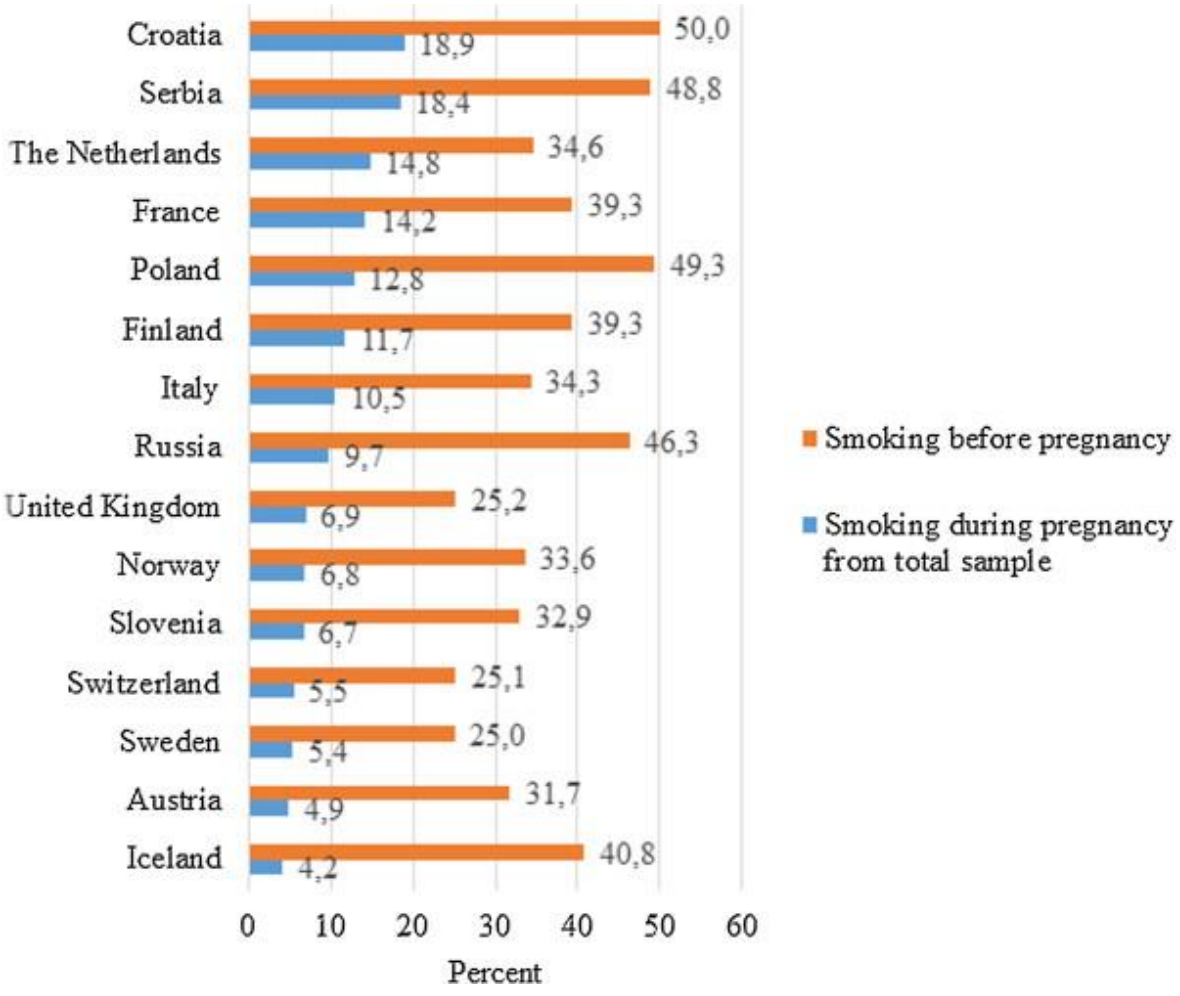
Prevalence of smoking and snuff use in pregnancy

Comparing data on the prevalence of maternal smoking on a global level is not unproblematic, because of the differences in maternal care systems, lack of national statistics in many countries, poor coverage of data and different periods in pregnancy when data is collected. Most data on tobacco use in pregnancy is based on self-reported information. Because of the social stigma related to smoking in pregnancy, there is a risk of underreporting of use, leading to misclassification of tobacco use.[47, 48]

The prevalence of maternal smoking in the US is approximately 15%, with great variation between states.[49] In the US, smoking in pregnancy is most frequent among American Indian/ Alaska Natives (26%), followed by non-Hispanics Whites.[49]

In the European countries, maternal smoking in early pregnancy varies from 5% to 20%.[50, 51] As many as 17% smoke in late pregnancy in France and 12% in the UK and Germany.[51, 52] In Eastern Europe, e.g. Poland, Greece and Croatia, the smoking prevalence in pregnancy ranges from 12% to 19%.[51]

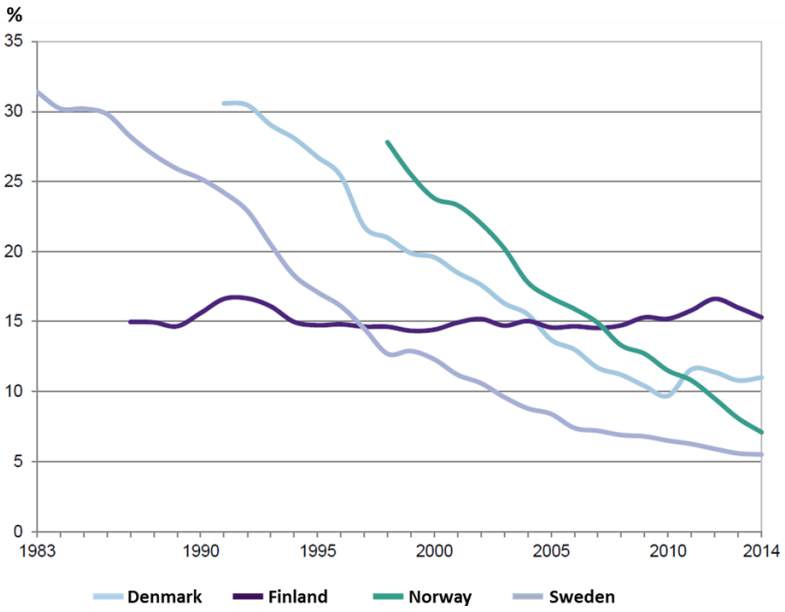
Figure 8. The prevalence of smoking during pregnancy in European countries



Source: Smedberg et al., 2014. Published from BMC Pregnancy Childbirth.

The prevalence of smoking before pregnancy in Sweden is 13.6%. However, the prevalence of smoking in early pregnancy has decreased from almost 30% in the mid-1980's to 5.5% in 2014.[6] The same is true for most Nordic countries except Finland, where the prevalence of maternal smoking in early pregnancy is still around 15%.[53]

Figure 9. The prevalence of maternal smoking in early pregnancy in the Nordic countries, 1983–2014



Source: Adapted from Perinatal statistics in the Nordic countries, THL, 2014

In Sweden, 4.6% use snuff three months before pregnancy, and 1.3% of women use snuff in early pregnancy. In late pregnancy, according to the Swedish Medical Birth Register, only 4% smoke and 0.7% use snuff.[6]

There are also large regional differences in tobacco habits within Sweden. In the northern counties, like Jämtland and Västernorrland, the proportions of smokers and snuff users in early pregnancy are similar. In contrast, in the southern part of Sweden, smoking in early pregnancy is 8-9%, whereas the prevalence of snuff use is less than 1%. The county of Stockholm has the lowest tobacco consumption in the country with only 3.3% smokers and 1.1% snuff users in early pregnancy.[6]

Maternal characteristics of tobacco use

Maternal smoking is associated with low socioeconomic status, low education level, not living with the father-to-be, having large number of children and inadequate prenatal care.[6, 49, 51, 54] A smoking partner increases the risk of continued smoking in pregnancy, as does a high level of pre-pregnancy smoking.[55]

According to the National Board of Healthcare in Sweden, as many as 16% of women with an education of 9 years or less smoked in early pregnancy, in comparison to 1.3% among women with more than 12 years of education. Approximately 20% of women below 20 years of age smoked in early pregnancy.[6]

Tobacco use and effects on pregnancy and the health of the newborn

Maternal smoking is associated with pregnancy complications like increased risk of spontaneous abortion,[56, 57] ectopic pregnancies,[3, 45] placenta dysfunction, placenta previa and placenta abruption.[1, 58, 59] Maternal smoking is also associated with increased risk of spontaneous and induced preterm birth, fetal growth restriction, stillbirth and oral cleft malformations.[2]

There is an increased risk of neonatal and infant mortality and morbidity in infants of smokers.[1, 2] Maternal smoking is also associated with increased risk of sudden infant death syndrome (SIDS).[1, 2]

Furthermore, prenatal exposure to smoke has also been associated with long term effects, such as respiratory problems and otitis in childhood.[2] Many studies have also shown associations between maternal smoking and behavioral problems in the offspring,[2, 3] but because of residual confounding it is difficult to infer causality.

In contrast to maternal smoking, snuff use during pregnancy has not been shown to be associated with placental dysfunction, such as placenta previa or placental abruption. [60] However, maternal snuff use has been associated with preterm birth[61, 62] and stillbirth.[60, 63] Baba et al. have also shown an association between snuff use in pregnancy and increased risk of being born small for gestational age (SGA).[64]

Spontaneous abortion and ectopic pregnancies

Spontaneous abortion (SAB) occurs in approximately 12% of all pregnancies and is defined as an involuntary termination of pregnancy before 20 weeks of gestation. Most SAB occur before the 12th week of gestation.[3] Maternal smoking and exposure to second-hand smoke is associated with increased risk of SAB.[56, 57] The mechanisms behind the effects of smoking are not clear, but placental insufficiency and fetal hypoxia from CO exposure and toxic effects of cadmium and cyanide in tobacco smoke have been suggested.[2, 44]

In ectopic pregnancy a fertilized egg is implanted outside the uterus, usually in the fallopian tube. It occurs in 1-2% of pregnancies.[65] Ectopic pregnancy is a risk factor for maternal mortality, subsequent infertility and/or recurrent ectopic pregnancies.[65]

The etiology of ectopic pregnancies is not fully understood but seems to involve motility of the fallopian tubes. The embryo remains within the fallopian tube because of structural or functional impairment of the tube, preventing implantation to occur.[2, 65] Suggested risk factors are history of previous ectopic pregnancy or SAB, sexually transmitted disease or surgical procedures affecting the fallopian tubes.[2, 65, 66]

The Surgeon General Report, 2014 concludes a causal relationship between smoking and ectopic pregnancy. There is evidence from animal studies that nicotine is playing a role in the mechanism behind ectopic pregnancy.[2, 66] There is a lack of knowledge about the association between snuff use and SAB or ectopic pregnancy.

Preeclampsia

Preeclampsia is a syndrome of reduced organ perfusion caused by vasospasm and endothelial dysfunction of the placenta, clinically defined as marked hypertonia and proteinuria, with an onset after 20 weeks of gestation.[2] Preeclampsia is a major cause of maternal and fetal mortality and morbidity globally and is associated with fetal growth restriction, stillbirth and placental abruption. It affects 3-7% of pregnant women.[67]

A two-stage model in the development of the disease is often discussed, where the first stage is characterized by defect implantation and formation of the placenta, leading to a hypoxic and dysfunctional placenta, inducing inflammation and endothelial damage. This results in the symptoms seen in the second stage with elevated blood pressure and proteinuria.[67]

Maternal smoking has a substantially protective effect and decreases the risk of developing preeclampsia.[68] However, if preeclampsia develops in smokers, the rates of adverse outcomes are increased substantially, which may occur as a result of synergistic effects of smoking and preeclampsia on the feto-placental circulation.[69] Snuff use does not protect against preeclampsia,[70, 71] implying that combustion products, such as CO with vasodilative properties, and not nicotine, are involved in the underlying protective mechanism. CO also reduces levels of anti-angiogenic protein soluble fms-like tyrosine kinas (sFlt-1), which in animal models initiate all symptoms of preeclampsia. Smokers have lower levels of sFlt-1 than nonsmokers.[2]

Placenta previa and placental abruption

Maternal smoking is associated with placenta dysfunction and antenatal bleeding, such as placenta previa and placental abruption, and the relationship is dose-dependent.[3, 58] The suggested mechanisms of placenta previa are related to chronic ischemia and hypoxia and consequent enlargement of placenta.[3]

The origin of placental abruption is multifactorial but related to placental dysfunction.[72] Potential risk factors are advanced maternal age, multiparity, previous placental abruption, hypertensive disorders, thrombophilia, previous cesarean section, trauma and uterine infections. Suggested underlying causes of abruption are vessel fragility and vascular malformation and impaired trophoblastic transformation.[3] Smoking has been proved to interfere with transformation of the spiral arteries and the thickening of the villous membrane in the placenta.[3]

Smoking-related placental abruption has been observed to be related to decidual necrosis at the periphery of the placenta, microinfarcts, atheromatous/fibrinoid changes in the placenta as well as with hypovascular and atrophic villi.[69, 73] Hypoxic changes, due to reductions in blood flow to the uteroplacental-fetal unit associated with cigarette smoking, may lead to placental infarcts and increased capillary fragility, in turn leading to arterial rupture and, subsequently, to placental abruption.[72, 73]

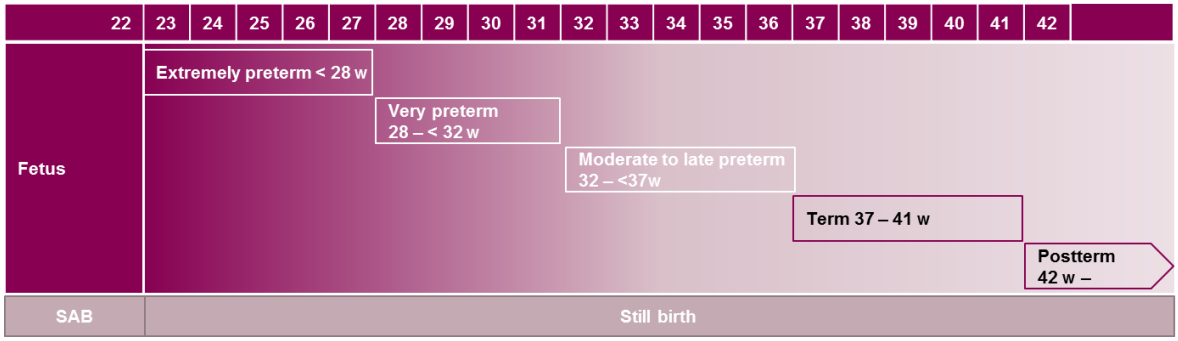
Smoking is supposed to impair collagen synthesis in placenta and induce inflammation in placental vessels.[1, 73] Studies of consecutive pregnancies show remaining but decreased risk when women stop smoking between pregnancies.[59]

Preterm birth

Preterm birth is defined as live birth before 37 weeks of gestation. Extremely preterm birth is defined as birth <28 weeks of gestation, very preterm birth 28 to <32 gestational week and moderately preterm birth 32 to <37 weeks of gestation. The highest morbidity and mortality is among the most preterm infants, born before 28 weeks of gestation.

More than 15 million babies are born preterm every year, and the incidence is increasing in most countries.[74] Preterm birth is the leading cause of perinatal morbidity and mortality in the developed countries, with an incidence of 11.1% globally.[75, 76] In the US, 12% of all babies are born preterm.[76] In Europe, preterm birth rates vary between 5-10%.[50] In Sweden, approximately 6% of all live born births are preterm.[6] Of live born singleton births in Sweden, 4.4% are born before 37 weeks; 0.2% are born extremely preterm, 0.6 % very preterm and 3.6% moderately preterm.[6]

Figure 10. Definition of preterm birth according to WHO



Preterm birth can be divided in spontaneous preterm birth (including spontaneous labor with intact membranes and spontaneous preterm premature rupture of membranes, PPROM) and induced preterm birth, i.e. induced labor or cesarean delivery for fetal or maternal indications.[77]

The cause of spontaneous preterm birth is multifactorial. Maternal history of preterm birth is a strong risk factor and suggested to be generated by the interaction of transgenic and environmental risk factors.[76] The role of ethnicity in preterm birth has been discussed and a variation in gestational length for different ethnic groups has been suggested; babies of African ancestry tend to be born earlier than babies of Caucasian ancestry.[76]

Previous preterm birth, multiple pregnancies, IVF pregnancies, intrauterine infection or inflammation and chronic diseases, such as hypertension, diabetes and thyroid disease are all associated with preterm birth.[76, 78] Preeclampsia, placental abruption, fetal growth restriction, maternal chronic disease and obesity are associated with medically indicated preterm birth.[77]

Maternal smoking has been casually related to preterm birth[1, 3, 45] and has been associated with both spontaneous and induced preterm birth.[79] The pathophysiological mechanisms are not clear, but smoking is associated with placental dysfunction, placenta previa and placental abruption as well as with fetal growth restriction, all associated with preterm birth.[2, 3]

Snuff use is also associated with preterm birth.[61, 62] However, snuff use is not associated with placental bleedings, placenta previa or placental abruption and the increased risk of impaired growth is moderate,[64] implying a different causal pathway for snuff and preterm birth than for that of tobacco smoke. The common substance in snuff and smoking is nicotine. In addition, tobacco smoke also contains numerous combustion products which may affect the risk of preterm birth, possibly through placenta dysfunction and subsequent fetal growth restriction.

Nicotine has vasoconstrictive properties, reducing the utero-placental blood flow[80] and may also affect the formation of the placenta in early pregnancy. Snuff use is a risk factor for early, but not late clinical stages of preeclampsia, suggesting that nicotine affects the formation of placenta,[71] which may play a role in the mechanisms behind preterm birth.

In addition, both combustion products of smoke and nicotine are supposed to affect the immune system,[81] and inflammation is a plausible explanation behind the increased risk of preterm birth. Inflammation and/or infection are known risk factors of preterm birth.[78, 82]

Fetal growth restriction

Both maternal smoking and maternal exposure to secondhand smoke are regarded causally related to fetal growth restriction.[2] A consistent finding is that infants of smoking mothers weigh 150-200 g less than infants of non-tobacco users.[3] Maternal smoking is also associated with SGA births, and the association is dose-dependent and correlated with maternal cotinine levels.[2, 3] How smoking affects fetal growth is not clear, but placenta dysfunction is believed to play a role. Maternal smoking is related to disturbances in the transformation of the spiral arteries and the thickening of the villous membrane forming the placenta, leading to placental dysfunction.[3] In addition, CO induces fetal hypoxia by binding to hemoglobin and inhibiting oxygen release. Nicotine is vasoconstrictive and has been supposed to decrease utero-placental blood flow with subsequent reduction in the delivery of oxygen and nutrients to the fetus.[80]

Animal studies of prenatal nicotine-exposure and effects on birth weight show conflicting results, but the majority show that nicotine impairs fetal and neonatal growth.[83] If nicotine has a major impact on fetal growth, smokeless tobacco and Swedish snuff should also be associated with low birth weight and SGA births, but most studies on snuff use or smokeless tobacco in pregnancy have shown very modest or no association with low birth weight.[70, 84, 85] However, Baba et al. found an association between snuff use and slightly increased risk of SGA birth, 1.38 OR (95% CI: 1.01-1.88), in comparison with that of nonusers.[64] The risk associated with smoking was higher, 3.21 OR (95% CI: 3.02-3.40).[64]

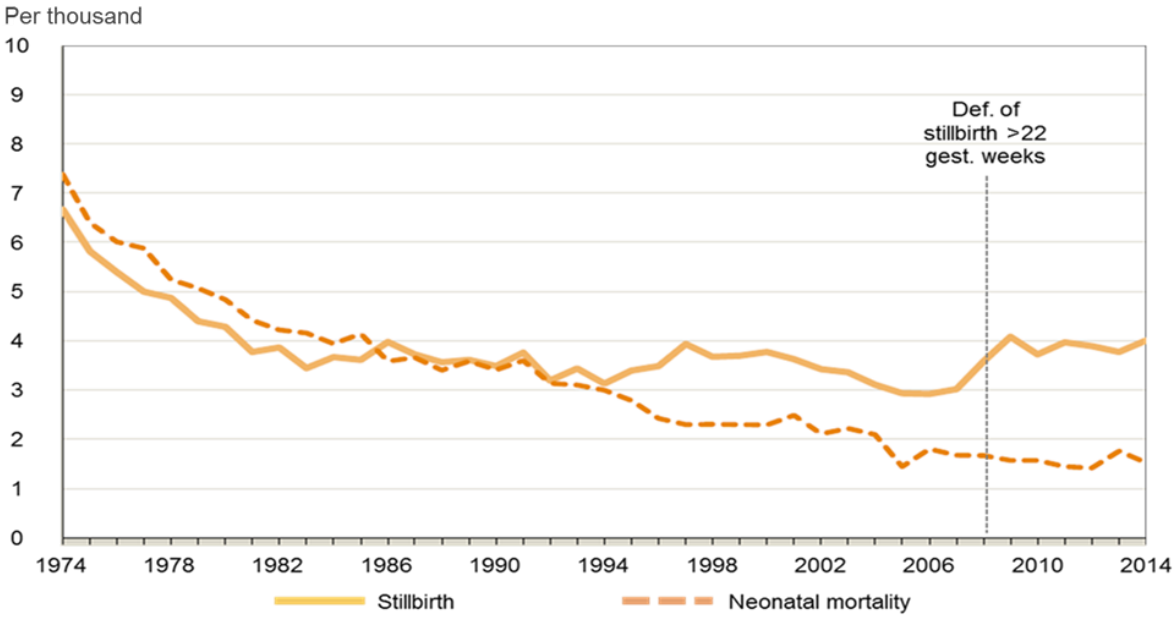
In addition, for women who had stopped using snuff before the antenatal booking, there was no increased risk of SGA birth, whereas women who stopped smoking before the antenatal booking had a lower, albeit increased risk of SGA birth compared to that of nonusers, OR 1.82(1.65-2.01).[64] Gupta et al. have shown decreased birth weight with use of mishri in pregnancy.[86] However, mishri and many other ST products contain numerous potentially toxic substances associated with low birth weight, such as PAH.[3, 23] The socio-demographic and cultural differences also make comparisons difficult to interpret.

To conclude, in humans the effect of nicotine on fetal growth seems to be modest in comparison to the effect of CO and other combustion products in smoke. Nicotine and CO and other combustion products may have synergistic effects on growth, explaining why smoking has a greater impact on growth than snuff use.

Stillbirth and neonatal and infant mortality

The estimated global stillbirth rate was 18.4/1000 live-born infants in 2015 (fetal age ≥28 weeks gestational weeks).[87] In Sweden, the incidence of stillbirth (fetal age ≥22 gestational weeks) is 4/1000 live born infants and the neonatal mortality is 1.5/ 1000 live-born babies.[6] As a consequence of a changed definition of stillbirth from fetal age ≥28 weeks to fetal age ≥22 weeks, the incidence of stillbirth in Sweden increased in 2008.

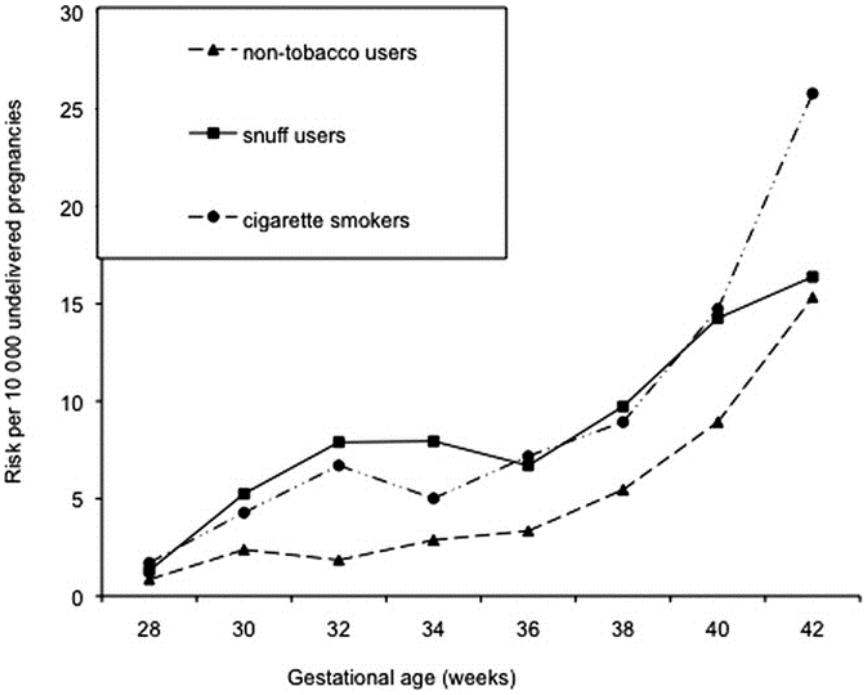
Figure 11. Incidence of stillbirth and neonatal mortality, 1974 -2014



Source: Adapted from National Board of Health: Pregnancies and Deliveries, 2015

The majority of stillbirths are preterm, but the risk of stillbirth increases with increasing gestational age. Risk factors for stillbirth are chromosomal and fetal abnormalities, high maternal age, obesity, intrauterine growth restriction, preeclampsia and placental bleedings.[88, 89] Both maternal smoking and use of snuff in pregnancy are associated with an increased risk of stillbirth.[63]

Figure 12. Risk of stillbirth per 10 000 pregnancies by gestational week



Source: Wikström et al. Epidemiology, 2010. Published with kind permission from Elsevier.

Maternal smoking is primarily associated with preterm stillbirth. Several studies have shown that the risk of stillbirth is mediated through increased risk of placental bleeding and intrauterine growth restriction.[88] Wikström et al. have shown that when women with preeclampsia, placental bleeding and/or small for gestational age deliveries were excluded from analysis, the smoking-related risk of stillbirth was attenuated, whereas it remained among snuff users.[60] Contrary to maternal smoking, snuff use in pregnancy is not associated with placental bleeding, and only a modest association between snuff use in pregnancy and fetal growth restriction has been found.[64] Importantly, women who stopped smoking or using snuff in early pregnancy were not at increased risk of stillbirth compared to that of non-tobacco users.[63]

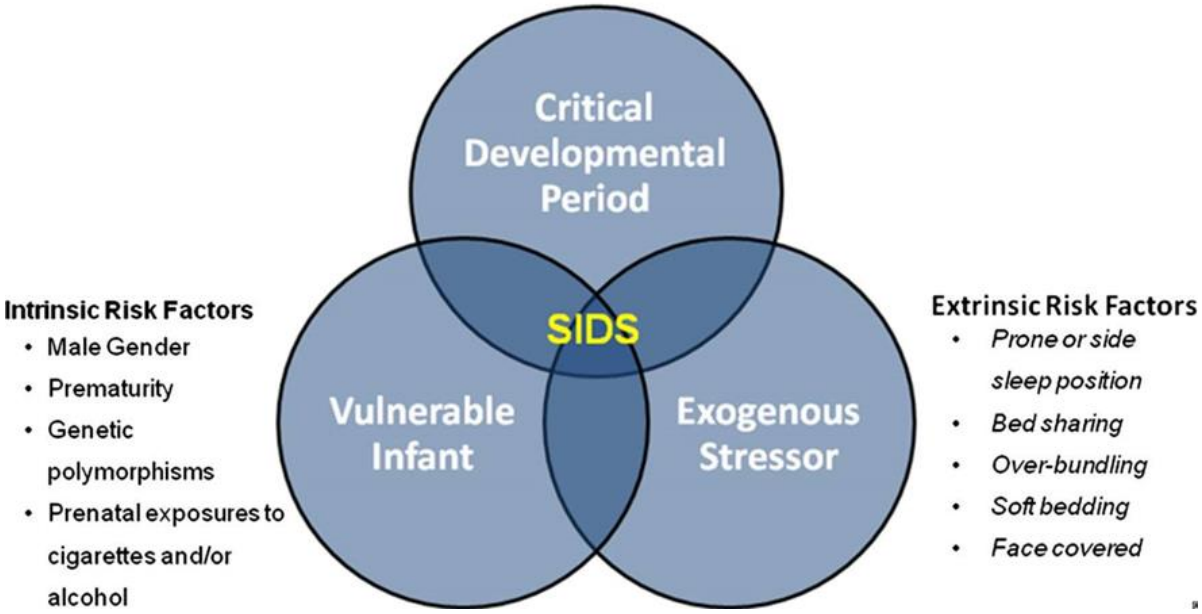
There is evidence that defect early placentation increases risk of stillbirth.[90] Nicotine possibly affects early placentation.[71] These findings suggest that nicotine is involved in the underlying mechanism of stillbirth, but in smokers combustion products may also play a role. In addition, Gupta et al. have shown increased risk of stillbirth after prenatal exposure to smokeless tobacco.[91]

In contrast to maternal smoking, snuff use is not associated with increased risk of early neonatal mortality.[63] However, Baba et al. showed that when adjusting for gestational age, the risk of early neonatal mortality decreased, suggesting that the early neonatal mortality is mediated through preterm birth. Maternal smoking is also associated with increased infant mortality, which is believed to be mediated by the increased risk of preterm birth and growth restriction[92] as well as with the increased risk of sudden infant death syndrome. [54]

Sudden Infant Death Syndrome

Sudden infant death syndrome, SIDS, is defined as a sudden unexplained death of a child below one year of age. A number of risk factors related to SIDS have been identified, such as prone position of sleep, bed sharing and overheating.[93] Since 1992, when the recommendation for non-prone sleeping position was launched, there has been a considerable decrease in SIDS cases.

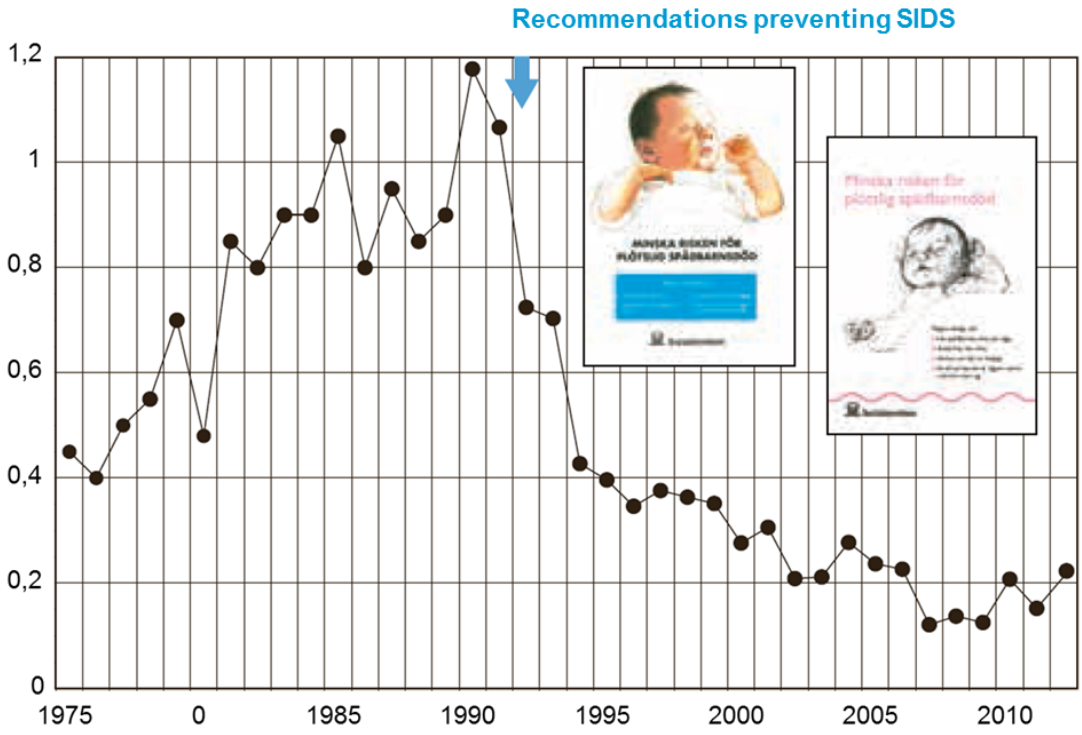
Figure 13. Factors associated with increased risk of Sudden Infant Death Syndrome



Source: Trachtenberg et al., Pediatrics. 2012 Apr; 129(4): 630–638.

A causal relation between maternal smoking, postnatal exposure to tobacco smoke and SIDS has been established.[2, 3] There is almost a threefold increased risk of SIDS among infants exposed to smoke prenatally, and the proportion of SIDS cases attributable to tobacco smoke is 23.2 according to Dietz et al., 2010.[54]

Figure 14.The incidence of SIDS and correlation with preventive recommendations



Source: Adapted from the National Board of Health and Welfare: Minska risken för plötslig spädbarnsdöd, 2014

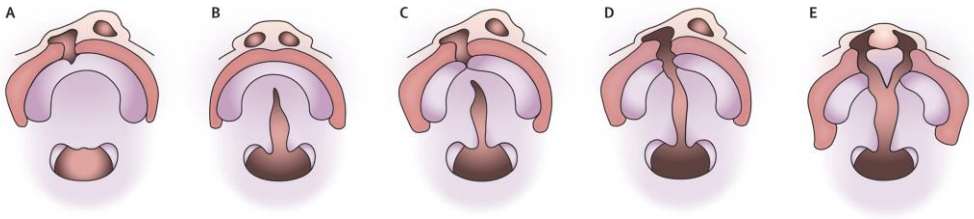
SIDS is associated with disturbed cardiorespiratory control[94] with blunt response to hypoxia and an impaired arousal.[95, 96] In the postnatal period, the cardiorespiratory system goes through maturation, which is a critical period for managing hypoxic stress, explaining why most cases of SIDS occur in this period.[94, 97]

Maternal smoking is associated with disturbed cardiorespiratory control, such as neonatal apneas and impaired arousal.[98-101] Several studies on prenatal nicotine exposure in animal studies have shown disturbances in the autonomic nervous system and disturbed cardiorespiratory control,[102, 103 104] which suggests that nicotine is involved in the mechanisms behind the smoking- related risks of SIDS. [3]

Oral cleft malformation

With an incidence of 1.7/1000 live-born, oral cleft malformations is one of the most common malformations.[105] Non-syndromic orofacial clefts include cleft lip, cleft lip and palate and isolated cleft palate. Oral cleft lip, with or without cleft palate (CL/P) and isolated cleft palate (CP) are embryological distinct entities with different epidemiological characteristics.[106]

Figure 15. Schematic presentation of different types of non-syndromic orofacial clefts



(A) Cleft lip and alveolus. (B) Cleft palate. (C) Incomplete unilateral cleft lip and palate. (D) Complete unilateral cleft lip and palate. (E) Complete bilateral cleft lip and palate.

Source: Mossey et al., Lancet 2009. Published with kind permission from Elsvier. Reprinted with permission from:Shaw WC. Orthodontics and occlusal management. Oxford: Butterworth-Heinemann, 1993

The etiology of oral cleft malformations is largely unknown and multifactorial, with large ethnic and genetic variation. Proposed environmental risk factors are maternal smoking, alcohol consumption and lack of vitamins, such as folic acids and zinc.[105-107]

CL/P and CP occur in the early trimester in the fourth to eighth gestational weeks as the result of disturbed fusion of the facial prominences. The earlier the interruption of fusion occurs, the greater the defect.[105] Moreover, CL/P has a different pathophysiological origin than CP. Cleft lip, with or without cleft palate is the result of interrupted fusion of the primary palate, while isolated oral cleft is the result of inhibited formation of the secondary palate.[105, 106]

Maternal smoking has been causally related to maternal smoking. There are also findings suggesting an interaction between maternal smoking and genetic polymorphism.[108-110] Maternal smoking is also associated with insufficient levels of vitamin C and zinc.[111-113] Vitamin deficiency is supposed to influence risk of oral cleft malformations,[105] and a few studies have found interactions between maternal smoking, vitamin supplements and risk of oral cleft malformations.[114]

Animal studies and in vitro studies suggest that nicotine inhibits the fusion of the facial prominences.[115-117] A Danish Medical Birth Register study found an association between NRT and oral cleft malformations and limb defects.[118] However, compliance with NRT in pregnancy is poor;[11] women continue to smoke using NRT. Thus, the results should be interpreted with caution.

Congenital malformations

Maternal smoking is not strongly associated with malformations, nor is nicotine in animal studies. Hackshaw et al., 2011, concluded in a meta-analysis that maternal smoking was associated with the following congenital abnormalities in addition to oral cleft malformations: clubfoot, gastroschisis and atrial septal heart defects, anal atresia, craniosynostosis, limb reduction defects and cryptorchidism.[2, 119] These findings were supported by a later Danish register based cohort study. [120]

Table 1. Summary of a systematic review of maternal smoking during pregnancy and its relationship with specific congenital malformations.

Outcome	No. of studies published, 1959-2010	OR (95% CI)
Orofacial clefts	38	1.28 (1.20-1.36)
Clubfoot	12	1.28 (1.10-1.47)
Gastroschisis	12	1.50 (1.28-1.76)
Congenital heart defects	25	1.09 (1.02-1.17)
Craniosynostosis	5	1.33 (1.03-1.73)
Anorectal atresia	7	1.20 (1.06-1.36)

Source: Adapted from Hackshaw et al., 2011

Long term effects of tobacco use in pregnancy

Maternal smoking is associated with increased risk of asthma and respiratory problems in children.[2, 3, 121, 122] Furthermore, smoking in pregnancy is associated with overweight, hypertension and type-2 diabetes in the offspring.[123, 124] Maternal smoking is related to growth restriction, which is a known risk factor for hypertension, type-2 diabetes and obesity later in life.[125-128] Many studies have found associations between maternal smoking and neurobehavioral disorders.[2, 129] However, this is difficult to study because of the socioeconomic and psychiatric confounding related to nicotine addiction. Findings in animal studies of nicotine and neurobehavioral disturbances support the findings in human epidemiological studies.[130, 131]

Tobacco cessation in pregnancy

"*To cease smoking is the easiest thing I ever did. I ought to know because I've done it a thousand times.*" Mark Twain

The majority of women stop smoking before the antenatal booking without any professional guidance.[132] However, almost 50% continue smoking in pregnancy.[49] A smoking partner, a high level of smoking prior pregnancy and multiparity are the most important predictive factors of continued smoking in pregnancy.[133] Although the proportion of smoking in pregnancy is higher in young women, the quitting rate during pregnancy is also higher among teenage mothers.[134]

Nicotine replacement therapy has not proved efficient in randomized control studies, because adherence to medication was low.[11, 135] Psychosocial clinic-based interventions in the prenatal care setting have also shown modest results on cessation rates.[132] According to the Surgeon General 2014, there is evidence of tobacco control policies having effect on maternal smoke cessation.[2]

2.10 NICOTINE

Nicotine binds to nicotine-acetylcholine receptors (nAChR), which are present at neuromuscular junctions, in the peripheral and central nervous system and also in many non-neural cells in muscle, skin, lung and pancreas. [136]

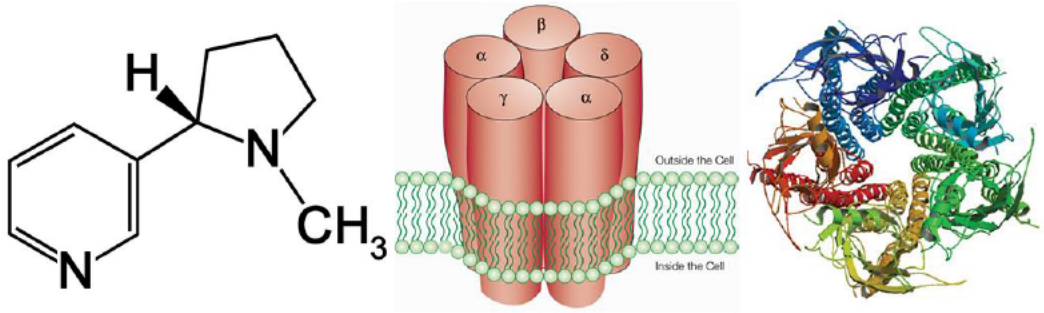


Figure16. Nicotine molecule and a schematic presentation of the nicotine-acetylcholine receptor.

The nicotine-acetylcholine receptor (nAChR) consists of five subunits forming a pore in the cell membrane and is a ligand-gated ion channel that mediates fast neurotransmission. The receptor consists of a combination of four related, but genetically and immunologically distinct, subunits forming a pentamer.

The variation of different types of subunits, combinations of subunits and the functional properties of different subunits explain the diversity of the physiological processes that the nAChR is involved in. The nAchR has metabolic and endocrine effects as well affecting the immune and nervous system. Furthermore, nAChR is also involved in control of endothelial function.[137]

Table 2.Physiological effects of nicotine

Cardiovascular	Endocrine and metabolic	Immune system	CNS-behavioral effects
Increased heart rate, cardiac contractility, and blood pressure	Adrenocorticotropic hormone, cortisol Catecholamine release	Immune suppressant	Relaxation or arousal
Cutaneous and systemic vasoconstriction	Release of growth hormone, vasopressin,	Affect T-cell signalling	Enhanced concentration
Increased muscle blood flow		Immune modulatory effects on the central nervous system by: 1) the HPA axis 2) direct effects on the autonomic nervous system.	Appetite suppression
Inhibition in prostacyclin synthesis, which affects endothelial cells and Platelet activation.			Alertness

Source:Adapted from Benowitz et.al.1988

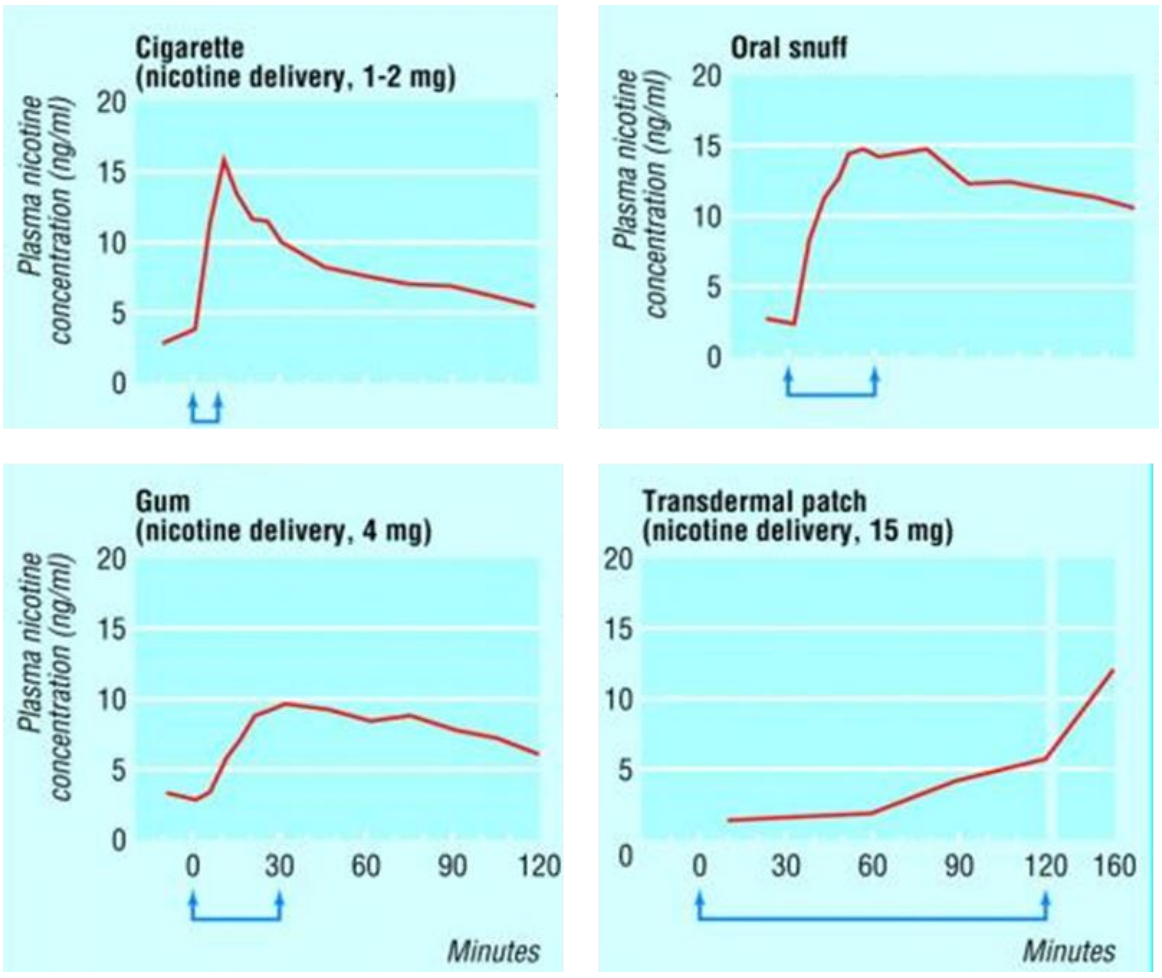
Pharmacokinetics of nicotine

Free nicotine, i.e. the non-ionized form of nicotine, easily passes cell membranes, including oral mucosa, pulmonary tissue, the blood-brain barrier, placenta and other tissues. It accumulates in breast milk[138-141] and amniotic fluid.[142] Nicotine is a weak base and the proportion of non-ionized nicotine increases with increasing pH-value. Oral moist snuff is therefore alkalized to facilitate the absorption of nicotine.

During cigarette smoking, nicotine reaches the brain within 10-20 seconds, with a peak plasma-concentration in 5 minutes.[136] During snuff use, nicotine is absorbed more slowly, but plasma-concentration peaks are similar to that of smoking. Whereas the plasma concentration of nicotine falls rapidly after smoking, the nicotine plasma-concentration of snuff users reaches a plateau after 30 minutes, and nicotine levels slowly decline after 1-2 hours. It has been speculated that the continued release of nicotine, also after use of oral snuff, comes from the mucus membranes.[143, 144]

NRT, such as gums and patches, has a substantially slower absorbance and lower peak plasma–concentrations, which is why the addictive potential is low.[145]

Figure 17. Plasma nicotine concentration from different sources of nicotine



Source: Adapted from Henningfield 1995 and Molneux, 2004.

Only a small amount of the nicotine in the cigarette or pouch of snuff is absorbed. Smoking behavior affects nicotine absorption through inhalation volume, number of puffs and number of covered ventilation holes in the filter of the cigarette.[146, 147] The amount of nicotine absorbed by the body during snuff use depends on how long the pouch is kept in the mouth and the properties of the snuff, such as the total nicotine content and the pH-value. The rate of absorbance and metabolism also varies substantially between individuals.[136]

As mentioned above, the pattern of tobacco use also affects nicotine plasma-levels. Whereas smoking gives high peaks of nicotine intermittently during the day, many snuff users have a pouch of snuff under the lip throughout the day which leads to a continuously high level of nicotine.

Nicotine metabolism

Nicotine is metabolized in the liver, primarily by cytochrome 2A6. It has a half-life of 2 hours and is subsequently eliminated by the kidneys. The primary metabolite of nicotine, cotinine, has a half-life of 18 hours and is the most frequently used biomarker of nicotine exposure.[136] There are major individual differences in nicotine and cotinine metabolism which are believed to affect the risk of addiction. Thus fast metabolizers appear to have a higher risk of addiction than slow metabolizers.[145]

Women have a higher metabolism of nicotine and cotinine than men. Women using oral contraceptives and pregnant women have a higher nicotine metabolism than non-pregnant women, not using oral contraceptives.[136] Nicotine metabolism is also elevated in pregnancy, which leads to a half-life of cotinine of 8-9 hours.[142, 148] In contrast, the nicotine metabolism in newborns is slower than in adults, with a half-life three times longer in infants. The cotinine metabolism seems to be the same as in adults, though.[136] This might explain why nicotine, which easily passes placenta, seems to accumulate in amniotic fluids.[80] Luck et al. found that fetal concentrations in cord blood were 15% higher than maternal levels at the time of delivery.[139]

Nicotine also easily passes into breastmilk, leading to a milk-to-maternal plasma ratio of 2.9.[141] Nicotine in breastmilk is swallowed by the infant, and there is insufficient knowledge about absorption of nicotine in the gastrointestinal tract of the infant.[141]

Effects of nicotine during development

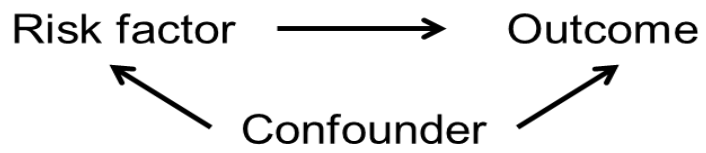
The effects of nicotine during development are very different from the effects in the adult. The nAChRs is present very early in the developing human fetus, as early as gestational week 4-5, and acetylcholine plays an important role in brain and lung maturation. Nicotine is a potent neuroteratogen and binds to nicotinic AChRs in the developing brain and lung, inducing apoptosis and affecting cell programming.[104 129, 131]

Prenatal nicotine exposure is also known to affect the cardiovascular, the endocrine and the immune system, inducing structural and even transgenetical changes that may have effect also in adulthood.[46, 149-153] In animal studies, prenatal nicotine-exposure induces lung hypoplasia, emphysema-like changes, such as decreased elastin and increased collagen synthesis in vascular endothelial cells and lung parenchyma.[102] The morphological changes are associated with increased airway resistance and hyper reactivity, observed in children exposed to maternal smoking, suggesting that nicotine is involved in the development of asthma and lung disease.[102] In addition, nicotine has effects on the nervous system and on the developing brain at doses that have no impact on fetal growth.[104] This is important in the discussion of NRT use in pregnancy.[131]

3 EPIDEMIOLOGICAL TERMINOLOGY

Bias is a systematic error in a study. **Selection bias** in a study stems from how study subjects are collected and/or from factors that influence study participation. **Information bias** or **misclassification** arises when there is a systematic error in the collecting of information. Misclassification of subjects of an exposure or outcome can be divided in *differential* and *non-differential* misclassification. The exposure bias is non-differential if the misclassification is unrelated to the outcome. If the exposure misclassification is related to the outcome, it is a *differential* misclassification. A *differential* misclassification is associated with under- or over -estimation of the effect on the outcome, whereas a *non-differential* misclassification tends to dilute the effect of the exposure on the outcome, resulting in a bias towards the null. **Recall bias** is a differential misclassification bias, where the exposure information is misclassified differentially for those with and without disease.

Confounding is a type of bias, that obscures or distorts the result of an association. A **confounding factor** is associated with both the exposure and the studied outcome. It may cause the outcome or be a proxy for the outcome but not be an effect of the outcome. A confounder is not an intermediate in the causal pathway between the exposure and the outcome.



Internal validity describes if the study or a test accurately measures what it is supposed to measure, which means lack of systematic errors such as bias, misclassification or confounding.

External validity or generalizability describes if the study is representative beyond the study population and if the result of the study is applicable not only to the study population, but also to the reference population.

Sensitivity is defined as the proportion classified by the test as having the disease among all in the population having the disease.

Specificity is defined as the proportion classified by the test as not having the disease among all in the population without the disease.

Positive predictive value is the probability of the disease among those with a positive test.

Negative predicted value is the probability of no disease among those with a negative test.

Receiver operating characteristics curve, ROC, describes the relationship between the sensitivity and specificity of a test and is used to decide the cut-off values for an optimal combination of sensitivity and specificity for a test.

4 AIM

The overall aim of this thesis was to study prenatal nicotine exposure, in the form of oral moist snuff and smoking, and its effects on the health of the newborn. The general research question was to investigate if it is safe to use snuff in pregnancy, and if cessation of snuff use or smoking during pregnancy influences the possibly adverse risks on the health of the newborn.

Specific research objectives:

Study I

- To investigate if maternal smoking or snuff use is associated with neonatal apnea.

Study II

- To study if smoking or snuff use in pregnancy is associated with increased risk of oral cleft malformations.
- To study if cessation of snuff use or smoking in early pregnancy influences the risk of oral cleft malformations in the newborn.

Study III

- To assess the relationship between maternal smoking or snuff use and extremely preterm birth.
- To study if cessation of snuff use or smoking influences the risk of extremely preterm birth.
- To investigate the association between tobacco use and spontaneous and medically indicated onset of preterm delivery.

Study IV

- To validate prospectively self-reported questionnaire data on snuff use in late pregnancy by quantifying cotinine in maternal urine and newborn meconium.
- To validate self-reported information on tobacco use in the Swedish Medical Birth Register in late pregnancy by cotinine in maternal urine and newborn meconium.
- To study if the levels of cotinine can be used to identify mothers with high nicotine intake.
- To investigate the relation between cotinine levels and neonatal birth characteristics, such as birth weight, length, head circumference and gestational age.

5 METHODOLOGICAL CONSIDERATIONS

The methods used in the individual papers are described in each paper and will not be explained in detail in this chapter.

5.1 DATA SOURCES

This thesis is predominately based on population-based studies in the Swedish Medical Birth Register (*paper I-III)* with linkage to other data sources from the National Board of Health, such as the Patient Register[154], the Education Register and the Total Population Register held by Statistics Sweden.[155, 156]

Study IV is a validation study of prospectively collected self-reported snuff use during pregnancy by the biomarker cotinine in maternal urine and in the meconium of the newborn. In addition, Medical Birth Register data for the cohort is linked to the cohort data.

The Medical Birth Register and other registers

The Swedish Medical Birth Register (MBR) was started in 1973 and is a population-based register that contains data on more than 98% of all births in Sweden, including maternal characteristics (demographic data, information on reproductive history and tobacco habits) and information on pregnancy, delivery and the neonatal period. Information on smoking in early pregnancy has been recorded since 1982, whereas tobacco use in late pregnancy was added in 1991. Snuff use was not reported to the MBR until 1999.

Data on tobacco use is based on prospectively collected self-reported information obtained by midwives at the antenatal booking in early pregnancy and in week 32. Tobacco use is categorized as no tobacco use, maternal smoking 1-9 cigarettes/day or >=10 cigarettes/day and snuff use (yes/no).[6]

Maternal diseases, complications during pregnancy or delivery and neonatal complications and diagnoses are classified according to the Swedish version of the International Classification of Diseases (ICD) system and reported at discharge from the delivery hospital.

Sweden has many other registers, such as the Register on Congenital Malformations, the Swedish Cancer Register, the National Cause of Death Register, the National Patient Register and the Prescribed Drug Register held by the National Board of Health.[154] Through the unique personal identification number (PIN), several registers and other data sources held by Statistics Sweden,[156] such as the Education Register, the Total Population Register and the Multi-Generation Register can be linked together.[155, 157]

The National Patient Register contains nationwide information on diagnoses and procedures and can be divided into the Inpatient Register, with coverage since 1987, and the Outpatient Register, with information on all hospital out-patient care since 2001.[154] Since 1997, both the MBR and the National Patient Register diagnoses are classified according to the 10th revision, ICD-10.

Validation of the Swedish Medical Birth Register

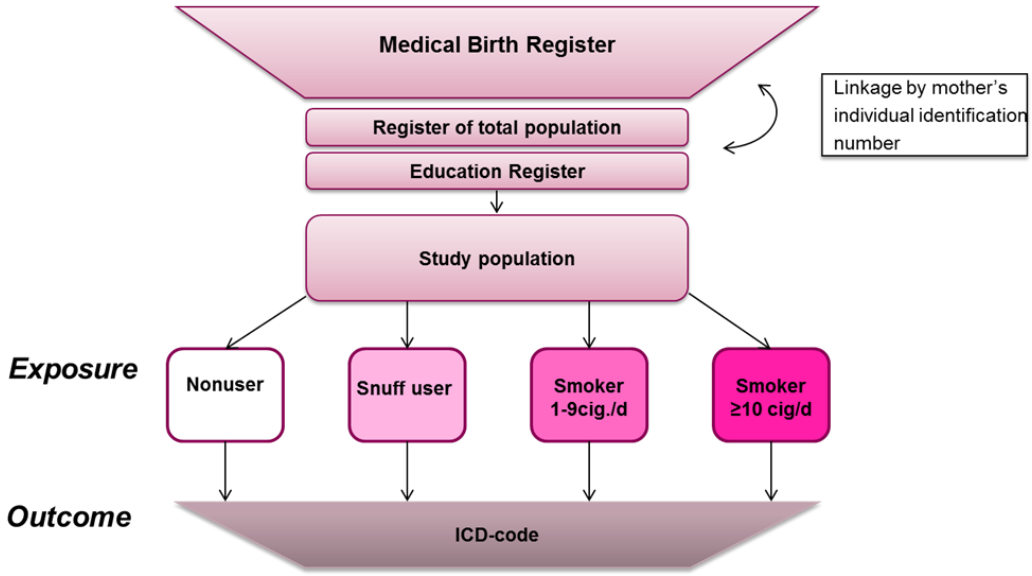
Since the start of the register in 1973, the MBR has been modified and variables added on several occasions, 1982, 1990, 1994 and 1998.[158] The MBR has been validated according to quality and coverage of data several times[159], the latest in 2002, and has been proved reliable with high coverage and data quality on most variables.[158] Smoking in early pregnancy has been missing in 4-9% throughout the years. However, smoking in late pregnancy had until 2000 a high percentage of missing information and was not useful in research.[158] According to the National Board of Health and Welfare, there was 4-5% missing information on smoking and snuff use prior pregnancy, in early pregnancy and in late pregnancy in the MBR 2014.[6] Information on drug and alcohol use in pregnancy is of poor quality.

Missing data on diagnoses of infants who have been transferred between hospitals and neonatal wards is still a problem.[158] Linkage to the Congenital Malformation Register, the Hospital Discharge Register or the National Patient Register can be necessary to ascertain certain diagnoses in epidemiological studies.[158]

5.2 STUDY DESIGN AND STUDY POPULATION, PAPER I-III

In *paper I-III*, tobacco exposure in the Medical Birth Register in early pregnancy was studied. *In paper I*, tobacco exposure was categorized as nonuser, snuff user, smoker 1-9 cigarettes/day and smoker ≥ 10 cigarettes/day.

Figure18. Schematic presentation of the study designs in *paper I-III*



What differed in the study design of *papers II* and *papers III* from *paper I* was the categorization of tobacco use. In *paper II* and *paper III*, tobacco use was categorized as: *no use* (i.e. no use three months prior pregnancy and in early pregnancy); *stopped using snuff* (i.e. snuff use three months before pregnancy but not in early pregnancy); *snuff use* (i.e. snuff use three months before pregnancy and in early pregnancy) *and stopped smoking* (i.e. smoking three months before pregnancy but not in early pregnancy); and *smoking* (i.e. smoking three months before pregnancy and in early pregnancy).

ICD-10 codes

For diagnoses of neonatal apnea and oral cleft malformations ICD-10 codes were used.

The American Association of Pediatrics[160] has defined infant apnea as "*an unexplained episode of cessation of breathing for 20 seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor and/or marked hypotonia*." Neonatal apnea unspecified was defined by the following ICD-10 codes: P 28.2 ("cyanotic attack"), P28.3 ("sleep apnea") and P28.4 ("other apnea of the newborn"). Sleep apnea was also analyzed separately as ICD-10 code P 28.3.

Oral cleft malformation was defined by the ICD- 10codes Q35-37. The different subtypes of oral cleft malformations were analyzed separately, as cleft lip with or without cleft palate (ICD-10 codes Q36 and Q37) and isolated oral cleft (ICD-10 Q35).

In *paper II*, the Patient Register was linked to the Medical Birth Register to ensure adequate coverage on congenital malformation diagnoses.

Inclusion and exclusion criteria

Exclusion criteria for all papers were missing information on tobacco use and dual use (snuff use and smoking). In the adjusted models missing data on co-variates was also excluded.

Only mothers of live-born infants were included in the studies I and III. In *paper II* and *paper IV* both singleton and multiple births were included.

In *paper I*, only mothers born in the Nordic countries were included.

In *paper IV*, smokers and dual users were excluded from the study.

Confounders and intermediate variables

In *paper I-IV*, co-variates were chosen because of their association with the exposure and the outcome in previous literature. Possible confounders were adjusted for in the adjusted analysis.

BMI is defined as: body weight/height ^{2}. Body mass index, BMI, was not adjusted for in *paper I* and *paper II* despite being a possible confounder associated with both exposure and outcome. There is a large number of mothers with missing information on the BMI variable in the MBR. When BMI was adjusted for in the regression model without affecting the estimate, BMI was not considered a confounder and was left out of the final analysis. In *paper IV*, maternal education and mother's country of birth were not possible to obtain.

Table 3. Maternal and birth characteristics used as co-variates in the different studies

Maternal and birth characteristics	Study I Apnea	Study II Oral Cleft Malformation	Study III Preterm birth	Study IV Neonatal growth characteristics
Maternal age	x	x	x	x
Height	x			
BMI			x	x
Parity	x	x	x	x
Cohabitant with father-to-be	x	x	x	x
Education	x	x	x	
Pre-pregnancy diabetes		x		
Preeclampsia		x		
Mother's country of birth		x	x	
Cesarean Section	x			
Small-for-gestational age	x			
Gestational age	x			x
Gender	x	x		
Single or multiple pregnancies		x		

5.3 STUDY DESIGN AND STUDY POPULATION, PAPER IV

Self-reported snuff use in pregnancy has not been validated previously. The objective with this study was to study the accuracy of self-reported snuff use but also to validate the data on snuff use in the Medial Birth Register.

Between 2005 and 2011, women were asked to participate in a prospective cohort study of snuff use during pregnancy. Antenatal clinics in seven counties in Sweden (Jämtland, Västernorrland, Västerbotten, Stockholm, Gotland, Gävleborg and Dalarna), chosen by their high prevalence of snuff use, took part in the study.

Inclusion occurred at the antenatal booking, and tobacco use was categorized as no use, snuff use or smoking. Only nonusers and snuff users were analyzed further.

Figure 19. Schematic presentation of the study design, *paper IV*

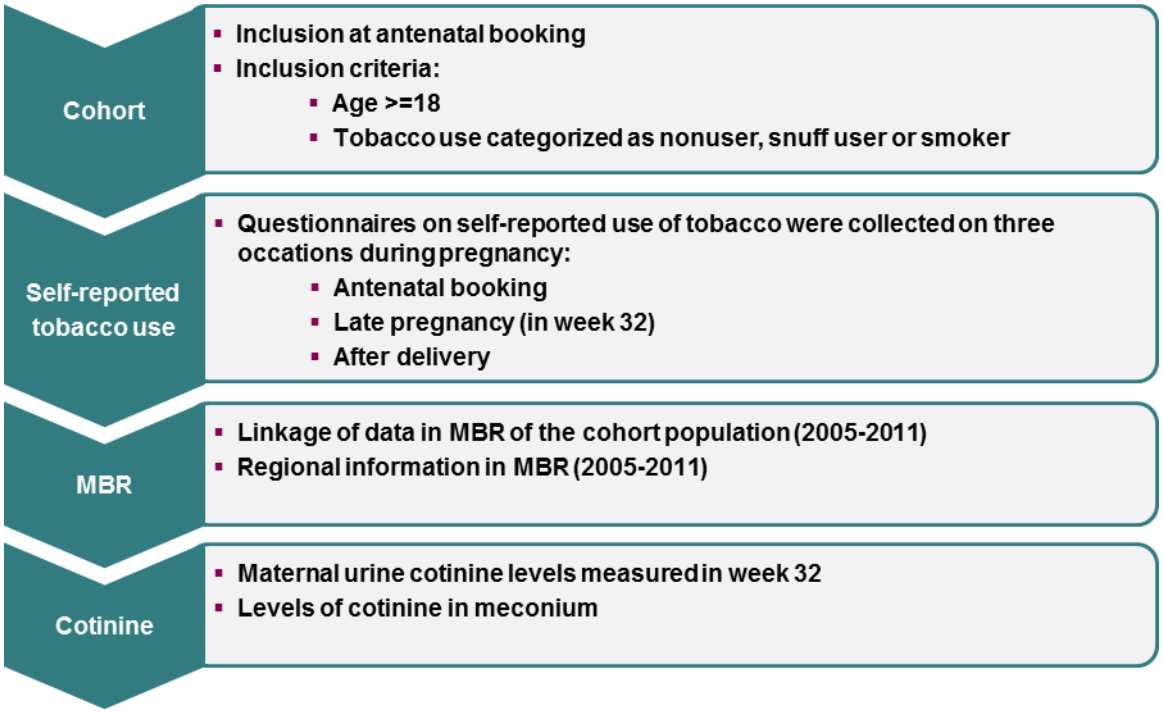
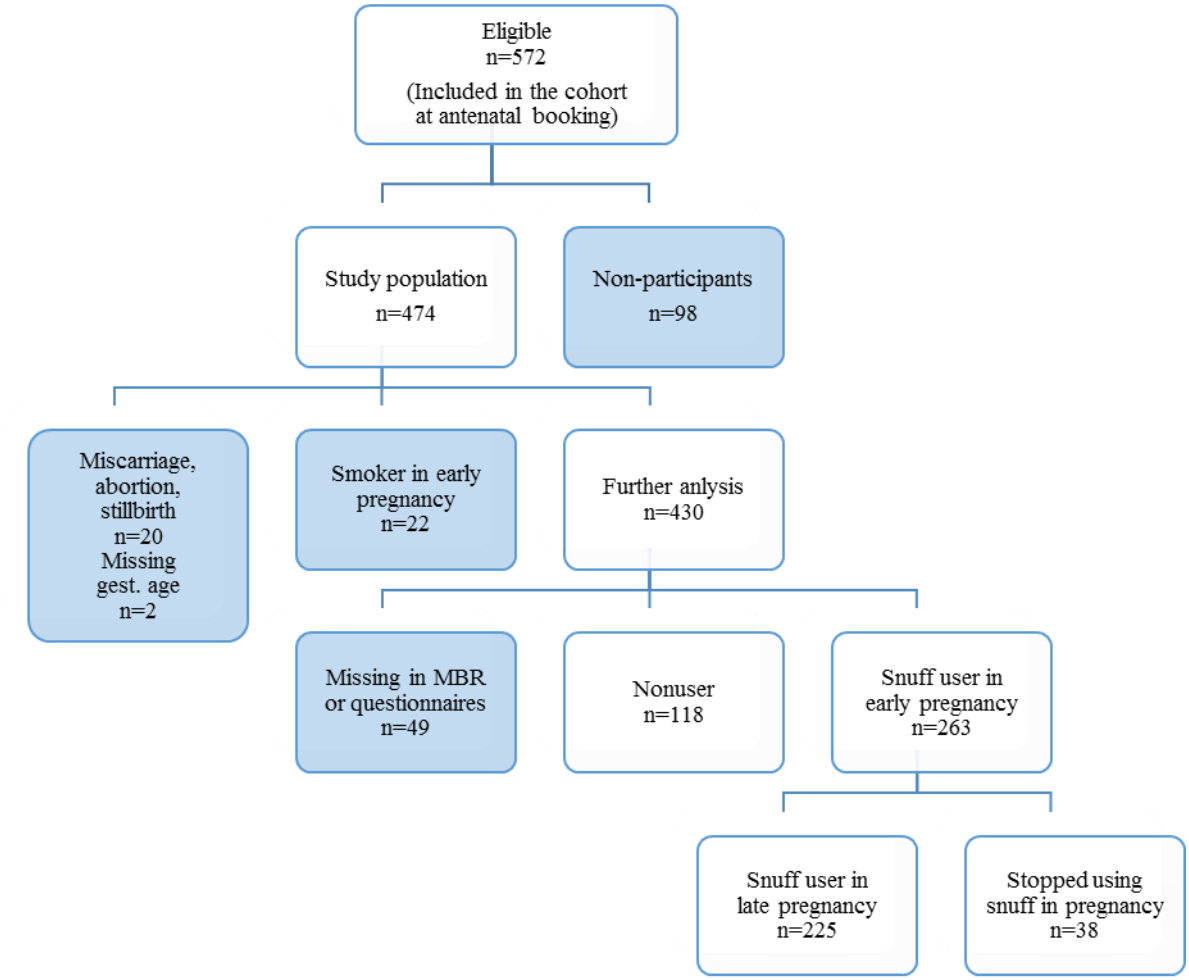


Figure 20. Schematic presentation of the study population



Self-reported snuff use through questionnaires

The participants were asked to report their tobacco use through questionnaires at antenatal booking, in late pregnancy and immediately after birth. The participants self-reported daily use of tobacco for every gestational week, including brand of tobacco used, use of nicotine replacement therapy (NRT) and exposure to second-hand smoke (SHS). Snuff use was reported as number of pouches/day each gestational week and categorized as follows: no use, 1-2, 3-4, 5-6 or ≥7 pouches/day. See Appendix A

Self-reported snuff use registered in the Medical Birth Register

We obtained information from medical records from the antenatal and delivery clinics on the participants and data from the Medical Birth Register (MBR). To assess if the study population was representative of the population, we also obtained data from the MBR for all births the corresponding years and geographic regions. As mentioned previously, tobacco use in the MBR was categorized as no use, snuff use, smoking 1-9 cigarettes/day and smoking >=10 cigarettes/day.

Analysis of cotinine

Maternal urine cotinine was collected in gestational week 32. In addition, cotinine in the meconium of the newborn was analyzed.

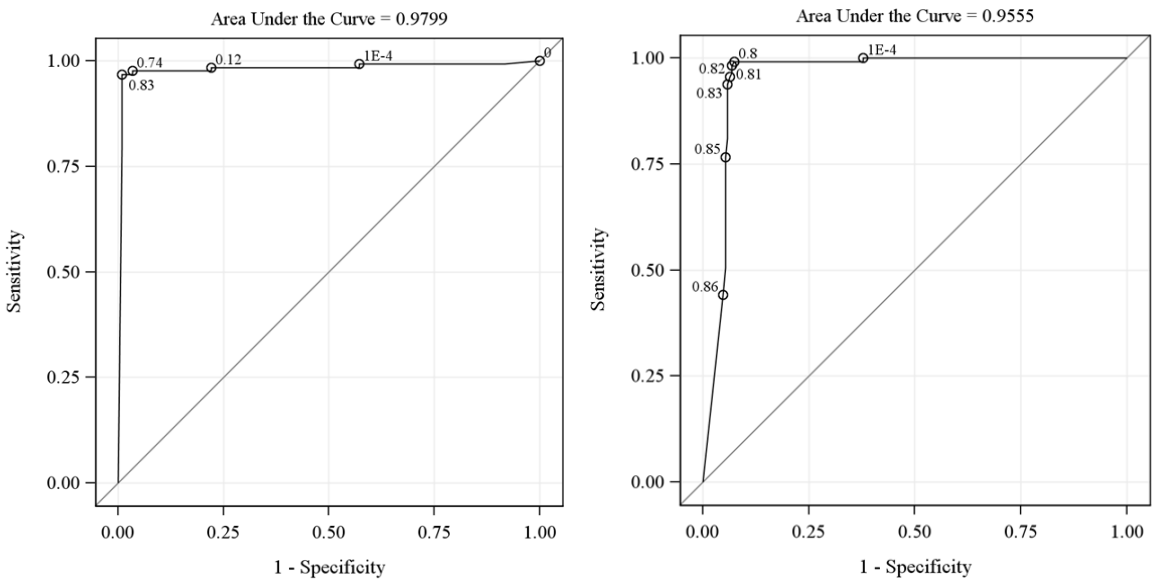
To determine cotinine in urine and meconium, a procedure based on high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used.[161] Meconium was first extracted with methanol. Further purification of the meconium methanol extracts and urine was done with solid phase extraction. Standard curves were created using seven calibrators at 0, 5, 15, 30, 50, 75 and 100 ng/mL, and a least square linear regression was generated with correlation coefficients > 0.99.

The lowest limit of quantification (LOQ) was 1 ng/ml for cotinine in urine and meconium.

The cut-off level of cotinine to distinguish tobacco exposure from no exposure was chosen by visually assessing the receiver operating characteristics (ROC) curve. Urine cotinine level ≥15ng/ml was defined as snuff use and urine cotinine <15 ng/ml was categorized as no use. In meconium, cotinine level ≥3 ng/ml was defined as snuff use and cotinine level <3ng/ml was categorized as no use.

Figure 21. ROC for snuff use and maternal urine cotinine (a) and meconium cotinine (b)

a) **b)**



5.4 STATISTICAL METHODS

The statistical methods used in this thesis are described in detail in each paper.

Paper I-III

Associations between tobacco exposure and outcomes were evaluated using multivariable adjusted logistic regression models. Results were presented as odds ratios, OR, with 95% confidence interval. In order to adjust for the dependence introduced by the fact that mothers may contribute with more than one child, the Generalized Estimation Equation method was applied.

Paper IV

Spearman's correlation coefficient was used to calculate the correlation between cotinine values in maternal urine and in meconium.

To discriminate snuff users from nonusers, optimal urine cotinine and meconium cotinine cut-off levels were determined using receiver operating characteristic curve analyses that maximize total probability of correct classification. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and 95% confidence intervals were computed by the exact method, using binomial distribution. Moreover, the likelihood ratio (LR) for a positive test was calculated as the sensitivity/(1-specificity) and the LR for a negative test was calculated as the specificity/(1-sensitivity).[162]

The agreement between self-reported dose and cotinine levels was analyzed with non-parametric Wilcoxon signed rank test and Kruskal-Wallis test.

Linear regression was used to analyze the associations between cotinine levels and birth outcomes.

All data in *paper I-IV* was analyzed using SAS version 9.2 (SAS Institute, Inc.; Cary, NC).

5.5 INFORMED CONSENT AND ETHICS

Papers I-III are population-based register studies and individual consent was not obtained from the participants. The participants gave informed consent on *paper IV*. The studies were all approved by the Research Ethics Committee at the Karolinska Institutet, Stockholm, Sweden (No 2005/223-31/2, 2006-835-32, 2011/895-32, 2011/1148-32, 02-405, 2009/1725-32).

6 RESULTS

6.1 MATERNAL CHARACTERISTICS

Maternal characteristics and tobacco cessation in MBR (papers I-III)

Our studies demonstrated a high level of correlation between maternal smoking and a low socioeconomic status and a low level of education. This was also seen, albeit less pronounced, among women using snuff in pregnancy. Moreover, snuff using women were to a greater extent teenagers, less educated and multiparous. A higher proportion of snuff using women were obese and not living with the father-to-be in comparison to that of nonusers. That snuff use is a Swedish habit was evident; as many as 94% of snuff users were born in the Nordic countries. Both maternal smoking and snuff use was associated with increased risk of cesarean section and preterm birth. Maternal smoking was also associated with increased risk of small for gestational age births, whereas the risk for snuff users was in comparison with that of nonusers.

Approximately 60% of snuff users and more than 50% of smokers stopped smoking before the antenatal booking. Tobacco cessation was most common among women giving birth to their first child. Among mothers who continued to use tobacco, a higher proportion were teenage mothers, multiparous and had less than eleven years of education in comparison to both nonusers and quitters. Among women who continued to smoke in pregnancy, 58% had less than eleven years of education. (*Paper 1 and II)*

Table 3. Tobacco use before pregnancy and in early pregnancy in the MBR, 1999-2009

Tobacco Use		**Before pregnancy**				
		Total No. (%)	**Nonuser (n)**	**Snuff user (n)**	**Smoker (n)**	**Missing (n)**
Early pregnancy	**Total No. (%)**	1,086,213 (100)	773,625 (71.2)	21,994 (2.0)	185,248 (17.2)	102,451 (9.4)
	Nonuser	917,900 (<4.5)	765,145	12,834	97,776	40,669
	Snuff user	11,461 (1.0)	1,237	8,859	732	36
	Smoker	92,092 (8.5)	953	74	85,144	5,626
	Missing	64,014 (5.9)	6,279	211	1,389	56,118

Table 4. Maternal and birth characteristics and tobacco use in MFR

Maternal and birth Characteristics	*paper I*, 1999-2006				*paper III*, 1999-2012			
	Nonuser N=503,460 **Rate %**	**Snuff user** N=7,599 **Rate %**	**Smoker 1-9 cig./day** N=41,391 **Rate %**	**Smoker ≥10cig./day** N=16,928 **Rate %**	**Nonuser** N=1,777,464 **Rate %**	**Snuff user** N=14,671 **Rate %**	**Smoker 1-9 cig./day** N=79,783 **Rate %**	**Smoker ≥10 cig./day** N=28,459 **Rate %**
Age								
≤ 19 years	1.1	1.9	6.4	3.6	1.4	2.0	6.2	3.8
Height					-	-	-	-
≤159 cm	8.6	11.3	11.8	12.4	-	-	-	-
BMI								
≥30	10.1	12.3	14.8	18.8	11.0	14.0	16.0	20.0
Multiparity *								
	4.2	7.3	8.0	18.5	18.4	22.6	23.1	39.2
Education								
≤9years	5.6	11.3	27.0	34.0	8.0	11.3	26.1	33.0
Not cohabitant with father-to-be								
	3.4	6.6	14.3	18.5	4.8	7.6	15.8	19.7
Mother's country of birth								
Nordic	-	-	-	-	80.1	94.0	82.4	85.3
Cesarean section								
Yes	15.4	18.2	16.5	16.6	-	-	-	-
Small for gestational age**								
Yes	2.2	2.4	4.7	5.8	-	-	-	-

*In *paper I* multiparity is defined as ≥4 children and in *paper III* multiparity is defined as ≥3 children, why figures are not comparable between papers.
**Small for gestational age (SGA) was defined as > 2 standard deviations below the mean birth weight for gestational age according to the sex specific Swedish fetal growth curve, Marsal.

Maternal characteristics and tobacco cessation in cohort (Paper IV)

Of the prospectively studied women, only 38 (14%) stopped using snuff during pregnancy, and the majority of these stopped between the first and second trimester. All quitters had stopped before 28th weeks of gestation. In agreement with the register studies mentioned above, women who ceased using tobacco during pregnancy were to greater extent <20 years and primiparous.

Few participants reported exposure to second hand smoke (SHS) in pregnancy. None of the nonusers reported daily exposure to SHS, whereas 9% reported exposure to SHS occasionally. Two nonusers reported continuous weekly, but not daily, exposure throughout pregnancy. Of the snuff users, 17% reported occasional exposure to SHS in early pregnancy and 24% in late pregnancy. Twenty-six women were exposed weekly throughout pregnancy and 10 were exposed daily.

Few participants used NRT in pregnancy, and none of the women who stopped using snuff in pregnancy were NRT users. Only 4 participants reported use of NRT in early pregnancy and 7 reported use in the second trimester, two of whom reported use also in the third trimester. All participants who reported use of NRT were also reporting simultaneous use of high doses of snuff.

6.2 NEONATAL APNEA

The most important risk factor for neonatal apnea *(Paper I)* was preterm birth, and the risk increased with decreasing gestational age. For very preterm infants (<32 gestational weeks), the adjusted OR was 139 (95% CI: 115-167). There was also a U-shaped association between parity and apnea, with increased risk of apnea in infants of primiparous and multiparous (≥4) women.

Snuff use in pregnancy was associated with a twofold increased risk of neonatal apnea, adjusted OR 2.2 (95% CI: 1.4-3.2), in comparison to that of nonusers. Maternal smoking was associated with lower risk than that associated with snuff use, but the risk increased with number of cigarettes smoked, adjusted OR 1.3 (95% CI: 1.0-1.7) and OR 1.5 (95 % CI: 1.1-2.1), for moderate and heavy smoking, respectively. However, the risk of neonatal apnea was no longer significant among infants of smoking mothers when adjusting for gestational age, SGA-births and cesarean section but was only slightly attenuated for snuff users.

Next, sleep apnea (ICD-10 P28.3) was analyzed separately. In infants of snuff users the adjusted OR was 2.5(1.4-4.4) and for moderate and heavy smokers adjusted OR was 1.2 (95% CI: 0.9-1.7) and 1.4 (95% CI: 0.9-2.1), respectively.

Table 5. Tobacco use and neonatal apnea UNS, **(**ICD-10 P28.2-28.4**),** n=931

Tobacco use	No.	Rate/ 1000	Odds ratio (95% Confidence Interval)		
			Crude	Adjusted model 1*	Adjusted model 2**
Nonuser	771	1.5	reference	reference	reference
Snuff user	26	3.4	2.2 (1.5-3.3)	2.2 (1.4-3.2)	2.0 (1.3-3.0)
Smoker					
1-9 cig./day	94	2.3	1.5 (1.2-1.8)	1.3 (1.0-1.7)	1.1 (0.9-1.4)
≥10 cig./day	40	2.4	1.6 (1.1-2.1)	1.5 (1.1-2.1)	1.1 (0.8-1.5)

**Adjusted model 1: adjustment for maternal characteristics, such as maternal age, height, parity, education, cohabitant with father-to-be **Adjusted model 2: adjustment for maternal characteristics as in model 1 and birth characteristics, such as cesarean section, SGA-births and gestational age.*

Table 6. Tobacco use and primary sleep apnea, (ICD 10 P28.3), n=416

Tobacco use	No.	Rate /1000	Odds ratios (95% Confidence Intervals)		
			Crude	Adjusted model 1*	Adjusted model 2**
Nonuser	320	0.6	reference	reference	reference
Snuff user	14	1.8	2.9 (1.7-5.0)	2.8 (1.7-4.9)	2.5 (1.4-4.4)
Smoker					
1-9 cig./day	56	1.4	2.1 (1.6-2.8)	1.7 (1.2-2.3)	1.2 (0.9-1.7)
≥10 cig./day	26	1.5	2.4 (1.6-3.6)	2.2 (1.5-3.4)	1.4 (0.9-2.1)

**Adjusted model 1: adjustment for maternal characteristics, such as maternal age, height, parity, education, cohabitant with father to be **Adjusted model 2: adjustment for maternal characteristics as in model 1 and birth characteristics as cesarean section, SGA-births and gestational age.*

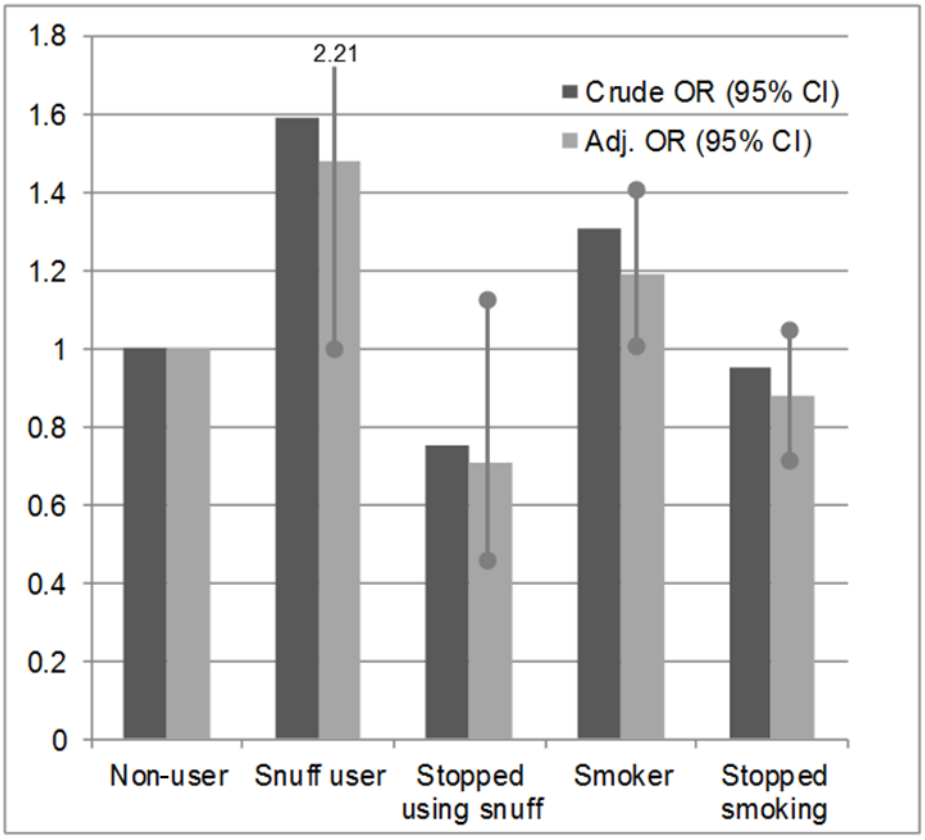
6.3 ORAL CLEFT MALFORMATION

The incidence of oral cleft malformations *(paper II)* was 1.8/1000 births. Maternal age ≥ 35 years, chronic hypertension, preeclampsia, male sex, multiple births and Nordic country of birth were all associated with an increased risk of oral cleft malformations.

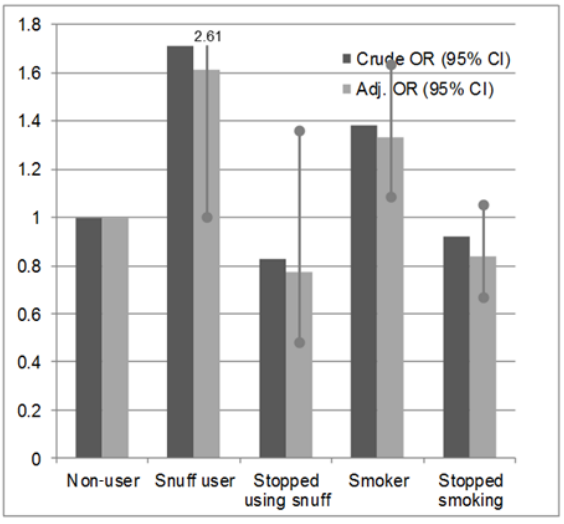
Both maternal snuff use and smoking were associated with an increased risk of oral cleft malformations, but varied between subtypes. Whereas risks of cleft lip with or without cleft palate were increased following maternal snuff use or smoking, no significant association was seen for isolated cleft palate. Infants of mothers who stopped using tobacco (snuff or cigarettes) before the antenatal booking were not at an increased risk of oral cleft malformations.

Figure 22. Maternal tobacco use in early pregnancy and risk of (a) oral cleft malformations, (b) cleft lip with or without cleft palate, (c) isolated cleft palate (CP). Odds ratios presented with 95% confidence interval

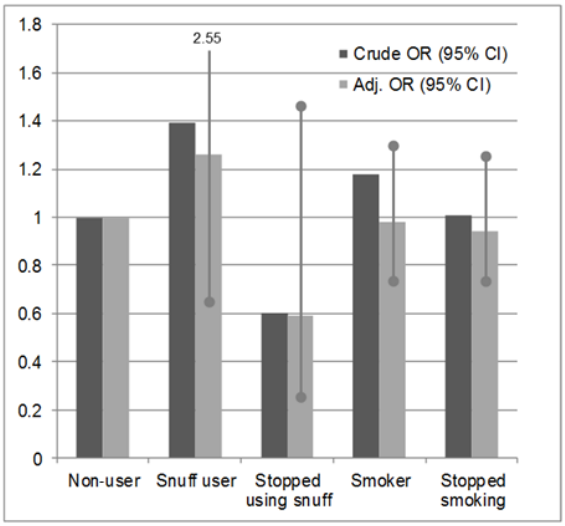
a) Oral cleft malformations



(b) Cleft lip with or without cleft palate



(c) Isolated cleft palate (CP)



6.4 EXTREMELY PRETERM BIRTH

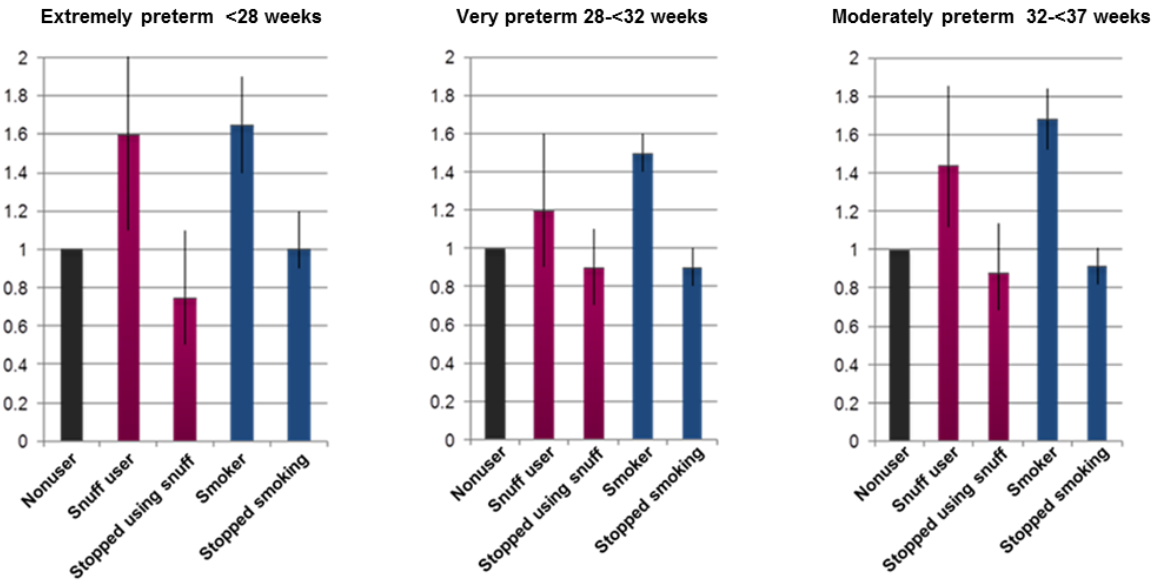
In the study population, 4.94% were born preterm (<37weeks), 4.26% of whom were moderately preterm, 0.45% very preterm (<32 weeks) and 0.23% extremely preterm (<28weeks). *(Paper III)*

Maternal age ≥ 35 years of age, primipara <12 years of education, BMI ≥30 and non-Nordic country of birth of the mother were all factors associated with an increased risk of preterm birth in the adjusted analysis.

Maternal smoking in early pregnancy was associated with all categories of preterm birth. The risk increased with the number of cigarettes smoked and with decreasing gestational age. An increased risk was seen both of spontaneous and medically induced preterm birth and across all gestational age strata. Previous smokers who had stopped smoking before the antenatal booking were not at an increased risk of preterm birth.

Maternal snuff use was associated with increased risk of extremely and moderately preterm birth, whereas the risk of very preterm birth was of borderline significance. Women using snuff three months before pregnancy but who had ceased using it at the antenatal booking were not at an increased risk. Snuff use was associated with medically induced extremely preterm birth and both spontaneous and medically indicated moderately preterm birth.

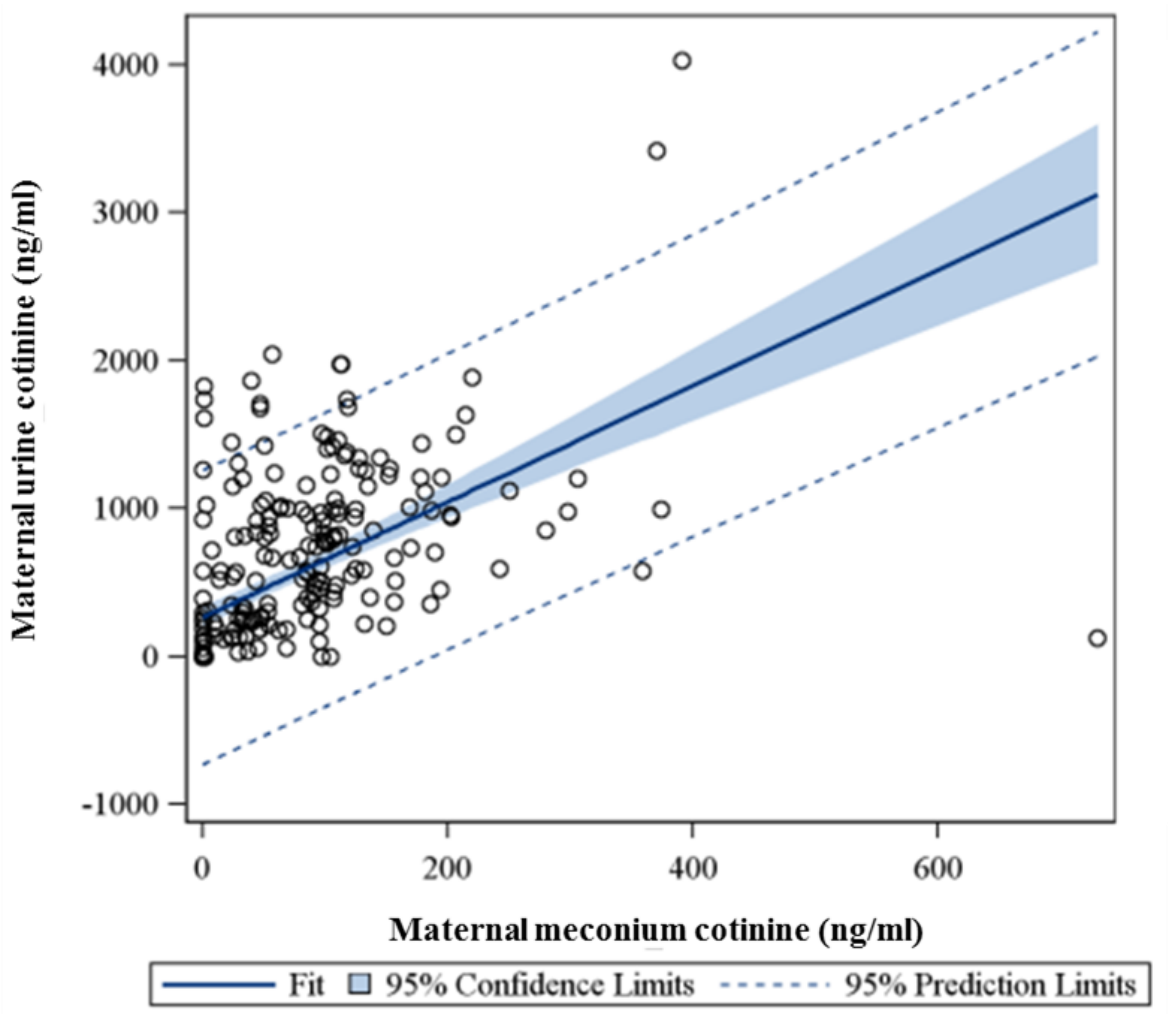
Figure 23. Maternal tobacco use in early pregnancy and risk of preterm birth. Odds ratios presented with 95% confidence interval.



6.5 ACCURACY OF SELF-REPORTED SNUFF USE

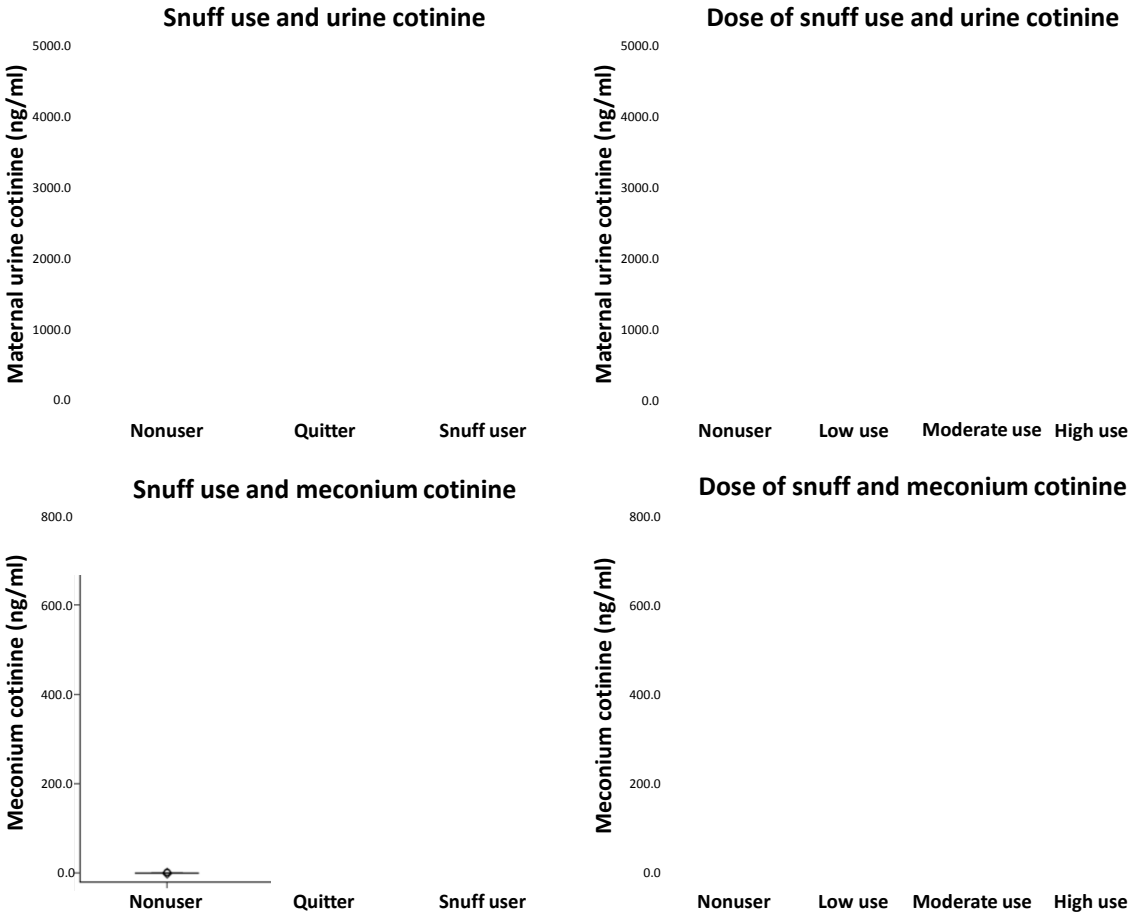
Self-reported snuff use in pregnancy was found to be valid (*paper IV*). There was a high agreement between self-reported use through questionnaires and maternal urine cotinine concentrations in late pregnancy. There was also a high agreement between self-reported snuff use in late pregnancy and cotinine in the meconium of the newborn. Furthermore, there was a correlation between maternal urine cotinine and cotinine in the meconium of the newborn, the correlation coefficient being 0.77 ($p<0.001$).

Figure 24. Correlation between the concentration of cotinine in maternal urine and in the meconium of the newborn.



There were also significant differences between low, moderate and high self-reported doses of nicotine and corresponding median cotinine levels in urine and meconium.

Figure. 25 Snuff use and cotinine levels in maternal urine and meconium



Misclassification in the Medical Birth Register

The information in the Medical Birth Register on snuff use in late pregnancy showed a high rate of misclassification. In the cohort, less than 15% stopped using snuff between the antenatal booking and week 32. However, according to the MBR register data, as many as 44% of the (same) cohort population were registered as nonusers in late pregnancy.

When validating the register data with cotinine levels in maternal urine, the sensitivity was only 50% (95% CI: 43-57) and the specificity was 98% (95% CI: 94-100). In late pregnancy, 45.5% of the MBR registered nonusers (i.e. registered non-users both in early and late pregnancy and registered quitters) had cotinine levels above cut-off level in maternal urine. As many as 77 % of participants who were registered as having ceased using snuff, i.e. registered as quitters, had maternal cotinine values above the cut-off level ≥15ng/ml.

Cotinine levels and neonatal growth outcomes

Of 381 participants in the study cohort, 15 (3.9%) women gave birth prematurely. Among snuff users, 11 (4.2%) infants were born preterm, and among nonusers 4 (3.4%) infants were born preterm. Among snuff users, two births were extremely preterm and two were very preterm, but they were all excluded because of missing information on cotinine as a consequence of the preterm birth. With them included, 4.9% were born preterm in the cohort, and the proportion of preterm birth among snuff users was 5.6%.

There was a significantly reduced gestational age of 3.3 days ($p<0.01$) for infants of mothers with cotinine levels in urine above cut- off, as compared to infants of mothers with cotinine levels below cut- off (Table 7a).

Table 7a. Urine cotinine levels and neonatal outcomes in paper IV, n= 307

Tobacco use	Crude			Adjusted*		Adjusted**	
	(95%CI)	mean difference	*p*	mean difference	*p*	mean difference	*p*
Gestational age							
Nonuser							
<3ng/ml	282(280-284)	ref		ref		-	-
Snuff user							
≥3ng/ml	278(277-280)	-4.13	<0.001	-3.32	<0.01	-	-
Birth weight							
Nonuser							
<3ng/ml	3,653(3,554-3,750)	ref		ref		ref	
Snuff user							
≥3ng/ml	3,588(3,513-3,662)	-66.1.	0.29	-99.2	0.11	-19.4	0.72
Length at birth							
Nonuser							
<3ng/ml	50.9(50.5-51.3)	ref		ref		Ref	
Snuff user							
≥3ng/ml	50.5(50.2-50.8)	-0.39	0.11	-0.44	0.08	-0.02	0.91
Head circumference							
Nonuser							
<3ng/ml	35.3(35.1-35.6)	ref		ref		ref	
Snuff user							
≥3ng/ml	35.2(35.0-35.4)	-0.06	0.67	-0.10	0.57	-0.11	0.49

Crude means calculated only for individuals with information on maternal characteristics and urine cotinine. Only participants who were categorized as nonusers or snuff users by both urine cotinine levels and self-reported information through questionnaires were included. Only singleton births were included.
**Adjusted for maternal characteristics, such as maternal age, bmi, co-habitant with father-to-be and parity.*
***Adjusted for maternal characteristics and gestational age.*

Infants with cotinine above cut-off level in their meconium also had a significantly reduced lower mean adjusted gestational age (-2.6 days, p<0.05) than that of infants with cotinine levels below cut-off in their meconium (Table 7b).

Table 7b. Meconium cotinine levels and neonatal outcomes, in *paper IV*, n= 283

Tobacco use	Crude			Adjusted*		Adjusted**	
	(95%CI)	mean difference	*P*	mean difference	*p*	mean difference	*p*
Gestational age							
Nonuser <3ng/ml	281.(280-284)	ref		ref		-	-
Snuff user ≥3ng/ml	278(277-280)	-3.61	<0.01	-2.63	<0.05	-	-
Birth weight							
Nonuser <3ng/ml	3650(3553-3748)	ref		ref		ref	
Snuff user ≥3ng/ml	3585(3502-3667)	-65.4	0.32	-98.8	0.12	-32.1	0.56
Length at birth							
Nonuser <3ng/ml	51.0(50.6-51.4)	ref		ref		ref	
Snuff user ≥3ng/ml	50.5(50.2-50.8)	-0.46	0.07	-0.47	0.07	-0.19	0.40
Head circumference							
Nonuser <3ng/ml	35.3(35.1-35.6)	ref		ref		ref	
Snuff user ≥3ng/ml	35.3(35.1-35.5)	-0.37	0.85	-0.05	0.77	-0.07	0.67

*Crude means calculated only for individuals with information on maternal characteristics and cotinine in meconium. Only participants who were categorized as nonusers or snuff users by both meconium cotinine levels and self-reported information through questionnaires were included. Only singleton births were included.*Adjusted for maternal characteristics, such as maternal age, bmi, co-habitant with father-to-be and parity.**Adjusted for maternal characteristics and gestational age.*

There were no significant differences in mean birth weight, birth length or head circumference adjusted for gestational age between newborns of women with maternal urine cotinine levels over and below cut-off , nor were there differences between newborn meconium cotinine levels over and below cut-off (cotinine level ≥ 3ng/ml) (Table 7 a and b).

7 DISCUSSION

"If younger adults turn away from smoking, the industry will decline, just as a population which does not give birth will eventually dwindle." RJ Reynolds researcher, 1984

The focus of this thesis is prenatal nicotine exposure in the form of oral moist tobacco, snuff and cigarette smoking and effects on the health of the newborn.

The rationale for the included studies was to study the differences between the effects of smoking and snuff on the health of the newborn. The adverse effects of smoking in pregnancy have been thoroughly investigated,[1, 45] and, likewise, animal studies have shown that prenatal nicotine is hazardous to the developing fetus.[83, 163] However, whether maternal tobacco use in the form of snuff is safe during pregnancy had not been elucidated.

Neonatal apnea

Maternal snuff use was associated with a twofold risk of neonatal apnea. This is the first study of snuff use and neonatal apnea, but previous studies on maternal smoking have found an association with neonatal apnea.[98] The risk of sleep apnea was increased almost threefold in infants of snuff users in comparison to that of nonusers.

The risk of apnea associated with maternal smoking was explained by the increased risk of preterm birth among smokers. In contrast, in infants of snuff users the risk was not only higher than in that of smokers, it also remained significant after adjustment for gestational age.

Neonatal apnea is a sign of disturbances in the cardiorespiratory system and is suggested to be associated with an increased risk of SIDS. Preterm birth is a strong risk factor for neonatal apnea, and the risk increases with decreasing gestational age.[164] Prematurity and maternal smoking are regarded as the most important risk factors for SIDS,[165] particularly since the back to sleep campaign was introduced.[93]

SIDS is a rare event, less than 0.2/1000 live born infants yearly in Sweden,[166] and the prevalence of snuff use in pregnancy is 1.1%. The association between snuff use and SIDS has not been possible to study because of lack of power.

SIDS is suggested to be related to a disturbed autonomous control with decreased arousal. In addition, a blunted response to hypoxia with a disturbed pattern of breathing, apnea and bradycardia is associated with the mechanisms behind SIDS.[94, 95, 165, 167]

Preterm infants and infants prenatally exposed to smoking show similar disturbances in the neural control of the cardiorespiratory system, with vascular, cardiac and blood pressure hyperactivity.[168] These similar findings thus represent an effect of immaturity in the premature infant and a developmental programming effect in the nicotine-exposed infant. As nAChRs are present as early as gestational week 4-5 in fetal development,[137] nicotine may carry out teratogenic effects by binding these receptors, induce apoptosis and affect cell programming.[104]

Nordenstam et al. found decreased heart rate variability among infants of smokers and snuff users compared to that of nonusers, also implying disturbances in autonomic control. A decreased heart rate variability was found also in infants of snuff users and smokers who had ceased using tobacco during pregnancy,[169] indicating early programming effects of nicotine on the development of the nervous system.

Furthermore, animal studies of prenatal nicotine exposure have shown similar disturbances in the cardiorespiratory system as those observed in infants of maternal tobacco use. Findings in animal studies of prenatal nicotine exposure include abnormalities in the sympathovagal balance, with an increased frequency of apneas and a blunted cardiorespiratory response to hypoxia. Changes in breathing patterns, such as a higher breathing frequency and a lower tidal volume in the neonatal, have also been observed.[102, 103, 151, 153] In addition, prenatal exposure to nicotine leads to a decreased arousal and ability to resuscitate, as well as to an impaired catecholamine release from the adrenal medulla, followed by a reduced cardiac response to adrenergic stimulation in rat pups.[104]

As mentioned, the plasma concentration of nicotine remains at a high level for hours after snuff use, in contrast to the rapid decline after smoking.[136] Nicotine also accumulates in the amniotic fluid[80] and the dose of nicotine to which the fetus is exposed may therefore be higher in infants of snuff users. This may be an underlying explanation for the higher risk of neonatal apneas in infants of snuff users than in infants of smokers.

Smoking during pregnancy is associated with both increased risk of preterm birth and SIDS.[97, 165] The increased risk of SIDS in smokers may therefore be an effect of both an immature nervous system caused by preterm birth and the toxic effect of nicotine on the autonomous nervous system.

Tobacco smoke and nicotine also affect the immune system,[81] which may influence both the susceptibility to infections and the subsequent inflammatory response.[170] Both preterm infants and infants of smokers are more prone to get respiratory infections,[121, 122 171] causing a mild hypoxia. Although such infections in most cases are benign, they may induce apnea and bradycardia. It is possible that this may cause a serious and even fatal event in the more vulnerable nicotine exposed infant. [170]

Oral cleft malformations

The association between snuff use in pregnancy and oral cleft malformations has not been studied previously. However, the finding that maternal smoking was associated with a moderately increased risk of oral cleft malformations is in agreement with previous studies on maternal smoking.[106, 172, 173] The Surgeon General states in their report from 2014 that there is sufficient evidence to conclude that a causal relationship between maternal smoking and oral cleft malformations exists.[2]

In the present study, smoking and snuff use in pregnancy were associated with increased risk of CL/P, but in contrast to previous studies on smoking in the MBR there was no increased risk of isolated CP.[172, 174] Previous literature is not consistent as to whether smoking increases the risk of both CL/P and CP. Most previous studies have found a relationship between smoking and CL/P,[120, 175-177] and others between both CL/P and isolated CP.[114, 178] In a meta-analysis Little et al. found a 34% risk increase of CL/P and a 22% increased risk of CP associated with maternal smoking.[106]

The lack of an association between tobacco use and isolated CP in *paper II* may be due to lack of power. Smoking in pregnancy and exposure to second hand smoke has decreased considerably in Sweden since the end of the 90's when the previous studies in the MBR were performed.[6] Furthermore, there is evidence of a dose-dependent association with oral cleft malformations.[178] It is possible that the different results between the present study and previous studies in the MBR may be that the dose of nicotine required to cause damage is higher for isolated CP than for CL/P.

The finding that both snuff use and smoking in pregnancy increase risk of oral cleft malformations implies that nicotine is involved in the mechanism. The risk was higher for snuff users than for smokers, which further strengthens this hypothesis. The dose of nicotine to which the fetus is exposed is likely to be higher in infants of snuff users than it is in infants of moderate, and possibly also heavy, smokers. Findings in vitro and from animal studies have shown disturbances in the fusion of the facial prominences, which further supports the theory that nicotine is involved in the mechanism.[115, 116, 179, 180]

Extremely preterm birth

Extremely preterm birth is rare, with an incidence of 0.3%-0.4% yearly in Sweden.[6] However, morbidity and mortality of preterm birth is increasing with decreasing gestational age, and extremely preterm birth is the major cause of neonatal death in most developed countries.[74] Both snuff use and smoking have previously been associated with preterm birth.[62] However, the association with extremely preterm birth (<28 gestational weeks) has not been studied previously in snuff users.

Maternal snuff use was associated with increased risk of extremely preterm birth and moderately preterm birth, but the risk of very preterm birth was only borderline significant. Maternal smoking was associated with increased risk of preterm birth in all gestational ages.

The risk increase was higher for smokers than for snuff users. The association with preterm birth and smoking was dose-dependent across all gestational ages.

In addition, smoking in pregnancy was associated with both spontaneous and medically indicated preterm birth in all gestational ages. Maternal snuff use was associated with a twofold increased risk of medically indicated extremely preterm birth and a moderately increased risk of both spontaneous and medically indicated moderate preterm birth.

The risk of preterm birth was higher for smokers than for snuff users but also dose dependent, which implies that both combustion products and nicotine are involved in the mechanism underlying preterm birth. It is possible that combustion products and nicotine have synergistic effects, leading to a high risk of preterm birth in smokers.

Several mechanisms through which smoking may contribute to preterm delivery have been suggested and include dysfunctional placentation, leading to placental abruption[58, 59, 72] and defect collagen synthesis, leading to premature rupture of the membranes.[3] Furthermore, smoking increases the risk of fetal growth restriction,[1, 45, 83] possibly via placental dysfunction, which is an important indication for medically indicated preterm birth.

Snuff use has not been shown to be associated with placenta previa or placental abruption. [60] However, there is a lack of studies exploring the associations between snuff use and placenta previa and placental abruption. The association between snuff use and risk of fetal growth is modest,[63] implying that although nicotine affects fetal growth, the fetal growth restriction caused by smoking is also due to placental dysfunction caused by combustion products.

Another important indication for medically indicated preterm birth is pre-eclampsia. Maternal smoking protects against preeclampsia, supposedly through the vasodilative properties of CO.[68, 71] In contrast, snuff use in pregnancy does not protect against preeclampsia.[70, 71] Maternal snuff use is associated with preterm but not term preeclampsia,[70, 71] suggesting that nicotine is involved in the early stages of the disease.[71] Furthermore, nAChRs are expressed in the preeclamptic placenta in nonsmokers, implying that nicotine is involved in the development of pre-eclampsia.[181] However, it was not possible to explore the association between preeclampsia and extremely preterm birth in snuff users *in paper III*, owing to lack of power.

Human labor is supposed to begin with functional progesterone withdrawal leading to decidual activation.[77, 182] At term, decidual activation seems to be mediated by the fetal-decidual paracrine system. In many cases of early preterm labor, decidual activation rather seems related to intrauterine bleeding or an intrauterine infection.[77] Smoking also affects the immune system, increasing inflammation and the susceptibility to infections.[81] It is thus possible that nicotine may influence preterm birth by an effect on the immune system,[81, 137] but also through the effect on the endocrine system inhibiting progesterone release.[183]

In conclusion, there is evidence suggesting that both nicotine and combustion products are responsible for increased risk of preterm birth, but the underlying mechanisms remain largely unexplained. Importantly, mothers who had stopped using tobacco (smoking or snuff use) very early in pregnancy, before the antenatal booking, had no increased risk of giving birth preterm.

Validation of self-reported snuff use

Most epidemiological studies on the effects of maternal smoking are based on self-reported information on tobacco use. The information in the MBR on maternal tobacco use is also based on self-reported information, with the advantage of prospectively collected data minimizing risk of recall bias. However, maternal smoking, and to some extent also snuff use in pregnancy, is a social stigma. With increasing knowledge of the hazardous effects of tobacco use in pregnancy there is a risk of underreporting use, which in epidemiological studies increase risk of misclassification, leading to both under- and overestimation of risks.[48]

Validation studies using cotinine as the golden standard have shown conflicting results. Most of them have concluded that self-reported use is valid,[184-187] but some have found a considerable proportion of underreporting of tobacco use.[47, 188] George et al. showed that self-reported maternal smoking is valid in Sweden.[184]

Most validity studies are based on women participating in a study cohort. Taking part in research may influence women to answer more truthfully than they would otherwise. Therefore, women willing to participate in research may not be representative of the general population smoking in pregnancy. Mattson et al. conducted a study on maternal smoking in the MBR, using cotinine in serum samples from the umbilical cord at delivery as well as maternal venous samples in early labor, showing that maternal self-reported smoking was valid in the MBR.[185] A methodological problem with the study was that self-reported smoking in early pregnancy at the antenatal booking was validated with cotinine in serum at delivery. Women who changed their smoking habits in pregnancy or ceased smoking were not taken into account.

Self-reported maternal snuff use has not previously been validated. Moreover, there is no information in the MBR on dose of snuff use in pregnancy, whereas smoking is categorized as moderate (1-9 cigarettes/day) and heavy (≥10 cigarettes/day) smoking.

Maternal self-reported snuff use in late pregnancy proved valid in our cohort. Interestingly, only14% reported cessation of tobacco use during pregnancy. According to the MBR data on the same individuals, 45% stopped using tobacco during pregnancy. Likewise, when validating the MBR information in late pregnancy with cotinine in maternal urine, the sensitivity was as low as 50%, whereas specificity was high, 98%. Of the registered quitters in the MBR in late pregnancy,77% had cotinine levels over the cut-off.

Surprisingly, none of the women who ceased using snuff in pregnancy used NRT. In addition, the few women actually using NRT were reporting high use of snuff simultaneously. This supports previous results of NRT not being effective for tobacco cessation in pregnancy.[135]

A possible explanation for the misclassification in late pregnancy in the MBR is that health care personnel fail to ask about snuff use, especially in late pregnancy. England et al. concluded that a large proportion of obstetrician/gynecologists never or inconsistently screen patients for smokeless tobacco products in the U.S. There was also lack of knowledge of potential adverse effects in pregnancy.[189] Another possible explanation may be difficulties in filling in the register form correctly.

Methodological strengths and limitations

Papers 1-III are all population-based cohort studies based on data from the Medical Birth Register. Self-reported tobacco use is prospectively collected, minimizing the risk of recall bias. Maternal socioeconomic characteristics are possible to obtain through linkage to the Education Register and the Register of Total Population.

However, register studies have a number of limitations. There is always a risk of differential misclassification of tobacco use with self-reported use not confirmed by biomarkers. Smoking in early pregnancy in the Medical Birth Register has been validated and proved reliable, but snuff use has not been validated previously. Register studies do not reflect patterns of tobacco use or dose of tobacco. Snuff use is merely categorized as yes/no. Use of NRT or electronic cigarettes are not registered, there is no information on the tobacco habits of the father-to-be and no information on exposure to second hand smoking.

There is also a problem of unmeasured confounding or residual confounding in register studies. Possible confounders, such as maternal substance use, alcohol consumption in pregnancy, maternal vitamin substitution and medications, and other life-style factors are not possible to obtain from the register. Smoking was associated with low socioeconomic status and low level of education. Snuff use was not to the same extent associated with low socioeconomic status and low education as was the case with smoking, a finding consistent with a study by Kvalvik et al. describing female smokers and snuff users in Norway.[186]

Paper IV is a cohort study with prospectively collected self-reported data where pattern of use and the dose of snuff use may be studied. Information on NRT and second-hand smoking was also possible to obtain. In addition, the self-reported tobacco use in late pregnancy was validated by cotinine in maternal urine and cotinine in the meconium of the newborn. The cohort was linked to the MBR register data, and validation of the MBR data in late pregnancy for snuff use was also possible. By comparing the cohort with a regional cohort in the MBR the same years, it was possible to conclude that the cohort was representative of the population, with the exception of the proportion of snuff users, smokers and nonusers.

However, there are limitations in the study design. The women included in the study were informed that self-reported snuff use would be confirmed by cotinine, which may influence the accuracy of the self-reported use There is also a risk of selection bias, i.e. women willing to participate in a validation study on snuff use may differ from the general population on basis of exposure. Another limitation is the categorization of snuff use. The high-use cut-off was defined as more than 7 pouches a day. This turned out to be too low, as the range of pouches used daily in the high-use category was very wide, 7- > 20 pouches a day. Therefore, a dose dependency may have been blunted.

Moreover, some possible confounding factors, such as consumption of alcohol, medication and use of vitamins were not obtained through the questionnaires. Information on tobacco habits of the father-to-be was not obtained either.

An important limitation is that the accuracy of self-reported snuff use in early pregnancy was not possible to obtain, because maternal urine cotinine was not analyzed in early pregnancy. Most register studies on maternal snuff use and smoking are based on self-reported use in early pregnancy, why validation in early pregnancy would have been very useful.

Conclusion and further perspectives

Nicotine is highly addictive, and smoking cessation in pregnancy has been found very difficult to achieve. Neither psychosocial interventions, nor NRT have proved successful.[2, 11, 132, 135] Use of nicotine is generally regarded as safer than smoking, and as a consequence snuff and new nicotine products, such as electronic cigarettes, are marketed as a safer alternative than smoking.[13, 34, 129] The adverse effects of nicotine on pregnancy and the developing fetus are often neglected in discussions of e-cigarettes or snuff as a means of smoke cessation and harm reduction.[32, 34, 129]

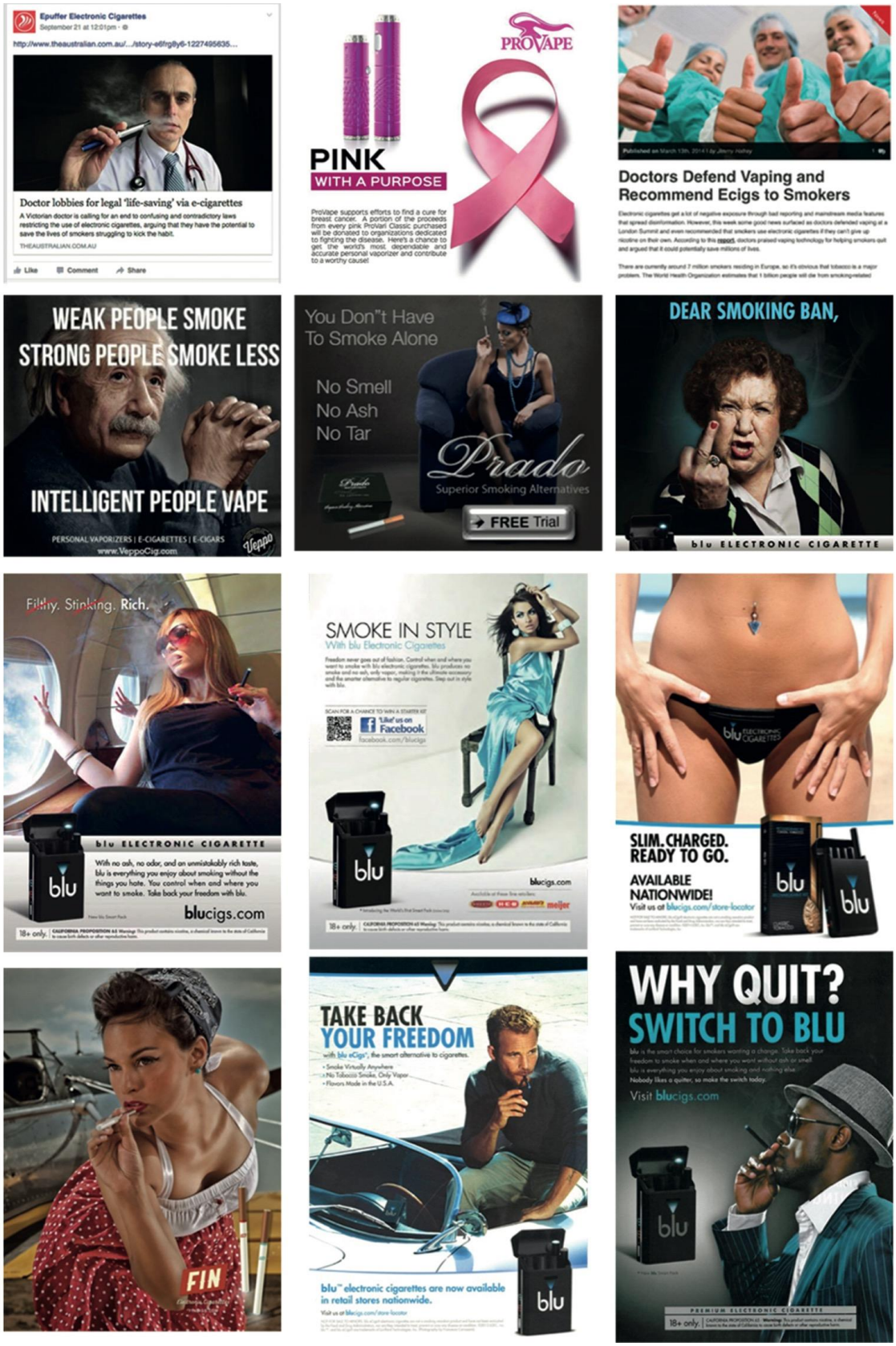
Because of population growth, the number of smokers is actually growing in the world despite decreasing prevalence in many countries. In some developing countries, smoking actually increases.[20] In developed countries a whole new market of nicotine and non-cigarette tobacco products, such as electronic cigarettes, has evolved, attracting young people in particular.[190] Among young people, aged 18 to 24, e-cigarettes are now more popular than conventional cigarettes.[191] E-cigarette use is strongly associated with the use of other tobacco products.[191] The use of new tobacco products and dual use of cigarettes and other nicotine sources place high demands on society and the health care professionals regarding preventive strategies. England et al. have shown that obstetricians seldom or never screen for nicotine products other than tobacco cigarettes and that the knowledge of adverse effects in pregnancy is limited.[189]

To conclude, snuff use in pregnancy has adverse effect on pregnancy and the health of the newborn. The mechanisms behind the harmful effects of smoking are not clear and for some conditions, such as fetal growth and possibly also preterm birth, both nicotine and combustion products seem to be involved. The risk increase of fetal growth restriction and preterm birth is higher for smokers than for snuff users, and there is evidence of combustion products affecting placental function.[2, 3] In contrast, the risks of adverse effects supposedly caused by nicotine, such as neonatal apnea and oral cleft malformation, seem to be higher in snuff users than in smokers.

Of those who cease smoking in pregnancy, the majority stop without counselling or use of NRT before the first visit to the prenatal clinic.[55, 133] However, among those who smoke at the antenatal booking, the majority continue to smoke throughout pregnancy. Pharmacological and psychological therapies have not proved effective for smoke cessation in pregnancy.[2, 132] Strategies for snuff cessation in pregnancy are lacking. In addition, because of lack of adherence to NRT, safety has been difficult to study.[135]

With growing knowledge about the detrimental effects of snuff, containing merely nicotine, caution is recommended regarding use of NRT and e-cigarettes in pregnancy. No safe level of prenatal nicotine exposure has been established.[192] Nicotine, in any form, is not to be considered safe and should be avoided in pregnancy.

Figure 26. Advertising for electronic cigarettes



Source: ***From the collection of Stanford Research Into the Impact of Tobacco Advertising (tobacco.stanford.edu).***

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10 APPENDICES

A. Questionnaires used in paper IV

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ORIGINAL ARTICLE

Snus and risk of gastroesophageal reflux. A population-based case-control study: the HUNT study

Tina Malene Lie[a], Maria Bomme[a], Kristian Hveem[b], Jane Møller Hansen[a] and Eivind Ness-Jensen[b,c,d]

[a]Department of Medical Gastroenterology S, Odense University Hospital, Odense C, Denmark; [b]HUNT Research Center, Department of Public Health and General Practice, NTNU, Norwegian University of Science and Technology, Levanger, Norway; [c]Upper Gastrointestinal Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; [d]Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway

ABSTRACT

Objective: Tobacco smoking is a risk factor for gastroesophageal reflux, but whether other tobacco products increase the risk is unclear. The aim of this study was to investigate if snus increases the risk of gastroesophageal reflux symptoms (GERS).

Material and methods: The study was based on the third Nord-Trøndelag health study (HUNT3), a population-based study of all adult residents in Nord-Trøndelag County, Norway, performed in 2006–2009. The association between self-reported severe heartburn/regurgitation and snus use was assessed by logistic regression.

Results: Compared to never snus users, daily snus users had a reduced risk of GERS (OR 0.77, 95% confidence interval [CI] 0.64–0.93), while previous snus users and those using <2 boxes of snus/month had an increased risk (OR 1.20, 95% CI 1.00–1.46 and OR 1.41, 95% CI 1.02–1.96, respectively). There was no association between age when starting using snus and GERS. Snus users who started using snus to quit or cut down on cigarette smoking, who started using both snus and cigarettes or cigarettes alone had an increased risk of GERS. Snus users <30 years of age had an increased risk of GERS (OR 1.49, 95% CI 1.02–2.16), while those aged between 50–60 and 60–70 years had a reduced risk (OR 0.67, 95% CI 0.49–0.93 and OR 0.51, 95% CI 0.28–0.94, respectively).

Conclusions: Daily snus users had a reduced risk of GERS. However, previous snus users and subgroups of snus users had an increased risk of GERS indicating reverse causality, such that snus use could increase the risk of GERS.

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Introduction

Snus is a form of smokeless tobacco that has been used for decades in the Scandinavian countries. It is consumed by placing a portion under the upper lip in front of the gingiva and is increasingly popular amongst young people.[1] The snus market is rapidly spreading worldwide and snus was recently launched in the US.[2,3] Previous studies have estimated that around 22% of men and 4% of women are using snus, and the prevalence of women using snus is increasing.[2,4,5]

The exposure time of snus is higher than for smoking; with an average duration time of 60–70 minutes per portion, giving twice as large the amount of nicotine.[6,7] It consists of tobacco, water, sodium chloride, sodium carbonate, humectants, and flavoring agents, and large amounts of these contents are swallowed into the digestive tract.[1,8,9]

Gastroesophageal reflux disease (GERD) is caused by abnormal backflow of gastric contents into the esophagus, producing symptoms and mucosal damage.[10,11] The main symptoms of GERD are heartburn and regurgitation, and the prevalence of GERD in Western adult populations is up to 30%.[12,13] GERD is associated with reduced health related quality of life [14] and work productivity,[15] and an increased risk of esophageal adenocarcinoma.[16] Genetic predispositions and lifestyle factors, including obesity and tobacco smoking, increase the risk of GERD.[17] Due to the existing evidence of tobacco smoking as a causal factor for GERD, snus has been proposed as a similar risk factor for the development of GERD.[17–20] A study by Aro et al. found an association between snus use and histological changes in the esophagus associated with GERD (basal cell hyperplasia and elongation of papillae).[21]

In general, the documentation of the health effects of snus is sparse, and the continuous product development of snus and changes in its ingredients makes interpretation of old research troublesome.[9]

The aim of this study was to investigate if snus is a risk factor for gastroesophageal reflux symptoms (GERS), using a large population-based study from Norway.

CONTACT Eivind Ness Jensen eivind.ness jensen@ki.se Upper Gastrointestinal Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, 171 76 Stockholm, Sweden

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Methods

Material

The Nord-Trøndelag health study (HUNT) is an on-going population based cohort study. Nord-Trøndelag is a county in the central part of Norway with about 135,000 inhabitants (in 2014). All residents above 20 years of age were asked to participate in three health surveys; HUNT1 (1984–1986), HUNT2 (1995–1997), and HUNT3 (2006–2008).[22] In HUNT1, 77,212 individuals (89% of the total population) participated and the corresponding numbers were 65,237 (70%) and 50,807 (54%) in HUNT2 and HUNT3, respectively. This makes HUNT the largest population based study in Norway, and also one of the largest internationally. Data on health-related items were gathered through self-reported questionnaires, clinical examinations, and blood samples of the participants.

As snus use was assessed in HUNT3 only, the present study is based on HUNT3. In addition, a short questionnaire for non-participants (the QNP) sent out in 2009 to those who did not participate in HUNT3 was also included ($n = 7591$).

Assessment of snus use

In HUNT3 and the QNP, the participants were asked, 'Do you use, or have you previously used snus?' with the possible answers 'no, never', 'yes, but I quit', 'yes, occasionally', and 'yes, daily'.

For those 'ever' using snus, HUNT3 included four additional questions regarding snus. The participants were asked about their consumption of snus per month, at what age they started using snus, their motivation to start using snus, and what type of tobacco they started with. These additional questions were not included in the QNP.

Assessment of GERS

GERS was assessed with the question 'To what degree have you had acid regurgitation or heartburn during the last 12 months' with the alternatives 'never', 'a little', or 'much' complaint. Previous validations of this question found that 95 – 98% of those reporting much complaint had symptoms at least weekly, thereby fulfilling the Montreal criteria for GERD.[11,23,24]

Those reporting much complaint were defined as the case group ('Severe GERS'), while those reporting never complaint were defined as controls ('No GERS'). Those reporting a little complaint were excluded from our analysis.

Assessment of co-variables

In the present study age, sex, tobacco smoking, body mass index (BMI), and physical exercise were taken into consideration as potential confounders.

The participants were asked, 'Do you smoke?' with the alternatives: 'no, I have never smoked', 'no, I quit smoking', 'yes, occasionally', or 'yes, daily'.

Trained personnel measured the weight and height of the participants in HUNT3, while weight and height were self-reported in the QNP. BMI was calculated as weight (kg)/height $(m)^2$ and categorized into normal weight (<25.0), overweight (25.0 – 29.9), and obese (≥30.0) according to the World Health Organization's classification.

Physical exercise was assessed by the question 'How often do you exercise?' with the options 'less than once a week', 'once a week', '2–3 times a week', or 'nearly every day', and further categorized into ≥ or < weekly, based on the self-reported frequency.

Statistical analysis

The risk of GERS among snus users compared to not snus users in HUNT3/QNP was estimated using multivariable logistic regression, reporting odds ratios (ORs) with 95% confidence intervals (CIs). We present crude ORs, age and sex adjusted ORs, and fully adjusted ORs, including adjustments for age, sex, smoking status, BMI, and physical exercise. The additional questions regarding snus were used in separate sub-analyses. As these additional questions only were included in HUNT3, the participants in QNP were excluded in these sub-analyses.

We tested the main model for interactions, and based on these results we performed analyses stratified by age and smoking status.

Statistical software: STATA 14.0 (StataCorp LP, College Station, TX)

Informed consent and ethical approval

All participants in HUNT gave written informed consent on participation that their data could be used in future medical research. Ethical approval was obtained from the Regional Committee for Medical and Health Research Ethics (reference: 2012/1290/REK midt) before the study was initiated.

Results

In total, 58,634 individuals participated in HUNT3 and the QNP and 2993 (5.1%) had severe GERS (cases) and 26,610 (45.4%) had no GERS (controls; Table 1).

Characteristics

The cases were older than the controls (mean age 54.3 and 50.1 years, respectively). The participation rate was higher among women than men, but the proportion of cases was higher among men than women (11.8% and 10.8%, respectively).

Daily snus use was less common among cases than controls (5.4% and 7.3%, respectively). On the other hand, previous snus use and occasional snus use were more common among cases. The cases using snus were older than the controls using snus (mean age 43.3 and 41.8 years, respectively). The mean age at the start of their snus use was similar for both groups. Snus consumption (boxes per month) was higher for cases than for controls. More cases than controls started using snus to quit or cut down on smoking, and

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Table 1. Characteristics of individuals reporting severe GERS (cases) and no GERS (controls) in HUNT3/QNP, compared with the total population.

	Cases with severe GERS HUNT3/QNP	Controls with no GERS HUNT3/QNP	Total population HUNT3/QNP
Number	2993 (5.1%)	26,610 (45.4%)	58,634 (100%)
Age, years			
Mean (s.d.)	54.3 (15.0)	50.1 (16.3)	51.8 (16.4)
Median (range)	55 (19 98)	50 (19 102)	52 (19 102)
Sex			
Women, no. (%)	1629 (54.4)	15,024 (56.5)	31,563 (53.8)
Men, no. (%)	1364 (45.6)	11,586 (43.5)	27,071 (46.2)
Snus status			
Never, no. (%)	2438 (81.5)	21,935 (82.4)	47,486 (81.0)
Previous, no. (%)	163 (5.5)	1179 (4.4)	2820 (4.8)
Occasionally, no. (%)	101 (3.4)	882 (3.3)	1998 (3.4)
Daily, no. (%)	161 (5.4)	1943 (7.3)	4201 (7.2)
Missing, no. (%)	130 (4.3)	671 (2.5)	2129 (3.6)
Age snus users, years			
Mean (s.d.)	43.4 (13.5)	41.8 (15.1)	42.3 (14.6)
Median (range)	43 (19 91)	41 (19 98)	42 (19 98)
Age starting with snus, years			
Mean (s.d.)	25 (12.3)	24.6 (11.5)	24.4 (11.6)
Median (range)	20 (7 70)	20 (8 80)	20 (7 80)
Missing, no. (%)	31 (8.3)	142 (4.8)	522 (7.0)
Snus consumption, boxes/month			
0, no. (%)	2176 (72.7)	17,865 (67.1)	41,575 (70.9)
$>0<2$, no. (%)	47 (1.6)	342 (1.3)	838 (1.4)
$\geq 2<8$, no. (%)	215 (7.2)	1811 (6.8)	4479 (7.6)
≥ 8, no. (%)	70 (2.3)	488 (1.8)	1333 (2.3)
Missing, no. (%)	485 (16.2)	6104 (22.9)	10,409 (17.8)
Motivation to start using snus			
None, no. (%)	191 (51.3)	1809 (61.6)	4380 (58.3)
Quit smoking, no. (%)	101 (27.2)	596 (20.3)	1664 (22.2)
Cut down on smoking, no. (%)	65 (17.5)	308 (10.5)	876 (11.7)
Missing, no. (%)	15 (4.0)	222 (7.6)	591 (7.9)
Tobacco type started with first			
Snus, no. (%)	72 (19.4)	694 (23.7)	1748 (23.3)
Snus and cigarettes, no. (%)	13 (3.5)	73 (2.5)	209 (2.8)
Cigarettes, no. (%)	213 (57.3)	1214 (41.4)	3351 (44.6)
Missing, no. (%)	74 (19.9)	954 (33.0)	2203 (29.3)
Smoking status			
Never smokers, no. (%)	977 (32.6)	12,351 (46.4)	24,521 (41.8)
Previous smokers, no. (%)	1176 (39.3)	7666 (28.8)	18,241 (31.1)
Current smokers, no. (%)	768 (25.7)	6008 (22.6)	14,335 (24.5)
Missing, no. (%)	72 (2.4)	585 (2.2)	1537 (2.6)
BMI, kg/m^2			
Mean (s.d.)	28.8 (4.5)	26.3 (4.2)	27 (4.4)
Median (range)	28 (14 51)	25.8 (12 55)	26.6 (12 56)
Missing, no. (%)	28 (0.9)	347 (1.3)	858 (1.5)
Physical exercise			
<Weekly, no. (%)	1284 (42.9)	9598 (36.1)	22,511 (38.4)
≥Weekly, no. (%)	1426 (47.6)	15,372 (57.8)	31,737 (54.1)
Missing, no. (%)	283 (9.5)	1640 (6.2)	4386 (7.5)

BMI: body mass index; GERS: gastroesophageal reflux symptoms (self reported degree of complaints with heartburn or acid regurgitation during the previous 12 months); HUNT3/QNP: the third wave of the Nord Trøndelag health study and the questionnaire to non participants (2006 2009).

more cases than controls started with cigarettes first. Compared to the controls, more cases were current or previous tobacco smokers, had a higher BMI, and exercised less frequently.

Associations

Compared to never snus users, daily snus users had a reduced risk of severe GERS; fully adjusted OR 0.77 (95% CI 0.64–0.93; Table 2). However, previous snus users had increased risk of severe GERS; fully adjusted OR 1.20 (1.00–1.46). Moreover, those using <2 boxes per month had increased risk of severe GERS; fully adjusted OR 1.41 (1.02–1.96), while there was no statistically significant association between snus use and use of ≥2 boxes of snus per month.

There was no association between age when starting using snus and severe GERS (Table 3). Compared to those reporting no specific motivation to start using snus, those snus users who started using snus to quit or cut down on smoking had an increased risk of severe GERS; adjusted OR 1.50 (1.13–1.99) and 1.88 (1.35–2.61), respectively. Snus users, who started with both snus and cigarettes, or cigarettes alone, also had an increased risk of severe GERS; fully adjusted OR 2.26 (1.17–4.35) and 1.76 (1.30–2.39), respectively.

Snus use interacted with age and tobacco smoking status. Stratified analyses showed that snus users <30 years of age had increased risk of severe GERS; fully adjusted OR 1.49 (1.02–2.16) (Table 4 and Figure 1), while those between 50–60 and 60–70 years of age had reduced risk; fully adjusted OR 0.67 (0.49–0.93) and 0.51 (0.28–0.94), respectively. Snus users who never or previously had smoked had a

Table 2. The odds ratio (95% confidence interval) of severe GERS compared with no GERS, by snus status (HUNT3/QNP) and snus consumption (HUNT3 only).

	Unadjusted	Adjusted for age and sex	Fully adjusted[a]
Snus status			
Never	Reference	Reference	Reference
Previous	1.24 (1.05 1.47)	1.33 (1.12 1.59)	1.20 (1.00 1.46)
Occasionally	1.03 (0.83 1.27)	1.23 (0.99 1.53)	1.21 (0.96 1.52)
Daily	0.75 (0.63 0.88)	0.84 (0.70 0.99)	0.77 (0.64 0.93)
Snus consumption, boxes/month			
0	Reference	Reference	Reference
<2	1.13 (0.83 1.54)	1.33 (0.98 1.82)	1.41 (1.02 1.96)
2 8	0.97 (0.84 1.13)	1.04 (0.89 1.21)	0.93 (0.78 1.10)
>8	1.18 (0.91 1.52)	1.34 (1.03 1.74)	1.16 (0.88 1.54)

[a]Adjusted for age, sex, smoking status, BMI, and physical exercise.
BMI: body mass index; GERS: gastroesophageal reflux symptoms (self reported degree of complaints with heartburn or acid regurgitation during the previous 12 months); HUNT3/QNP: the third wave of the Nord Trøndelag health study and the questionnaire to non participants (2006 2009).

Table 3. The odds ratio (95% confidence interval) of severe GERS compared with no GERS, by age starting using snus, motivation to start with snus and type of tobacco started with first, in HUNT3.

	Unadjusted	Adjusted for sex	Fully adjusted[a]
Age starting with snus			
<20	Reference	Reference	Reference
20 30	1.06 (0.80 1.39)	1.06 (0.81 1.40)	1.06 (0.78 1.44)
30 40	0.88 (0.61 1.29)	0.88 (0.60 1.28)	0.70 (0.45 1.07)
40 50	1.28 (0.87 1.89)	1.27 (0.86 1.87)	1.02 (0.66 1.56)
50 60	1.39 (0.83 2.32)	1.35 (0.81 2.26)	1.02 (0.59 1.79)
60 70	1.12 (0.44 2.90)	1.09 (0.42 2.82)	1.13 (0.42 3.00)
≥70	1.42 (0.17 1.90)	1.35 (0.16 11.30)	1.91 (0.22 17.01)
	Unadjusted	**Adjusted for age and sex**	**Fully adjusted[b]**
Motivation to start using snus			
None	Reference	Reference	Reference
Quit smoking	1.55 (1.20 1.99)	1.52 (1.17 1.98)	1.50 (1.13 1.99)
Cut down smoking	1.93 (1.42 2.61)	1.91 (1.40 2.59)	1.88 (1.35 2.61)
Type of tobacco started with first			
Snus	Reference	Reference	Reference
Snus and cigarettes	1.72 (0.91 3.25)	1.73 (0.92 3.28)	2.26 (1.17 4.35)
Cigarettes	1.77 (1.35 2.31)	1.72 (1.30 2.29)	1.76 (1.30 2.39)

[a]Adjusted for sex, smoking status, BMI, and physical exercise.
[b]Adjusted for age, sex, BMI, and physical exercise.
BMI: body mass index; GERS: gastroesophageal reflux symptoms (self reported degree of complaints with heartburn or acid regurgitation during the previous 12 months); HUNT3: the third wave of the Nord Trøndelag health study (2006 2008).

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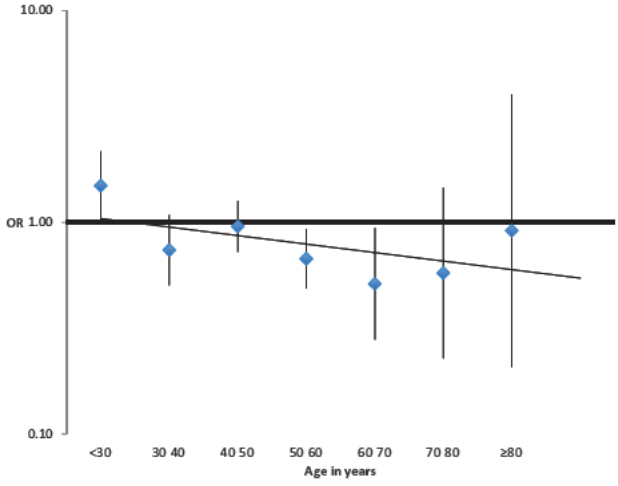


Figure 1. The odds ratio (OR) and 95% confidence interval (vertical lines) of severe gastroesophageal reflux symptoms (GERS; self reported degree of complaints with heartburn or acid regurgitation during the previous 12 months) compared with no GERS among snus users in different age categories in the third wave of the Nord Trøndelag health study (HUNT3) and the questionnaire to non participants (QNP) performed 2006 2009. Adjusted for sex, smoking status, body mass index, and physical exercise.

reduced risk of severe GERS; fully adjusted OR 0.75 (0.54–1.03) and 0.62 (0.48–0.79), respectively. Moreover, snus users who were occasionally or daily smokers seemed to have an increased risk of severe GERS, however, this association did not reach statistical significance; fully adjusted OR 1.39 (0.94–2.04) and 1.12 (0.78–1.61), respectively (Table 4).

Discussion

This large population-based observational study showed that daily snus users had a reduced risk of severe GERS compared with not snus users. However, previous snus users and subgroups of snus users had an increased risk of severe GERS: those consuming <2 boxes of snus per month, snus users who started with both snus and cigarettes or cigarettes alone, snus users who started using snus to quit or cut down cigarette smoking, and snus users <30 years of age. In snus users the risk of severe GERS was reduced with increasing age.

The large population-based study design facilitates subgroup analyses and reduces the risk of selection bias. However, it is known that those participating in population-based studies have higher socioeconomic status compared to those not participating. As diseases and health related risk factors are often related to socioeconomic status, this makes selection bias possible [25,26] and might weaken the external validity of the results. Except for lower average income and education, and the lack of a large city, the population in

Table 4. The odds ratio and (95% confidence interval) of severe GERS compared with no GERS among snus users, stratified by age and smoking status, in HUNT3/QNP.

	Unadjusted	Adjusted for sex	Fully adjusted[a]
Age stratified			
<30	1.37 (0.99 1.90)	1.44 (1.02 2.03)	1.49 (1.02 2.16)
30 < 40	0.98 (0.70 1.37)	0.80 (0.56 1.13)	0.74 (0.50 1.08)
40 < 50	1.11 (0.87 1.42)	0.96 (0.75 1.25)	0.96 (0.72 1.26)
50 < 60	0.85 (0.64 1.14)	0.76 (0.56 1.02)	0.67 (0.49 0.93)
60 < 70	0.57 (0.34 0.95)	0.65 (0.39 1.10)	0.51 (0.28 0.94)
70 < 80	0.58 (0.27 1.26)	0.70 (0.32 1.55)	0.58 (0.23 1.46)
≥80	0.80 (0.24 2.66)	0.82 (0.25 2.76)	0.91 (0.21 4.03)
	Unadjusted	Adjusted for age and sex	Fully adjusted[b]
Stratified by smoking status			
Never	0.62 (0.47 0.83)	0.76 (0.57 1.03)	0.75 (0.54 1.03)
Previous	0.60 (0.48 0.75)	0.63 (0.50 0.80)	0.62 (0.48 0.79)
Occasionally	1.24 (0.90 1.71)	1.33 (0.93 1.92)	1.39 (0.94 2.04)
Daily	1.32 (0.9 1.80)	1.14 (0.82 1.59)	1.12 (0.78 1.61)

[a]Adjusted for sex, smoking status, BMI, and physical exercise.
[b]Adjusted for age, sex, BMI, and physical exercise.
BMI: body mass index; GERS: gastroesophageal reflux symptoms (self reported degree of complaints with heartburn or acid regurgitation during the previous 12 months); HUNT3/QNP: the third wave of the Nord Trøndelag health study and the questionnaire to non participants (2006 2009).

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Nord-Trøndelag is representative of the Norwegian population at large.[27] The extensive questionnaires used in HUNT also allowed adjustments for possible confounders of the association between snus use and GERS. Alcohol use is associated with snus use and some studies have also found an association between alcohol use and reflux, while other studies have not found any association.[28] In our previous studies on GERS using the HUNT material, alcohol use was not associated with reflux [17,20] and, thus, we have not included alcohol use as a possible confounder in this study. All questionnaires used in this study were self-reported. Even if self-reported questionnaires are validated,[29] we cannot exclude possible information bias in our study.[30]

In HUNT, more men than women were using snus, current snus users were younger than not snus users, snus use was associated with lower education, higher alcohol consumption, and less physical exercise, and smoking rates were lower among daily snus users.[2]

According to the Montreal definition, GERD is 'a condition that develops when reflux of stomach contents causes troublesome symptoms and/or complications. (…) In population-based studies, mild symptoms occurring two or more days a week, or moderate/severe symptoms occurring more than one day a week, are often considered troublesome by patients'.[11] The HUNT study was initiated before the Montreal consensus and did not use this definition. However, the question used in HUNT has been validated and these validations have shown that participants reporting severe complaints in HUNT have at least weekly symptoms.[23,24]

We have no information available on whether the participants were taking anti-reflux medications. This could have reduced the proportion of participants reporting GERS. In addition, participants with GERS may also have developed Barrett's esophagus which has the potential to reduce symptoms. However, only a small percentage of the participants are expected to develop Barrett's so this should not have influenced the overall results.

In this study, we hypothesized that snus use would increase the risk of GERS. However, the overall result was that snus use seems to protect against GERS. We believe this could be explained by reverse causality, meaning that those with severe GERS because of snus stop their exposure to snus.[20] The results of the subgroup analyses support this assumption. Previous snus users developing GERS may have quit using snus as a result of snus inducing GERS. This is also supported by the declining trend from high risk of GERS among young snus users to low risk with increasing age. This is the opposite of the trend in prevalence of GERS seen in the total population.[24] If reverse causality is the explanation, our results will wrongfully suggest that snus has a protective effect against GERS and persons with a lower predisposition of developing GERS will continue using snus.

If snus increases the risk of GERS, one would presume an increasing prevalence with increasing doses; a dose-response relationship. However, in this study we found that those using <2 boxes per month were more likely to have GERS than those with a higher consumption. Again, if our theory of reverse causality is true, this may be explained by GERS inducing a reduced exposure to snus. One could argue that GERS induced by snus should disappear when quitting snus. However, individuals with GERS probably have several risk factors, including a genetic predisposition, but snus is maybe one factor which is recognizable as a trigger of GERS and probably easier to deal with than other risk factors. So, even if snus is stopped and this has an effect on GERS, it is unlikely that this would make all the complaints disappear. A previous study on this topic, the Kalixanda study, found histological changes associated with GERD in the mucosa of snus users, supporting an increased risk of GERS with snus use.[21] However, the study did not find any link between snus consumption and GERS. If our findings are not caused by reverse causality, snus may indeed have a protective effect against GERS and this makes our results highly controversial. In this case, the protective effect of snus could be explained by increased saliva section in snus users,[31] the relatively high pH (approximately 8.5) [8,32] or other unknown effects of the snus contents on the esophageal or gastric mucosa, e.g., creating a barrier effect or reducing acid secretion, respectively. However, these protective effects

would likely affect all age groups and cannot explain the reduced risk of GERS exclusively seen in the older snus users.

In conclusion, this study found that daily snus users overall had a reduced risk of GERS. However, subgroups of snus users had an increased risk of GERS and we propose that the overall result could be due to reverse causality and that snus use actually increases the risk of GERS. Further research is needed to conclude whether snus use is a risk factor for the development of GERS. A study combining a detailed questionnaire on reflux symptoms, anti-reflux medication and risk factors with objective investigations with endoscopy and pH-measurements may increase the evidence on this topic.

In 2017–2018, HUNT4 will be conducted and the same population as in HUNT3 will be re-invited to participate. This provides an excellent opportunity to follow snus users prospectively and to investigate further whether the reduced risk of GERS seen in this study is related to reverse causality or not.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. The present manuscript has not been published, simultaneously submitted, or already accepted for publication elsewhere.

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Do never smokers make up an increasing share of snus users as cigarette smoking declines? Changes in smoking status among male snus users in Norway 2003–15

Karl Erik Lund[1], Tord Finne Vedøy[1] & Linda Bauld[2]

Norwegian Institute of Public Health, Oslo, Norway[1] and Institute for Social Marketing and UK Centre for Tobacco and Alcohol Studies, University of Stirling, Stirling, UK[2]

ABSTRACT

Aims To examine how the relative size of six groups of male ever snus users (current and former users of snus who were current, former or never cigarette smokers) varied over time in Norway, and how these groups differ with regard to important measures of tobacco behaviour. **Design** Repeated cross sectional nationally representative surveys of tobacco use. The association between survey year and the six categories of ever snus use was examined using cross tabulation and multinomial logistic regression. Differences in tobacco behaviour across snus use categories were examined using logistic and ordinary least squares (OLS) regression. **Setting** Norway, 2003–15. **Participants** A total of 2067 males aged 15–79 years. **Measurements** The categories of ever snus use represented all six combinations of cigarette smoking (current, former or never) among current and former users of snus. The variables measuring tobacco behaviour were: order of product uptake (snus or cigarettes first), mean cigarette consumption, reduction from daily to occasional smoking, intention to quit cigarettes, future smoking identity and use of snus in latest quit attempt. **Findings** During the period 2003–15, the relative share of current snus users who had never smoked, and current snus users who were former smokers, increased. The share of dual users, and smokers who were former snus users, decreased. Among men who reported life-time experience with both products, a large majority had initiated their tobacco use with cigarettes. The average number of cigarettes smoked weekly was lower among dual users compared with current smokers who were former snus users or had never used snus. **Conclusions** During the period 2003–15 in Norway, which has a mature snus market, even though smoking has declined and the relative size of the category of never smokers among male users of snus has increased, the majority of snus users are still former or current smokers.

Keywords Nicotine alternatives, public health, smokeless, snus, tobacco, tobacco harm reduction.

Correspondence to: Karl Erik Lund, Norwegian Institute of Public Health, PO BOX 4404 Nydalen, Oslo 0403, Norway. E-mail: kelu@fhi.no
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INTRODUCTION

In his visionary paper in *Addiction* in 1991, the tobacco behavioural scientist Michael Russell asserted that alternative delivery systems of nicotine could make the virtual elimination of cigarettes a realistic future target [1]. However, Russell maintained that the quit smoking effect from new delivery systems would be limited to a specific historical stage in the phasing out of cigarette smoking. According to Russell, it was the potential of new nicotine delivery systems as long term alternatives to cigarettes for youth susceptible to smoking that would be their most important contribution in the cigarette endgame.

Working in an era before some of the alternative nicotine delivery products such as low nitrosamine snus were available, Russell held an optimistic view towards such products. In the contemporary tobacco harm reduction discourse, attitudes are split [2–6]. Opponents are particularly concerned about the uptake of snus or e-cigarettes among non-smokers compared to smokers, and how the user configuration of such products may change as the number of smokers continues to decline.

Observational and experimental studies suggest that the availability of snus influences smoking cessation. Empirical evidence from Norway and Sweden – where the use of snus is common – demonstrates that snus is the most frequently used method for stopping smoking after

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unassisted quit attempts [7–12]. Evidence also shows that former smokers make up the largest segment of snus users [13,14], that snus use is associated with higher quit rates compared to not using snus [15,16] and that snus might be more effective for smoking cessation than pharmaceuti cal nicotine [11,17,18].

Through its role in smoking cessation, evidence sug gests that snus has contributed to the decline in smoking in Sweden and Norway, particularly among males, who are far more likely to use this product than females [15,19–22]. However, although the majority of snus users today have had prior experience with cigarette smoking, there is a growing concern that future snus use will consist increasingly of people who have no experience with ciga rettes [23–25].

The first aim of this paper is to examine the size and temporal variations of six groups of ever snus users (cur rent or former users who were current, former or never smokers) during the period 2003–15 in Norway—a coun try with a mature snus market.

Our second aim is to examine if these six groups differ with regard to measures of tobacco use that are important for population health. For comparison, we also included smokers who have never used snus.

METHODS

Sample

Our study population included male ever users of snus who had participated in annual cross sectional surveys of to bacco behaviour conducted in Norway from 2003 to 2015. The total number of male respondents was 8157 and age ranged from 15 to 79 years with a mean of 44.4. Of these, 2067 were ever snus users (see Table S1 in the Supporting information for descriptive statistics). Females were not included because the uptake of snus in females has occurred at a much lower rate and is still concentrated among young adult women.

The surveys and interviews were carried out by Statis tics Norway. For each year, a simple random sample of 2000 (3000 in 2015) respondents was drawn from the National Population Register by Statistics Norway and in terviews were carried out by telephone [26,27]. The mean response rate for the period was 62.1%.

Measures

Regarding snus use, respondents were asked if they used snus daily, occasionally or not at all. Respondents without current (daily or occasional) use were asked if they had used snus previously. These questions were used to identify our study population of ever snus users—males who cur rently or formerly used snus.

For smoking status, respondents were asked: 'Do you smoke?'. Respondents answering 'yes' were then asked if they smoked daily or occasionally. Current smokers were defined as those who smoked cigarettes daily or occasionally.

Respondents who answered that they did not smoke at the time of the survey were asked if they had smoked daily or occasionally earlier in their life time. Respondents an swering 'no' were categorized as never smokers. Respon dents reporting 'yes' were identified as former smokers.

Based on the questions above, we were able to identify six groups of ever snus users through combinations of cur rent (daily or occasional) or former use of snus and current/former/never use of cigarettes. The six groups were (1) current snus users who were current smokers (dual users), (2) current snus users who were former smokers, (3) current snus users who had never smoked, (4) former snus users who were current smokers, (5) for mer snus users who were former smokers and (6) former snus users who had never smoked. We also identified a contrasting group of men who currently smoked, but who had never used snus (7).

Tobacco use was measured by seven items. Dual users and current smokers were asked to state the average num ber of cigarettes consumed per day (for daily smokers) or per week (for occasional smokers). Frequencies among daily smokers were multiplied by seven and combined with frequencies among occasional smokers.

Dual users and current smokers were then asked if they had an intention to stop smoking within the next 6 months. Possible answers were 'yes' and 'no'.

Dual users and current smokers who smoked occasion ally were also asked if they had previously smoked daily.

Respondents with prior regular use or current regular use of both products were asked: 'Which tobacco product did you start to use first—snus or cigarettes?'. Response categories were 'cigarettes first', 'snus first' and 'about the same time' (within 3 months). Respondents were dummy coded as either 'snus first' or 'cigarettes first'.

Men who had had a life time experience with both products were also asked if they had used snus at their lat est quit attempt (if any). Possible answers were 'yes' and 'no'.

Finally, all respondents were asked if they viewed them selves as smokers 5 years into the future. Response catego ries were: 'definitely yes', 'probably yes', 'probably not' and 'definitely not'.

Analyses

To address our first aim, we calculated the fraction and 95% confidence intervals of men in each snus use category across four time periods (2003–05, 2006–08, 2009–11 and 2012–15) (Table 1). Possible linear temporal trends

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Table 1 The percentage and 95% confidence intervals of respondents in each of the six ever snus user groups across four periods Norwegian men 15–79 years 2003–15

	Dual users of snus and cigarettes (I)	*Current snus users, former smokers (II)*	*Current snus users, never smokers (III)*	*Former snus users, current smokers (IV)*	*Former snus users, former smokers (V)*	*Former snus users, never smokers (VI)*	*Total*	*n*
2003–2005	33 0 (28 8–37 2)	14 5 (11 4–17 7)	16 0 (12 7–19 2)	22 4 (18 7–26 1)	8 9 (6 4–11 5)	5 2 (3 2–7 2)	23 3 (21 5–25 1)	482
2006–2008	25 6 (21 5–29 6)	22 9 (19 0–26 8)	15 9 (12 5–19 3)	13 9 (10 7–17 1)	15 2 (11 9–18 6)	6 5 (4 2–8 8)	21 6 (19 8–23 4)	446
2009–2011	24 3 (20 2–28 5)	23 4 (19 3–27 5)	21 0 (17 0–24 9)	9 2 (6 4–11 9)	13 5 (10 2–16 8)	8 7 (6 0–11 4)	20 1 (18 4–21 8)	415
2012–2015	18 9 (16 1–21 8)	25 1 (22 0–28 3)	24 9 (21 7–28 0)	8 1 (6 2–10 1)	11 2 (8 9–13 5)	11 7 (9 4–14 1)	35 0 (33 0–37 1)	724
Total	24 7 (22 9–26 6)	21 8 (20 0–23 6)	20 1 (18 4–21 8)	12 9 (11 5–14 4)	12 0 (10 6–13 4)	8 5 (7 3–9 7)	100	–
Linear trend[a]	–5 0 (–7 1 to –2 9)	3 8 (1 6–6 0)	6 2 (3 8–8 6)	–12 2 (–15 4 to –9 0)	–0 8 (–4 0 to 2 3)	10 1 (6 1–14 0)	–	–
n	511	451	415	267	248	175	–	2067

[a]Calculated from a multinomial logistic regression model where year was entered as a continuous variable with 1-year intervals Coefficients show percentage change with 1-unit change in year (semi-elasticities)

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in the relative size of each of the snus use categories were examined using a multinomial regression model with the six snus use categories as dependent variable. Independent variables were year (continuous with 1 year intervals), age (continuous with 1 year intervals), education (four categories: 'primary', 'secondary, 'tertiary' and 'other') and geographical region (six categories). The slope coefficient was presented as percentage change with 1 unit change in year (semi elasticities).

To address our second aim, we constructed a set of regression models to examine the seven measures of tobacco behaviour across the six groups of ever snus users and among current smokers without experience with snus (Table 2).

The following seven measures of tobacco behaviour were recoded into dummy variables and treated as dependent variables in the set of logistic regression models: 'used cigarettes before snus', 'used snus before cigarettes', 'had intentions to quit cigarettes within 6 months', 'would definitely be smoke free in 5 years', 'had reduced from daily to occasional smoking' and 'used snus in latest quit attempt'. The average number of cigarettes per week was modelled using ordinary least squares (OLS) regression.

Independent variables were identical to those used in the multinomial regression model described above, but included an additional variable identifying the six categories of snus use and the contrasting group of never snus users/current smokers. Coefficients and 95% confidence intervals were reported as marginal mean probabilities multiplied by 100 (proportions).

All data analysis was performed in Stata version 13.

RESULTS

In this nationally representative sample of 8157 male Norwegians, our study population of ever snus users comprised 2067 individuals (25.3%), while our contrasting group of never snus users/current smokers included 1411 respondents (17.3%) (for descriptive statistics, see Table S1 in the Supporting information).

The percentage of current snus users/never smokers among ever snus users increased from 16.0% in 2003 05 to 24.9% in 2012 15 (Table 1, column III). The underlying linear trend showed a 6.2% increase per year during the period. The annual increase in the percentage of never smokers who were former snus users (column VI) was somewhat higher (10.1%). However, this segment comprised only 11.7% of ever snus users in the latest period. In sum, never smokers (columns III and VI) made up 36.6% (11.7 + 24.9%) of ever users of snus in 2012 15, an increase from 21.2% in 2003 05.

The percentage of dual users among ever snus users decreased from 33.0 to 18.9% from 2003 05 to 2012 15 (column I). The percentage of current snus users who had quit smoking increased from 14.5 to 25.1%, but this increase occurred mainly between the first and second periods (column II). The segment of men who had quit both snus and cigarettes remained stable over time, and comprised only 11.2% by the end of the study period (column V). Another relatively small group was current smokers who had quit using snus (8.1% in 2012 15). This was the group with the highest relative annual change (−12.3%) (column IV).

When we examine the seven measures of tobacco behaviour within groups, we find that in the four groups where respondents reported life time experience with both products (Table 2, columns I, II, IV and V), a majority ranging from 67.6 to 77.4% had initiated their tobacco use with cigarettes. Only between 19.6 and 29.8% had started with snus.

The average number of cigarettes smoked weekly was significantly lower among dual users (57.0, column I) than among never snus users/current smokers (80.9, column VII) and current smokers who had quit snus (79.6 column IV).

Moreover, a significantly higher proportion of dual users (29.7%) reported that they had reduced from daily to occasional smoking than was the case among never snus users/current smokers (14.6%) and among current smokers who at the time of the survey had quit snus (17.2%).

No significant differences were observed in the proportion who answered that they had intentions to stop smoking within the next 6 months between dual users (45.8%, column I) and never snus users/current smokers (43.1%, column VII). However, a significantly higher proportion of dual users (43.8%) reported that they would definitely be smoke free in 5 years' time compared to never snus users/current smokers (32.2%) and current smokers who had quit snus (32.2%, column IV).

A total of 75.4% of dual users had used snus in their latest quit attempt (column I). Among current snus users who had succeeded in stopping smoking completely (column II), 82.7% reported that snus was used in their final quit attempt.

DISCUSSION

Our study has shown that in a country with a mature snus market, the majority of snus users still derive from the smoking population, even if smoking has been declining rapidly. By the end of our study period, never smokers made up just above one third of the respondents who had been using snus on a regular basis either currently or previously. However, we also observed a marked increase in the

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Table 2 Order of product uptake smoking intensity intentions to quit presumed future smoking status and use of snus in latest quit attempt Marginal mean probabilities and 95% confidence intervals Norwegian men aged 15–79 years 2003–15

	Ever snus users						*Never snus users*
	Dual users of snus and cigarettes (I)	*Current snus users, former smokers (II)*	*Current snus users, never smokers (III)*	*Former snus users, current smokers (IV)*	*Former snus users, former smokers (V)*	*Former snus users, never smokers (VI)*	*Current smokers (VII)*
Used cigarettes before snus	70 3 (66 5–74 1)	67 6 (63 4–71 7)	–	77 2 (71 2–83 2)	77 4 (71 9–82 9)	–	–
Used snus before cigarettes	25 7 (22 1–29 4)	29 8 (25 7–33 8)	–	21 1 (15 2–26 9)	19 6 (14 4–24 8)	–	–
Average number of cigarettes per week	57 0 (51 5–62 5)	–	–	79 6 (72 0–87 1)	–	–	80 9 (77 6–84 3)
Reduced from daily to occasional smoking	29 7 (25 8–33 7)	–	–	17 2 (12 7–21 7)	–	–	14 6 (12 8–16 4)
Intentions to quit cigarettes within 6 months	45 8 (41 5–50 1)	–	–	47 9 (42 0–53 9)	–	–	43 1 (40 5–45 7)
Definitely smoke free in 5 years	43 8 (39 6–48 1)	86 0 (82 9–89 2)	94 7 (92 6–96 8)	32 2 (26 7–37 7)	90 3 (86 7–94 0)	96 0 (93 1–98 9)	32 2 (29 8–34 6)
Used snus in latest quit attempt	75 4 (71 0–79 9)	82 7 (78 3–87 1)	–	30 1 (23 9–36 3)	48 0 (40 6–55 3)	–	–

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fraction of never smokers among ever snus users during the study period of 12 years.

Consequently, as smoking declines due partly to the availability of snus the relative share of never smokers among snus users is likely to continue to increase. If so, the main effect of snus in tobacco harm reduction will be increasingly in reducing smoking initiation, while its effect on smoking cessation may eventually diminish, as pointed out by Russell in 1991 in relation to alternative nicotine delivery devices [1].

As observed in other studies [15], an increase in snus use has taken place in parallel with a decline in smoking among young adults in Norway. The inverse and very high correlation between snus use and cigarette smoking could be interpreted in line with Russell's predictions. Thus, the availability of snus might have produced a shift in tobacco preferences and contributed to lower smoking initiation among young adults, particularly males.

Snus use among former smokers

In line with previous studies [14,17], our data demonstrate that former smokers make up a sizable and increasing seg ment among ever snus users (Table 1, columns II and V). This increase apparently contradicts Russell's assumption that the quit smoking effect from new delivery systems of nicotine would diminish over time. An explanation might be that the rapid increase in snus use in Norway started less than 2 decades ago, and coincided with a ban on all in door smoking in public places. This means that men who replaced cigarettes with snus are still relatively young, still count among former smokers and that it will take some time before they disappear from the study population. When this cohort effect eventually decreases, the fraction of former smokers among snus users may diminish, in line with Russell's prediction.

It is important to note that former smokers who are current or former snus users did not necessarily stop smoking because they used snus. Among former smokers who also had quit snus at the time of the survey, only 48.0% (Table 2, column V) reported that snus was the method they had used when finally quitting smoking. However, among former smokers who continued to use snus, 82.7% reported that snus was used at the final quit attempt (column II). These findings suggest that the frac tion of former smokers among snus users can only indicate the role of snus in smoking cessation.

However, our data suggest that snus might have served as an exit from cigarettes for a number of smokers. Given the share of ever snus users who were former smokers, and the large difference in health risks associated with these products, the anticipated transition from cigarettes to snus might have made a significant positive contribution to the public health effect.

Additional use of snus among smokers

There was no difference in intention to quit smoking in the next 6 months between dual users of snus and cigarettes and smokers who had never used snus (Table 2, columns I and VII). This is consistent with findings from Sweden [28], and more recently from the United States [29]. How ever, expectancies of being smoke free 5 years into the fu ture were significantly more prevalent among dual users compared to never snus users/current smokers (columns I and VII). Thus, we observed no empirical evidence in sup port of delayed smoking cessation among dual users in our study.

Consistent with observations in Sweden [12], it has been argued that alternative harm reduction strategies other than smoking cessation, such as smoking reduction, have been important motives for additional snus use in Norway. In line with this, approximately one third of dual users reported that they had changed smoking status from daily to occasional smokers, in contrast to only one in six among never snus users/current smokers (Table 2, col umns I and VII). However, given the cross sectional nature of the data, it cannot be discerned if the switch from daily to occasional smoking occurred before or after initiation of snus use among dual users.

A systematic review of health risks related to dual use from 2014 concluded that dual users of cigarettes and snus smoke fewer cigarettes, on average, than do never snus users/current smokers [30]. Findings in two papers pub lished after that date have been inconsistent [31,32].

There is also some evidence that unsuccessful attempts to quit smoking using snus lead to reduced smoking inten sity [11,18,33]. Our study showed that a majority (75.4%) of dual users had, in fact, used snus in their latest quit smoking attempt, and that dual users consumed on aver age fewer than 23.9 cigarettes per week than never snus users/current smokers (Table 2, columns I and VII). How ever, the health gain from reduced cigarette consumption is modest [34].

In sum, our data indicate that additional snus use among smokers is not likely to impact negatively upon pub lic health compared to exclusive cigarette use. Moreover, for a wide variety of health end points, evidence does not suggest any special hazard associated with dual use of snus and cigarettes compared to smoking only, according to a systematic review [30].

Former snus use among current smokers

The fastest declining group in our study population com prised respondents who have quit snus, but were current smokers at the time of the survey. This combination might imply that snus had served as a gateway to smoking. How ever, the order of product uptake in this group does not

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support such an interpretation. Instead, a majority (77.2%) had started their tobacco use with cigarettes (Table 2, column IV). If a gateway mechanism existed, it would be operating only among the 21.1% in this group who reported that snus was their first product.

Moreover, neither smoking intensity nor intention to quit within the short or long term in this group differ from never snus users, current smokers. Therefore, when assessing the overall public health effect from snus, the de cline in size of this segment probably does not play an im portant role.

The tipping point

What proportion of never smokers relative to smokers among users of an alternative nicotine product would mark the tipping point from a possible net gain to a possible net loss for public health? This question is of contemporary interest, particularly in a context where nicotine delivery products other than snus (i.e. electronic cigarettes) are growing in popularity.

To answer this question we need to have information about the configuration of snus users over time, as we have tried to identify in our study. In addition, we need informa tion about the relative risk difference between snus and cig arettes. According to qualitative assessments of risks by groups of tobacco researchers, low nitrosamine smokeless tobacco products pose less than one tenth the risk of ciga rettes [35,36].

Using relative risk estimates, Gartner *et al*. [37] assessed the potential for net population level harm given different rates of snus uptake by current smokers, ex smokers and people who have never smoked. According to their model, 14 25 individuals who have never smoked would need have to started using snus to offset either the health gain from every tobacco user who uses snus rather than ciga rettes or the health gain from every smoker who switches to snus. According to our data, the number of snus users who were former smokers was relatively large, while the number of never smokers among snus users, although in creasing, is far from being of the order of magnitude sug gested by Gartner *et al*.

However, in an imaginary situation where the preva lence of smoking has declined to a minimum, any public health benefit from the availability of snus will depend upon the ratio between (a) snus users who in the absence of snus would have become smokers and (b) snus users who, in the absence of snus, would have remained completely tobacco free.

Follow up studies of snus initiation among non smokers with and without traits for smoking susceptibility at baseline could shed some light on this. Currently, results from cross sectional studies indicate that primary snus users [11] overlap to some degree with smokers as well as non smokers with regard to susceptibility characteristics [38 43]. This suggests that a certain fraction of those who used snus before cigarettes would have become smokers even if snus was not available. Conversely, it also suggests that a certain fraction of never smoking snus users would probably have remained tobacco free in the absence of snus. Our aim is to address these questions in fu ture research with a prospective design.

Strengths and limitations

In evaluating our study findings, some strengths and limi tations should be noted. Strengths that lend confidence to our findings include a population based methodology, a ro bust data pool (n 8157), an acceptable and relatively sta ble response rate (average 62.1%) and standardized measures of tobacco behaviour.

However, all behavioural characteristics were based upon self report and may be affected by under or over reporting, or misclassification due to imperfect recall. Note also that the questions used to define former use were not identical for snus and cigarettes. The study findings are probably also product , culture and diffusion stage specific, and the generalizability of our results to other pop ulations might be limited.

Our study focused only on males, as the uptake of snus in females has occurred at a much lower rate and is still concentrated among young adults. Future studies should assess trajectories of use of snus and other nicotine containing products among women and girls.

In addition, differences between groups in the distribu tion of age or education could undermine our findings. To control for this, we calculated marginal mean probabilities of being in each cell of Table 1 using the same multinomial regression model used to calculate temporal trends (not shown). Results were nearly identical with the percentages reported in Table 1. We also checked for possible temporal variations in the seven measures of tobacco behaviour by calculating the percentage change in each measure with a 1 unit change in year in each group (not shown). The re sult showed little, if any, change over time (at most 3.5% per year).

There have been some attempts to quantify the public health effects from snus use [44], and more recently use of e cigarettes [45,46], using simulation models with a va riety of constructed scenarios of user patterns.

To understand the complex calculus of the overall pop ulation health impact of snus one has to operationalize the complex states and transitions that must be examined si multaneously and dynamically over time. Our data derive from cross sectional studies, and therefore raise concerns about selection bias and unmeasured or insufficiently con trolled confounders, precluding causal inference. However, given the lack of prospective data to uncover the overall

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population level effects of snus on tobacco use behaviour and health outcomes, cross sectional data can shed some light on these processes. The fact that our results are based on real world observations should be considered a strength.

CONCLUSION

In Norway, with a mature snus market, even though smoking has been declining rapidly and the relative size of never smokers among users of snus has increased, the majority of snus users are still those who have been smokers.

Declaration of interests

KEL has served as an unpaid expert witness in five court cases for private and governmental committees against the tobacco industry. TFV has been an unpaid advisor to Norwegian Health authorities for questions relating to changes in tobacco use. In addition to her University post, LB holds the CRUK BUPA Chair in Beverley Research for Cancer Prevention at Cancer Research UK.

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switched to snus, various dissolvable tobacco products, dual use, or tobacco abstinence. *Regul Toxicol Pharmacol* 2015; **71**: 186–97.
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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1 Descriptive statistics, men 15–79 years, 2003–15.
Table S2 Coding of snus status groups.
Table S3 Output from regression models for Table 1.

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Polycyclic Aromatic Hydrocarbon Biomarker Levels among Smokers who Switch to Oral Nicotine

Ellen Meier, PhD
Rachel Isaksson Vogel, PhD
Steven Carmella, PhD
Viviana Paiano, MS
Stephen S. Hecht, PhD
Dorothy K. Hatsukami, PhD

Objectives: We examined levels of the urinary polycyclic aromatic hydrocarbon (PAH) biomarker *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) in smokers who switched to either snus or nicotine gum. We also examined differences in PheT levels as a function of whether smokers used both their assigned product (eg, snus or nicotine gum) and cigarettes, or switched completely to product. **Methods:** Cigarette smokers (N = 308, 46.4% women) were randomized to receive snus or nicotine gum and were instructed to use only their assigned product for 12 weeks. Urine samples for analysis of PheT were collected at baseline and 4 weeks after receiving product. **Results:** All participants showed reductions in PheT regardless of product and whether they continued smoking while using product ($p < .003$); however, reductions were greater among those who completely switched to nicotine gum or snus. No differences emerged between smokers provided with nicotine gum versus snus in PheT levels after 4 weeks of use ($p > .05$). **Conclusions:** Switching to snus or nicotine gum appears to result in similar reductions in smokers' levels of urinary PheT with the greatest reductions among those who avoid smoking completely.

Key words: oral nicotine; smokers; tobacco substitution; biomarkers; carcinogens
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Snus, with lower levels of carcinogens compared to traditional smokeless tobacco (SLT) in the United States (US), has been considered as a harm reduction tool because of its link to decreases in smoking and related diseases, primarily in Sweden.[1-3] Smokers who switch to snus display similar oral cancer and cardiovascular disease risk profiles as those who quit tobacco completely.[4] Whether this is the case for smokers who use both cigarettes and snus, and how these risk profiles compare to nicotine gum, is less clear.

Polycyclic aromatic hydrocarbons (PAH) and tobacco specific nitrosamines (TSNA) are considered to be among the most important carcinogenic constituents of tobacco products.[5] These constituents are typically formed during tobacco curing, processing, and/or manufacturing, and incomplete combustion of organic materials predominantly found in fire-cured tobacco, but not during storage (ie, snus is refrigerated for preservation of "freshness," not to prevent production of PAH).[6] Exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-buta-

Ellen Meier, Postdoctoral Fellow, Tobacco Research Programs, University of Minnesota, Minneapolis, MN. Rachel Isaksson Vogel, Assistant Professor, Gynecologic Oncology, University of Minnesota, Minneapolis, MN. Steven Carmella, Professor, Masonic Comprehensive Cancer Center, Minneapolis, MN. Viviana Paiano, Research Associate, Masonic Comprehensive Cancer Center, Minneapolis, MN, United States. Stephen S. Hecht, Professor, Masonic Comprehensive Cancer Center, University of Minnesota, Minneapolis, MN. Dorothy K. Hatsukami, Professor, Tobacco Research Programs, Psychiatry, University of Minnesota, Minneapolis, MN.
Correspondence Dr Meier; meier109@umn.edu

none (NNK; a TSNA) and *N′*-nitrosonornicotine (NNN; a TSNA) can be assessed by measuring the sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (total NNAL) and the sum of unchanged NNN and its glucuronides (total NNN), respectively. PAH exposure can be assessed by measuring the phenanthrene metabolite *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT). Phenanthrene is not considered carcinogenic, but its levels correlate with those of carcinogenic PAH. Additionally, PheT is found at higher concentrations in smokers than non-smokers, mimics metabolic pathways of carcinogenic PAH, and has been identified as a useful biomarker of PAH metabolic activation.[7,8] Thus, PheT is a good proxy for estimating PAH exposure among tobacco users.

Snus contains multiple PAH (eg, phenanthrene, benzo[*a*]pyrene) believed to be the causative agents of lung and other cancers,[7] but generally at lower levels than traditional SLT. Research has shown that smokers who switch to US snus show significant reductions in measured benzo[*a*]pyrene.[9] This trend is also seen in smokers who continue to smoke while switching to snus.[9] As a result, snus has been suggested as a potential reduced harm alternative to combusted tobacco for those smokers unable or unwilling to quit.[10] Research has not yet examined how these patterns of PAH reductions compare to patterns seen when smokers switch to nicotine gum, particularly when they continue to smoke cigarettes. Additionally, nicotine gum has been shown to be effective at reducing smoking,[11,12] yet it has received little consideration in the US as a potential harm reduction tool for smokers unwilling or unable to quit cigarettes completely or who need to use nicotine on a long-term basis.

The current study examined smokers' levels of urinary PheT across a 4-week period when they switched to snus or nicotine gum. Additionally, we examined differences in exposure to PheT among smokers who used both their assigned snus/nicotine gum and cigarettes, or switched completely to their assigned product. The results of this study will help to characterize the risk profile of using snus as compared to nicotine gum, particularly as it may differ among those who completely switch to snus/nicotine gum compared to those who use both cigarettes and snus/nicotine gum.

METHODS

Participants

This study is a secondary data analysis of a randomized control trial examining switching behavior with snus and nicotine gum during a 12-week treatment period and is described fully in the parent article.[13] We limit our reporting to the baseline period and the fourth week of treatment at which time samples for biomarker analysis were collected.

A convenience sample of adult smokers from the Minneapolis/St. Paul, Minnesota and Eugene, Oregon areas were recruited via Internet and local media advertisements from May 2010 to May 2013. Eligible participants were: (1) men and women 18-70 years old; (2) smoking ≥10 cigarette per day (CPD) for the past year; (3) exhaled carbon monoxide (CO) ≥10 ppm or if CO < 10 ppm, then Nic Alert® urine dipstick test = 6 (indicates urine contains >1000 ng/mL of cotinine and regular tobacco user);[14] and (4) interested in completely switching to snus or nicotine gum. Smokers were excluded if they: (1) had unstable/untreated physical or mental condition(s); (2) had contraindications for snus/nicotine gum; (3) regularly used other nicotine/tobacco products; or (4) were pregnant or nursing (for women).[13]

Procedures

Interested participants completed an initial phone screen and eligible participants were invited to the research clinic for an orientation visit. Upon arriving at the clinic, participants provided consent and completed additional screening measures (ie, full assessment of medical and tobacco history, nicotine dependence, exhaled CO test to verify smoking status, pregnancy testing). Eligible participants were scheduled for a subsequent visit one week later (baseline week 0) and instructed to continue smoking as they normally do for the next week (baseline smoking period). Participants recorded their CPD using an Interactive Voice Response (IVR) system. This automated system called participants daily and allowed participants to call in as well.

After the baseline smoking week, participants returned to the clinic (week 0) and entered the 12-week treatment period. During the clinical trial, participants attended 2 weekly visits, followed by 10 weeks of biweekly visits. Participants were randomized to receive either Camel snus (Reynolds American Inc.) or Nicorette nicotine gum (4mg;

Table 1
Baseline Demographics and Smoking History of Participants by Study Arm (N = 308)

	Gum		Snus		p-value
Age (Years), Mean±SD	155	44.8±12.6	153	43.1±12.3	.24
Women	76	49.0%	67	43.8%	.36
Non-Hispanic Whites	131	84.5%	121	79.1%	.22
Education					.91
Some High School	7	4.5%	9	5.9%	
High School Graduate Or Equivalent	43	27.7%	46	30.1%	
Some College/2-Year Degree	79	51.0%	70	45.8%	
College Graduate/4-Year Degree	19	12.3%	21	13.7%	
Graduate	7	4.5%	7	4.6%	
Cigarettes Per Day, Mean±SD	155	18.0±6.8	153	17.6±6.4	.55
Age Smoking First Cigarette (Yrs), Mean±SD	154	14.6±4.5	151	13.8±3.6	.10
Age Becoming a Regular Smoker (Yrs), Mean±SD	154	18.1±5.8	151	17.2±3.7	.11
Number Of Quit Attempts					.85
0-2	56	36.4%	54	35.8%	
3-5	58	37.7%	53	35.1%	
6-10	21	13.6%	26	17.2%	
11-20	19	12.3%	18	11.9%	
FTND (Fagerström Test for Nicotine Dependence), Mean±SD	155	5.0±2.0	152	5.1±2.0	.58

GlaxoSmithKline) for the rest of the treatment period. Participants in the snus group could select one of 2 flavors – Winterchill (2.5 mg nicotine/pouch) or Robust (2.6 mg nicotine/pouch). Participants were provided approximately 90 pieces of product for the first 2 weeks of the trial and subsequent amounts were adjusted according to use. Participants were welcome to return to the clinic prior to scheduled visits for additional product if needed.

Participants were instructed to avoid smoking and only use their assigned product, at least 6-8 pieces per day for about 30 minutes per pouch/piece, and more if necessary. Products were used up to 6 weeks and then participants were instructed to reduce the amount of use gradually during the subsequent 6 weeks. At each visit during the clinical trial, participants completed outcome measures and received approximately 5 minutes of behavioral counseling for smoking avoidance. During counseling, smoking avoidance successes/failures and problem solving for smoking avoidance were discussed. At the baseline and week 4 visits, participants brought a first morning urine sample to be analyzed for biomarkers of exposure. Week 4 was chosen as the biological sampling period because it was prior to instructions to reduce the use of the assigned product and was of sufficient duration to minimize the effects from smoking.[15] Participants earned up to $360 for study completion.

Measures

At baseline, participants completed demographic and smoking history questionnaires (eg, age of first cigarette, number of quit attempts), the Fagerström Test for Nicotine Dependence,[16] and Timeline follow-back interviews (TLFB) to assess tobacco use during the past 14 days. After the baseline visit, use of product (pieces per day) and cigarettes was obtained using an IVR system allowing for daily assessment of consumption. TLFB was used at visits when IVR data was unavailable (eg, missed calls). Biomarkers of exposure were assessed via urine collection at weeks 0 (baseline) and 4. This study examines urinary PheT across treatment groups (snus versus nicotine gum) and use status (product only versus product and cigarette use).[13]

Biochemical Analyses

PheT was analyzed by gas chromatography – tandem mass spectrometry as previously described.[17] Intraday precision was 2.9%, interday precision was 3.7%, and accuracy was 95%.

Table 2
PheT (pmol/mg Creatinine) by Product and Use Status from Baseline to Week 4

Product Use Status	Gum						Snus						Snus vs. Gum p-value[a]
	Baseline PheT		Week 4 PheT		Change PheT		Baseline PheT		Week 4 PheT		Change PheT		
	N	Mean (SD)	N	Mean (SD)	Mean (SD)	p-value	N	Mean (SD)	N	Mean (SD)	Mean (SD)	p-value	
Study Product Only[b]	55	2.95 (2.50)	54	1.52 (1.88)	**-0.88 (0.71)**	**< .001**	51	2.54 (2.52)	51	1.25 (1.45)	**-0.76 (0.67)**	**< .001**	.87
Study Product + Cigarettes	92	2.61 (1.73)	92	2.21 (1.53)	**-0.23 (0.58)**	**< .001**	93	2.88 (2.24)	90	2.65 (3.65)	**-0.22 (0.66)**	**.003**	.80
Used Cigarettes Only[c]	3	4.85 (4.31)	3	4.00 (3.06)	**-0.12 (0.29)**	-	5	3.38 (1.18)	5	2.20 (0.71)	**-0.43 (0.59)**	-	-
No Product/ Cigarettes Use Reported[c]	5	1.76 (1.02)	5	2.01 (1.51)	**-0.06 (0.81)**	-	4	2.11 (1.00)	4	0.85 (0.78)	**-0.91 (0.31)**	-	-
Total: All Participants	155	2.74 (2.09)	154	2.00 (1.73)	**-0.45 (0.70)**	**< .001**	153	2.76 (2.29)	150	2.11 (3.02)	**-0.43 (0.70)**	**< .001**	.83

Note.
a Comparison of week 4 values by study arm, adjusted for baseline values and site.
b CO verified (<6ppm)
c Analyses were not run due to small sample sizes.

Statistical Analyses

Participants' baseline characteristics, including demographics and smoking history among those with available PheT data, were described and compared by randomization group (nicotine gum versus snus). Discrete variables were analyzed using Pearson's χ^2 test or Fisher's exact test and continuous variables were analyzed using t-tests and Wilcoxon Rank Sum tests as appropriate.

Participants were divided into status based on their cigarette and study product use at week 4; specifically, "Study Product Only" or "Study Product + Cigarette." Participants who reported 0 cigarettes via the IVR/TLFB, using at least one piece of study product, and demonstrated a CO < 6ppm were classified as "Study Product Only" (N = 106). Participants who reported using both their study product (at least one piece) and at least one cigarette in week 4 were classified as "Study Product + Cigarette" users (N = 185). Anyone who avoided all nicotine products (eg, cigarette and nicotine gum; N = 9) or were using cigarettes only (N = 8) were included in the full group comparisons, but not separately by status due to small numbers.

PheT was analyzed on the natural log scale to ensure normality. Change in PheT levels between week 4 and baseline within group were analyzed using paired t-tests. Differences in PheT levels at week 4 were analyzed by randomization group using linear regression models with fixed effects for baseline PheT levels, site, treatment, nicotine gum/snus use status (nicotine gum/snus only versus nicotine gum/snus and cigarette use), and an interaction between treatment and product use status. Pairwise comparisons were adjusted for multiple comparisons using a Bonferroni correction and p-values < .05 were considered statistically significant.

RESULTS

Participant

Of the 308 participants who provided urine samples at week 4, 46.4% were women with a mean age of 43.9 years (SD = 12.4). There were no baseline differences between smokers randomized to the nicotine gum versus snus conditions in age, sex, education, CPD, or nicotine dependence. Table 1 shows the complete baseline demographic and smoking history data by product group.

Urinary PheT

Table 2 summarizes the PheT levels by treatment group and product use status. All gum (gum only, $p < .001$; gum and cigarettes, $p < .001$) and snus (snus only, $p < .001$; snus and cigarettes, $p = .003$) groups demonstrated significant reductions in PheT from baseline to week 4. Pairwise comparisons at week 4 revealed significant status differences. Gum only and snus only groups demonstrated significantly greater reductions in PheT than statuses comprising of smokers who used both their study product and cigarettes (all $ps < .001$). No differences emerged between gum only and snus only users ($p = .87$). Among those who used both study product and cigarettes, no differences emerged between gum and snus groups ($p = .80$). Smokers assigned to the gum and snus groups, who continued to smoke cigarettes, reported smoking similar cigarettes per week at week 4 (27.2 and 28.6 cigarettes per week, respectively).

DISCUSSION

All participants showed reductions in urinary PheT regardless of whether they used only the product or they continued to smoke while using the product; however, this reduction was greater among those who completely switched to their assigned product. There was no difference between smokers provided with gum or snus on PheT levels at the end of the 4-week period. Thus, switching to snus or nicotine gum appears to have similar effects on smokers' levels of PheT. Important to note, even participants assigned to nicotine gum had measureable levels of urinary PheT, which is expected. Nicotine gum does not contain PAH; however, most individuals are exposed to some PheT in their food and environment. Thus, even nonsmokers often have measureable urinary PheT.

Similar to previous research, smokers who switched to snus showed reductions in PAH, as measured by PheT, even if they continued to smoke cigarettes.[9] However, this is just one biomarker and it is important to consider these results in conjunction with previous studies and the parent study of this current paper. That is, results are mixed as to whether switching to snus reduces exposures to NNK. Older studies show reductions in urinary total NNAL among smokers who switched to Marlboro or Camel snus;[9,18] however, when compared to nicotine gum, Camel snus resulted in less total NNAL reduction.[18] The present study's parent study showed significant reductions in total NNAL when smokers switched to nicotine gum, but not when they switched to snus.[13] Authors of the parent study noted that mixed findings could be due to an increase in measured NNK/pouch during the time in which these studies were conducted. In regards to switching to snus or nicotine gum completely versus partially (ie, continuing to smoke cigarettes), the parent study showed that those who completely switched to nicotine gum demonstrated significantly greater reductions in total NNAL than those who switched only partially; no differences emerged between use groups among smokers assigned snus.[13] Collectively, switching to snus may result in some reduced exposure to toxicants, but benefits are greater among smokers who switch to nicotine gum.

There are several limitations to the current study. First, the small sample size prevents examination of other potential important predictors of exposure (eg, quantity of product used, dependence, sex). Second, participants were provided specific instructions for product use and may not represent naturalistic use. Third, a measure assessing other potential exposure to PAHs was not included (eg, dietary), although there is no reason to believe that differences in dietary exposures would occur across product assignments. Despite these limitations, this paper improves upon our knowledge regarding the toxicant exposure experienced by smokers who switch to snus and how this profile compares to nicotine gum.

In the context of overall exposure to harmful constituents examined in this study and the parent study,[13] switching to snus appears to reduce smokers' exposure to some harmful constituents (ie, PAH as measured by PheT) at similar levels as nicotine gum, but not all, ie, greater reductions in total NNAL with nicotine gum (parent study).[13] These findings suggest that it is important for the US FDA Center for Drug Evaluation and Research to consider nicotine gum a potential harm reduction tool, particularly for smokers who are unwilling or unable to quit. This could mean encouraging use of nicotine gum as an alternative to smoking, while maintaining the larger goal of quitting nicotine use altogether. Researchers have proposed a

similar harm reduction model in the United Kingdom.[19] Some suggested changes include selling nicotine gum everywhere cigarettes are sold, thereby increasing the availability of these products, reducing nicotine gum costs, informing the public about the relative harm of different nicotine products,[19] and allowing modified risk claims on medicinal nicotine.

IMPLICATIONS FOR TOBACCO REGULATION

Nicotine gum and snus results in similar reductions in urinary PheT. Both nicotine gum and snus could be beneficial as harm reduction tools; however, in the context of overall exposure to harmful constituents (eg, PAH and tobacco-specific nitrosamines), nicotine gum may be superior to snus.

Human Subjects Statement

All procedures were approved by each site's institutional review boards.

Conflicts of Interest Statement

The authors declare no conflicts of interest.

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Original investigation

Prenatal Exposure to Snus Alters Heart Rate Variability in the Infant

Felicia Nordenstam MD[1], Bo Lundell MD, PhD[1], Gary Cohen PhD[2], Mesfin K. Tessma MD[3], Pauline Raaschou MD, PhD[4], Ronny Wickström MD, PhD[5]

[1]Department of Women's and Children's Health, Pediatric Cardiology Unit, Karolinska Institute, Stockholm, Sweden; [2]Department of Women's and Children's Health, Neonatal Unit, Karolinska Institute, Stockholm, Sweden; [3]Department of Learning, Information, Management, Ethics, Karolinska Institute, Stockholm, Sweden; [4] Department of Medicine, Clinical Epidemiology Unit, Karolinska Institute, Stockholm, Sweden; [5]Department of Women's and Children's Health, Neuropediatric Unit, Karolinska Institute, Stockholm, Sweden

Corresponding Author: Felicia Nordenstam, MD, Department of Pediatric Cardiology C8:34, Eugeniav.23, Astrid Lindgren's Children's Hospital, Karolinska University Hospital, 171 76 Stockholm, Sweden. Telephone: 08-51777041; Fax: 08-51777778; E-mail: felicia.nordenstam@ki.se

Abstract

Introduction: Maternal use of smoked tobacco during pregnancy causes significant morbidity and mortality in the human infant including alterations in autonomic control with increased risk of sudden infant death syndrome. We hypothesized that maternal snus (smokeless tobacco) use during pregnancy affects autonomic cardiac regulation in the infant, as measured by heart rate variability (HRV) and the low frequency and high frequency ratio (LF/HF ratio).

Methods: A prospective observational study of 56 infants of women who used snus (n = 23) or cigarettes (n = 13) during pregnancy versus tobacco- and nicotine-free controls (n = 19). The nicotine dose was estimated by questionnaires at 4 timepoints pre- and post-natally. The infants' urine cotinine concentration and HRV during 2 hours of sleep were studied 1–2 months after birth.

Results: LF/HF ratio was higher in snus (mean 3.31; 95% CI 2.78–3.83) and smoke (3.51;2.54–4.47) compared to controls (2.15; 1.76–2.54, p = .002). Early prenatal nicotine exposure "without" any further exposure increased the LF/HF ratio (3.19; 2.55–3.84, p = .02). Continuous prenatal nicotine exposure "without" postnatal exposure was also associated with a residual increase in LF/HF ratio (4.40; 3.38–5.42, p < .001). There was no difference between infants exposed to smokeless versus smoked tobacco, suggesting a common constituent (nicotine) altering autonomic cardiac regulation.

Conclusion: Infants to mothers who used snus during pregnancy showed lower vagal activity with an increased LF/HF ratio compared to controls, and similar to infants of smokers. Even early prenatal exposure to snus has a lasting impact on autonomic cardiac regulation suggesting a fetal "re-programing" of the developing autonomic nervous system.

Implications: The results indicate that smokeless tobacco (Swedish snus) affects the developing autonomic nervous system during gestation. Even if exposure is interrupted during the first or second trimester, effects in autonomic cardiac regulation are seen in the 1–2 month-old infant. This underlines the importance of abstaining from all types of tobacco use during the whole pregnancy. Our findings may also have more general relevance to other routes by which nicotine can be delivered to a fetus and newborn.

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Introduction

Tobacco use by pregnant women is a global public health hazard with both short- and long-term effects on the fetus and child. Exposure to tobacco and nicotine by pregnant women differs across cultures and between continents, and includes smoking, use of chewing tobacco and snus as well as use of nicotine replacement products such as patches, gums, and e-cigarettes. The use of tobacco alternatives to cigarettes and a combination of different nicotine sources are increasing amongst youth in the United States and may produce a different pattern of nicotine addiction in the near future.[1] In the industrialized world, the use of oral, smokeless tobacco during pregnancy is rare, except in Sweden and Norway, where oral moist snuff (Swedish snus) use amongst pregnant women is not uncommon.[2,3] While 1.3% of pregnant women use snus in Sweden overall, there are regional differences, with more than 5% reported in some northern parts (Swedish National Board of Health and Welfare 2014).[4]

The detrimental effects of maternal smoking during pregnancy and postpartum (eg, increased rates of preterm birth, intrauterine growth retardation and sudden infant death syndrome [SIDS]) are well known.[5,6] Although cigarettes contain many toxins, nicotine is the proposed linkage between maternal smoking and increased risk of SIDS.[7] Nicotine passes through the placenta and exposes the fetus to nicotine levels comparable to or even higher than those in the pregnant woman herself.[8] Swedish snus is pulverized or powdered tobacco that contains water, sodium carbonate, sodium chloride, moisturizer, flavoring, and nicotine.[9] It is placed behind the upper lip as a loose powder or sealed in small pouches. Although snus delivers lower concentrations of some harmful chemicals, for example, nitrosamines and metals, than other tobacco products, it does deliver high doses of nicotine.[9,10]

Studies from Swedish health registers show that adverse neonatal outcomes such as preterm birth,[11] stillbirth[12] and neonatal apneas[13] are associated with prenatal exposure to snus. Lower birth weight[14,15] has also been described, although the risk of being born small for gestational age (SGA) is not as pronounced as for active maternal smoking during pregnancy.[3,15,16] Animal studies indicate that nicotine is, by and large, partly responsible for these adverse effects,[17] but discrepancies between the adverse effects of smoked tobacco versus snus on neonates suggest that nicotine may not be the only culprit.[15,18] In humans, comparative data on the effects of prenatal nicotine exposure via routes other than smoking are extremely limited.[17,19] This is an important and troublesome knowledge gap, particularly because nicotine replacement therapy (NRT) is approved by some authorities as a smoking cessation strategy in pregnancy, despite concerns about its lack of safety or efficacy.[20]

In this study we have made use of the prevalence of snus usage amongst pregnant women in Sweden to investigate the likely effect of prenatal and postnatal exposure to nicotine on infant HRV. HRV is a functional marker of autonomic cardiorespiratory control. Small fluctuations in the time interval between heartbeats (the interval between the R peaks of QRS complexes; R-R interval) of an ECG recording are measured and expressed as discrete bands across a frequency spectrum. The high frequency (HF) component is generally thought to represent efferent vagal activity, whilst low frequency (LF) power is more complex, modulated by both vagal and sympathetic influences. The LF/HF ratio is considered to be an indirect index of overall sympatho-vagal balance.[21] Altered cardiac autonomic regulation has long been thought to be a "marker" of increased risk for SIDS in infants of smoker mothers.[22–24] We therefore studied HRV of 4–10 week-old infants to investigate whether maternal use of smokeless tobacco (snus) during pregnancy is also associated with altered cardiac autonomic regulation.

Methods

Study Design

This prospective observational study is a part of a larger Swedish project studying the effects on the health of the infant of perinatal smokeless tobacco (Swedish snus) exposure. Healthy pregnant women (n = 55) were included prospectively at their first antenatal appointment. All women included in the study thereafter completed standardized questionnaires at the time of inclusion, again at gestational week 32, after delivery and at the time their infants were tested at 1–2 months after birth. Upon inclusion, women were grouped into snus users (n = 23), smokers (n = 13) and nicotine-free controls (n = 19). Dual users were excluded from the analysis.

To investigate the effects of "prenatal exposure without postnatal exposure," the tobacco-exposed groups were merged into groups by time of exposure: the first comprising infants with only early prenatal exposure (until gestational week 6–26); and the second comprising those infants exposed continuously during the entire period of gestation. Children whose mothers did not use tobacco postnatally, or who were formula-fed by snus-using mothers, were labelled as not postnatally exposed.

In addition, infants with "prenatal and (ongoing) postnatal exposure" were compared to nicotine-free controls and the prenatal exposure only group. The smoke group was exposed postnatally by smoke from the mother and by breast milk if breast fed. Second-hand smoke was considered prevalent if the mother was exposed to passive smoking.

At 4–10 weeks after delivery, mother and infant presented to the nursery at their hospital. A urine sample from the infants was collected for cotinine analysis. A portable ECG recorder (LifeCard CF, Spacelabs Healthcare Limited, Hertford, UK), connected with three channel electrodes, was used to record a digital 24-hour ECG (sampling frequency 1024 Hz). All recordings were made at home.

Questionnaires

Questions included medications and health status of the mother and infant as well as the type and pattern of tobacco use. The questionnaires also covered mothers' exposure to second-hand smoking (hours per day) and maternal use of alcohol and drugs. The reported average daily snus intake numbers (0–1, 2–3, 4–5, 6–7, >7 portions) were converted to mean nicotine dose (mg/day) taking into account the nicotine content of different brands. Regular snus brands contain ~8 mg/portion whereas "mini" portions contain ~4 mg/portion. Cigarettes were defined accordingly (<1, 1–5, 6–10, 11–15, 16–20, >20) with an estimated nicotine dose of 2 mg/cigarette.[25,26] Daily doses were estimated for each week during pregnancy and after the baby was born. Doses were defined as high if >56 mg/day for snus or >40 mg/day for smokers.[27] The different ways of absorption and individual differences in smoking a cigarette or in the amount of time used for each snus portion makes it difficult to directly compare the estimated nicotine doses between the two tobacco groups.[25,26]

Cotinine Analysis

Following preparation, urine samples were analyzed by mass spectrometry (UHPLC-MS/MS in MRM mode). Measuring range was from 5–1000 ng/mL for all analyses. For urine analysis of cotinine, precision at 7 and 700 ng/mL was 8.3 and 4.3 CV%. An internal

standard (cotinine-d_4 from Sigma Aldrich, Stockholm, Sweden) was prepared using blank urine and urine analyses and a 50 µL human urine sample was mixed with 100 µL internal standard.

ECG Recordings

The digital ECG recordings were edited and a HRV analysis was made with Impressario software (Version 7 Del Mar Reynolds Medical, Hertford, UK). All 24-hour recordings were reviewed by a single analyst (FN) to ensure accurate detection of all QRS complexes (crucial because the quality of the HRV analysis depends upon accurate labeling of the QRS complexes). Editing was made on the full 24-hour data file and a 2-hour artifact-free period during night-time sleep (between 10:00 PM and 6:00 AM) was selected for frequency domain analysis.

At least 22 hours of edited data from the 24-hour recording were required for acceptable detection of extra systoles and arrhythmias. Complexes classified as ectopic or noise were rejected.

We performed spectral analysis of 5-minute epochs during the 2-hour period of sleep. Early beat-to-beat variation was set to < 40%.[21] The interval between the R waves in every normal QRS complex was measured; only normal-to-normal R-R intervals were used the HRV analysis (ie, extra systoles were excluded).

For the analysis of the frequency domain indexes, beat-to-beat fluctuations were transformed to the frequency domain by fast Fourier transformation, and the specific measures were computed as the square root of the areas under the power spectrum, expressed as ms^2/Hz.

Spectral indexes over three standard frequency regions of interest was determined; very low frequency (VLF, 0.017–0.05 Hz), low frequency (LF, 0.05–0.150 Hz), and high frequency (HF, 0.15–0.5 Hz). Total power (all frequencies between 0.017–0.5 Hz) was also determined as well as the ratio between low and high frequency (LF/HF). The spectral analysis was made in accordance with the standards of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[21]

Ethical Approval

This study was approved by the local ethics committee in Stockholm, and written consent was obtained from the mother at the time of inclusion.

Statistics

Descriptive statistics were presented as mean and standard deviations and median (range) for numerical variables, or as frequencies for categorical variables. Differences between subject characteristics of the groups were examined using chi-square test or Fischer's Exact test for categorical variables and one-way ANOVA for continuous variables. Further analysis with Tukey's post hoc pairwise comparison was made if the overall ANOVA test was significant. A two-way ANOVA was used to examine the interaction between nicotine group and the sex of the infant. Simple main effects were used to evaluate heterogeneous effects in the presence of significant interactions. Effect size was examined by difference in mean, 95% confidence interval, partial eta-squared (η^2), *R*-squared, and the Spearman correlation coefficient. The Mann–Whitney *U*-test was used because of deviations from normality in the estimated nicotine exposure and urine cotinine levels. Spearman's correlation was used to examine the relationship between maternal nicotine exposure and measured levels of cotinine in the urine of the infants. Multiple linear regression was employed to control for potential confounders. The dependent variable was HRV and relevant clinical and demographic explanatory variables were included in the model, including maternal age, sex of the infant and breast feeding. We followed a systematic approach to identify the explanatory variables. At first, simple linear regression was used to examine the relationship between the predictor variables and the outcome variable in univariate models. After completing the univariate analyses, we selected variables for the multivariate analyses. Any variable whose univariate test had a *p*-value < .1 was considered as a candidate for the multivariate model, along with variables of known clinical importance. Variables identified were entered into a multivariate model. Potential outlier and multi-collinearity problems were checked. Residual plots, normal probability plot and Cook's distance were used to assess model assumptions. A Cook's distance value (D) > 1 was taken as a criterion to constitute a strong indication of outlier problems and $D > 4/n$ a criterion to indicate a possible problem, where n was the sample size. A Variation Inflation Factor (VIF) greater than 4 was considered as the cut-off criterion for deciding when a given independent variable displayed "too great" a multicollinearity problem. SPSS for Windows version 23 (IBM Corp, Armonk, NY) was used for all statistical analysis. The level of significance was specified at 0.05.

To attain 80% power and considering a 5% significance level and expecting a mean difference in LF/HF of 1.15 (between control and exposed groups) with a standard deviation of 0.95 and design effect of 1.5, we estimated that 54 subjects would be sufficient.[28]

Results

Participants

The 55 healthy pregnancies resulted in 56 infants, including one twin pregnancy (born preterm, week 34). Except for preterm birth, there was no morbidity or hospitalization reported for any of the infants. We did not observe significant differences between the groups except that tobacco-exposed mothers were younger than controls ($p = .009$), and that the estimated nicotine exposure of snus versus smokers during the first trimester and at the time of examination differed ($p < .001$ and $p = .009$, respectively). There was no reported use of drugs or alcohol in any of the groups. Descriptive data are displayed in Table 1.

Nicotine Consumption

Eleven women stopped using tobacco during pregnancy: six smokers quit during gestational week 6–10, and five snus users quit during gestational week 6–26. None of the mothers in the study changed their source of tobacco during the study, and none used nicotine gums or patches. In addition to maternal nicotine consumption, the postnatal exposure of the infants also depended on passive smoking and whether the child was breast fed or not. The reported maternal nicotine use was highly correlated to the urine cotinine levels of the infants (Spearman's correlation coefficient = 0.88, $p < .001$, in Supplementary Figure 1).

Cotinine Concentration in Urine

All controls had cotinine levels < 0.7 ng/mL, the snus group ranged from 0–408 ng/mL and the smoke group ranged from 0–129 ng/mL. All the infants in the snus and smoke groups without any reported postnatal exposure had cotinine levels < 0.7 ng/mL.

Electrocardiogram

Two ECG recordings were lost (one from the snus group due to technical problems and one from the smoke because the mother

disconnected the recording device), resulting in 54 recordings available for analysis. We did not observe statistically significant differences between controls, snus or smoke groups in R-R intervals (min, max or mean); the results are displayed in Table 2. There were occasional extra beats found in all groups, mostly supraventricular extra systoles and occasionally ventricular extra systoles, but no other arrhythmias were detected.

Heart Rate Variability

Spectral analysis of HRV showed that infants in the two tobacco-exposed groups had a comparable LF/HF ratio, which was significantly higher than that of the control group.

One-way ANOVA showed the statistically significant difference in LF/HF ($F_{2,51}$ = 6.97, p = .002, partial η^2 = 0.22). The main differences for infants in the snus group (mean difference = 1.16, 95% CI = 0.29–2.02, p = .006) and the smoke group (mean difference = 1.36, 95% CI = 0.32–2.4, p = .007) were statistically significant higher compared to controls. However, we did not observe any difference between infants in the snus and smoke groups (mean difference = 0.20, 95% CI = −0.8 to 1.2, p = .88) (Table 2 and Figure 1).

We also observed a difference in HF ($F_{2,51}$ = 3.2, p = .049, partial η^2 = 0.11) with a higher HF activity in the control group compared to smoke group (mean difference = 64, 95% CI = 0.14–128, p = .049). We were unable to detect any respiratory sinus arrhythmia (RSA) peak up to and including 0.8 Hz in the HF area in any of the groups.

Two-way ANOVA revealed a significant interaction between the tobacco group and the baby's sex (p = .03), which suggests that the effect of tobacco on LF/HF was heterogeneous. The mean differences for both the snus group (mean difference = 1.65, 95% CI = 0.56–2.7, p = .003) and smoke group (mean difference = 2.7, 95% CI = 1.4–4, p < .001) compared to controls were higher among boys. We did not observe significant difference amongst girls, with a difference of (0.84) amongst snus compared to controls (95% CI, -0.9–1.8, p = .76).

Table 1. Characteristics of Participants in Control, Snus and Smoke Group, Means and (*SD*)

	Control	Snus	Smoke
Women (*n* = 55)	*n* = 19	*n* = 23	*n* = 13
Age mother (years)	33.5 (4.7)	29 (6.3)	27.8 (4.8)
Weight mother (kg)	64.1 (9.6)	66 (11.3)	63.2 (9.1)
Height mother (cm)	167.4 (4.1)	165.5 (5.7)	164.8 (5.4)
Parity (*n*)	1.2 (0.5)	1.2 (1.1)	0.4 (0.5)
Nicotine first trimester (mg/day)	0	42.3 (11–56)	20 (6.7–40)
Nicotine dose at examination (mg/day)	0	17.5 (0–56)	2 (0–40)
Vaginal/cesarean delivery (*n*)	15 (79%)/4 (21%)	20 (87%)/3 (13%)	9 (69%)/4 (31%)
SHS exposure during pregnancy (*n*)	0	2 (9%)	3 (23%)
SHS exposure postpartum (*n*)	0	1 (4%)	2 (15%)
Infants (*n* = 56)	*n* = 19	*n* = 24	*n* = 13
Gestational (week)	40 (0.9)	39.1 (2.0)	39.9 (1.1)
Birthweight (g)	3727 (264)	3426 (555)	3501 (670)
SGA/LGA (*n*)	0/2 (10%)	0/0	2 (15%) /2 (15%)
Age at examination (week)	6 (1.4)	5.8 (1.6)	6.3 (1.5)
Weight at examination (g)	4833 (447)	4860 (665)	4841 (758)
Boy/girl (*n*)	7 (37%)/12 (63%)	12 (50%)/12 (50%)	5 (38%)/8 (62%)
Cotinine urine (ng/mL)	0.07 (0.2)	59.2 (95.9)	29.6 (44.0)
Breast fed/formula (*n*)	19 (100%)/0	19 (79%)/5 (21%)	11 (85%)/2 (15%)
Postnatal tobacco exposure (*n*)	0	14 (58%)	7 (54%)

SGA = Small for gestational age; LGA = large for gestational age.
Characteristics of women are data from the first visit to the maternity center. Second-hand smoke (SHS), defined as maternal exposure.

Table 2. Heart Rate Variability in Control, Snus and Smoke Groups and Prenatal Exposure "without" Postnatal Exposure (Prenatal Early Exposure and Continuous Prenatal Exposure Groups), in mean (*SD*)

	Control *n* = 19	Snus *n* = 23	Smoke *n* = 12	*p* ANOVA	Control *n* = 19	Prenatal-early *n* = 8	Prenatal-continuous *n* = 7	*p* ANOVA
max RR (ms)	733.5 (73.6)	728.3 (99.4)	670.6 (92.4)	.13	733.5 (73.6)	685.6 (108.4)	709.8 (95.4)	.42
min RR (ms)	303.5 (43.4)	304.4 (32.9)	296.9 (38.8)	.85	303.5 (43.4)	302.7 (44.1)	294.6 (22.9)	.88
mean RR (ms)	466.8 (26.5)	460.5 (36.7)	458.8 (24.6)	.73	466.8 (26.5)	462.7 (32.2)	438 (14.6)	.056
sd RR (ms)	40.6 (8)	39.9 (11.2)	37.2 (6.3)	.59	40.6 (8)	34.9 (7.6)	35.4 (6.7)	.14
VLF (ms^2)	322.8 (170.6)	362.5 (269.8)	314.4 (122.6)	.76	322.8 (170.6)	317.2 (160.1)	272.8 (81.4)	.76
LF (ms^2)	249.4 (100)	294.4 (198.4)	217.6 (101.9)	.34	249.4 (100)	217.4 (121.5)	231 (125.7)	.78
HF (ms^2)	136.2 (87.1)	96.9 (68)	72.2 (46.7)	.049	136.2 (87.1)	76.4 (57.1)	51.8 (23.1)	.023
total power (ms^2)	708.3 (309.4)	753.8 (500.8)	604.3 (232.9)	.57	708.3 (309.4)	611 (323.8)	555.7 (189.3)	.46
LF/HF	2.15 (0.81)	3.31 (1.2)	3.51(1.51)	.002	2.15 (0.81)	3.20 (0.77)	4.40(1.1)	< .001

HF = High frequency; LF = low frequency; VLF = very low frequency.

On merging the two tobacco groups for prenatal-early exposure ($n = 8$), prenatal-continuous exposure ($n = 7$) and controls ($n = 19$), we found statistically significant differences between the groups for the dependent variable LF/HF ($F_{2,31} = 18.06$, $p < .001$, partial $\eta^2 = 0.54$) and HF ($F_{2,31} = 4.3$, $p = .023$, partial $\eta^2 = 0.22$), respectively (Table 2). There was a higher LF/HF ratio in the early prenatal group than in the control group (mean difference = 1.04, 95% CI = 0.15–1.9, $p = .02$). The prenatal-continuous exposure group had an even higher LF/HF ratio than that of controls (mean difference = 2.24, 95% CI = 1.3–3.2, $p < .001$), with the difference significant compared to prenatal-early exposure group (mean difference = 1.2, 95% CI = 0.1–2.3, $p = .037$). The HF activity of the prenatal-continuous group was lower than that of the control group (mean difference = −84.4, 95% CI = −163.2 to −5.6, $p = .03$). The results of prenatal tobacco exposure are shown in Table 2 and Figure 2.

Infants with both pre- and postnatal exposure (total $n = 20$: snus $n = 13$, smoke $n = 7$) had higher LF/HF compared to controls (mean difference 0.94, 95% CI 0.06–1.81, $p = .033$), with no significant difference versus prenatal-only exposure ($p = .206$).

With respect to the acute dose–response relationship at the time of examination, we found no statistically significant relationship between cotinine levels in infants' urine or the reported nicotine dose and any HRV values.

We did not observe a statistically significant relationship between LF/HF and birth weight, baby's sex, mother's weight, parity, gestational week at delivery, infants' age and weight at examination in a multiple linear regression model. Simple linear regression revealed that breast feeding was a significant predictor of LF/HF (β = −0.53, 95% CI = −1.03 to −0.3, $p = .04$), accounting for 8% of the variance in LF/HF. However, multiple linear regression displayed non-significant findings when LF/HF was regressed on relevant clinical and demographic variables. We did not observe violations of assumptions of ANOVA and regression analysis and multicollinearity and outlier problems.

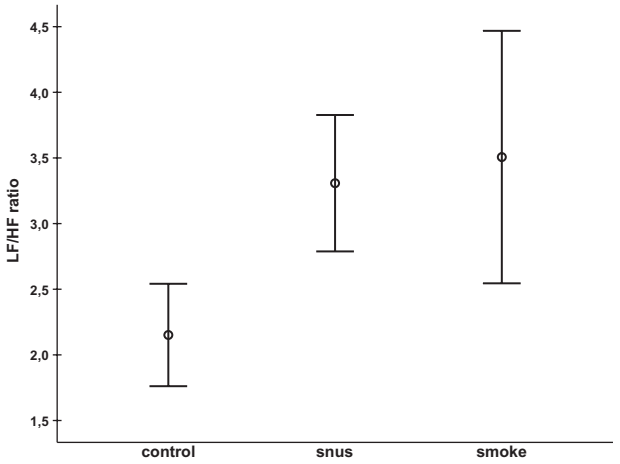


Figure 1. HRV in control, snus and smoke groups. LF/HF ratio in original groups based upon inclusion, mean and 95% CI. LF/HF ratio: low frequency/high frequency ratio made by spectral analysis of heart rate variability.

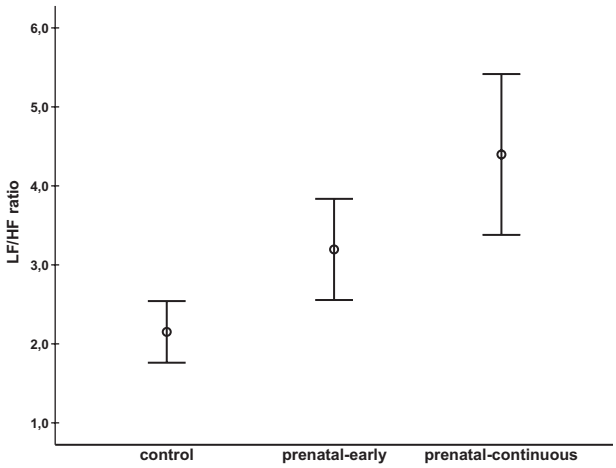


Figure 2. HRV in prenatal exposure without postnatal exposure. LF/HF ratio in control, prenatal early exposure without any further exposure and prenatal continuous exposure (whole gestation without postnatal exposure), mean and 95% CI. The snus and smoke groups are merged together in the exposure groups.

Discussion

We studied HRV in infants of mothers who used snus during pregnancy, which, to our knowledge, has not been done previously. Studies have shown reduced HRV (especially in parasympathetic activity) of infants exposed to cigarette smoke and those at higher risk of SIDS. In this study we demonstrate effects of prenatal tobacco exposure on cardiovascular regulation that extended well beyond the neonatal period. We found a higher LF/HF ratio in both snus- and smoke-exposed groups compared with controls, which was partly explained by lower HF activity, suggesting less vagal activity. The lack of RSA peak usually found in the HF area is conceivably due to the peak frequency of RSA lying above the 0.8 Hz cut-off or also possibly because RSA is low-to-absent at this early age, as suggested by human and animal data.[23,29,30] There was no significant difference between LF/HF ratios of the two tobacco-exposed groups, which suggests that nicotine may be the common constituent of tobacco mediating these effects regardless of the precise route by which it is absorbed. Cotinine concentrations in the urine of the infants of each group showed a good correlation with the reported nicotine doses by the mother and the highest values were observed in the infants of snus users.

Power spectral analysis of beat-to-beat variations in heart rate provides an indirect index of the autonomic modulation of the cardiovascular system. In the fetus and infant, sympathetic drive tends to dominate, with a gradual increase in parasympathetic activity and progressive decrease in sympathetic modulation of the autonomic system with advancing age.[31] Altered autonomic cardiorespiratory regulation is associated with an increased risk of SIDS. Infants who later succumbed to SIDS appeared to show reduced overall heart rate variability,[22] and lower variability due to reduced RSA during sleep.[32] An increased LF/HF ratio has been shown in infants born to smoking mothers during REM sleep.[33]

Nicotine per se may be of importance for the infants' health, as a large body of experimental animal data supports the concept that prenatal and perinatal nicotine exposure is detrimental to postnatal autonomic control. This autonomic dysfunction may be involved in SIDS, although the exact mechanism is not fully understood. Nicotine-exposed rat pups show enhanced expression of cardiac M2-muscarinic cholinergic receptors involved in inhibitory autonomic actions.[34] Mice lacking the b2-nAChR subtype exhibit autonomic control deficits (including unstable breathing, impaired arousal and catecholamine biosynthesis), much like nicotine-exposed mice.[35] Furthermore, nicotine-exposed animals exhibit

impaired cardiac regulation of sino-atrial reactivity,[7] augmented inhibitory cardio-vagal neural control,[36] and changes in medullary neurotransmitter receptors and altered heart rate variability.[23] Thus, a number of pathophysiological effects of prenatal nicotine exposure may interact.

We also found an increased LF/HF ratio in infants exposed to tobacco before but not after birth. This has been difficult to study before because most infants born to smokers receive a combination of prenatal and postnatal exposure. Most animal studies also include a combination of prenatal and perinatal exposure. Our study included infants whose exposure effectively ceased before or at birth, either because mother ceased tobacco use during pregnancy, or chose not to breast feed (snus group). The persistence of a relatively high LF/HF ratio for several months after exposure effectively ended suggests that there is a residual impact on autonomic system function. This may be due either to delayed maturation[29] or an altered trajectory of development, a finding which is supported by several studies. Human fetuses exposed early-on to tobacco show persistent changes in the expression of nicotinic and muscarinic acetylcholine receptors in the brainstem and cerebellum.[37] Infants of smokers who experience a combination of prenatal and postnatal exposure exhibit persistent mild dysfunction in blood pressure control during sleep.[38] Rat studies reveal long-lasting effects of prenatal nicotine exposure into adolescence and adulthood, such as persistent cholinergic hypoactivity and an altered sensitivity to nicotine.[39] The high levels of nicotine cholinergic receptors in the brainstem in mid-gestation, and rapid changes in the profile of receptor subtype expression during late gestation suggested that these are periods of particular vulnerability to nicotine,[40] in addition to any changes that occur earlier, for example, during the first trimester.[41] Our finding that autonomic regulation may be altered even if the infant is only exposed during first trimester suggests there is probably no time-point during development when exposure is "safe," that is to say, when the autonomic system is unaffected by constituents of tobacco, such as nicotine. Although we cannot establish causality, it is conceivable that prenatal nicotine exposure exerts a programming effect, altering the normal development of the autonomic regulation.

Boys may be more vulnerable to prenatal and postnatal tobacco exposure than girls, the former exhibiting a more pronounced rise in LF/HF ratio. This finding is supported by earlier studies of maternal smoking and secondhand exposure on RSA in infants (exposed boys had lower RSA vs. exposed girls).[42] Boys have an increased risk of SIDS,[43] and other studies also support the hypothesis that sex differences in HRV are associated with common risk factors such as preterm birth and intrauterine growth retardation.[44,45] Sex differences in HRV of healthy children is much more controversial.[46,47]

Our study has a number of strengths: it was a prospective study from early gestation to infancy, we conducted a high quality HRV analysis and carefully documented tobacco exposure at four longitudinal time points, and used an objective biomarker measures of nicotine exposure (cotinine analysis of the infants' urine). There were, however, limitations, the most important of which was the relatively small size of the groups, which also limits stratified analysis. Although we cannot definitively rule out the possibility of differences between the smoke-exposed and snus-exposed groups, our data suggest that this is unlikely. Secondly, the infants' actual nicotine exposure was estimated at only one time-point (ie, cotinine in urine at the time of investigation). Although highly correlated to the mothers' reported dose, a single urinary estimate may not be truly representative of the level of exposure at other times, or throughout gestation. Finally, although we did take into account passive smoking and the use of alcohol and other drugs, it is possible that there might be other factors in addition to nicotine that influence HRV.

In conclusion, we demonstrate an effect on autonomic regulation in infants of mothers using snus during pregnancy, in terms of an increase in LF/HF ratio comparable to that of infants of active smokers. Even very early prenatal exposure to tobacco has a long-lasting impact on autonomic cardiac regulation. It is apparent that tobacco can affect the developing infant regardless of the form, or route by which, or time-point during development when exposure actually occurs. Our data consequently emphasize the importance of abstaining from all forms of tobacco use during pregnancy, and suggest that there are no safe forms or safe periods for its use during pregnancy. Our findings may also have more general relevance to other routes by which nicotine can be delivered to a fetus and newborn, including NRT for smoking cessation.

Supplementary Material

Supplementary data are available at *Nicotine & Tobacco Research* online.

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Declaration of Interests

None declared.

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Research: Epidemiology

Use of Swedish smokeless tobacco (snus) and the risk of Type 2 diabetes and latent autoimmune diabetes of adulthood (LADA)

B. Rasouli[1], T. Andersson[1,2], P.-O. Carlsson[3], V. Grill[4,5], L. Groop[6], M. Martinell[7], K. Midthjell[8], P. Storm[6], T. Tuomi[9] and S. Carlsson[1]

[1]Epidemiology Unit, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, [2]Center for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, [3]Department of Medical Sciences, Uppsala University, Uppsala, Sweden, [4]NTNU Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, [5]Department of Endocrinology, Trondheim University Hospital, Trondheim, Norway, [6]Department of Clinical Sciences in Malmö, Lund University, Malmö, [7]Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden, [8]HUNT Research Centre, Department of Community Medicine and General Practice, Norwegian University of Science and Technology, Levanger, Norway and [9]Department of Medicine, Helsinki University Central Hospital and Research Program for Diabetes and Obesity, University of Helsinki, Helsinki, Finland

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Abstract

Aims It has been suggested that moist snuff (snus), a smokeless tobacco product that is high in nicotine and widespread in Scandinavia, increases the risk of Type 2 diabetes. Previous studies are however few, contradictory and, with regard to autoimmune diabetes, lacking. Our aim was to study the association between snus use and the risk of Type 2 diabetes and latent autoimmune diabetes of adulthood (LADA).

Method Analyses were based on incident cases (Type 2 diabetes, n = 724; LADA, n = 200) and population-based controls (n = 699) from a Swedish case–control study. Additional analyses were performed on cross-sectional data from the Norwegian HUNT study (n = 21 473) with 829 prevalent cases of Type 2 diabetes. Odds ratios (OR) were estimated adjusted for age, BMI family history of diabetes and smoking. Only men were included.

Results No association between snus use and Type 2 diabetes or LADA was seen in the Swedish data. For Type 2 diabetes, the OR for > 10 box-years was 1.00 [95% confidence interval (CI), 0.47 to 2.11] and for LADA 1.01 (95% CI, 0.45 to 2.29). Similarly, in HUNT, the OR for Type 2 diabetes in ever-users was estimated at 0.91 (95% CI, 0.75 to 1.10) and in heavy users at 0.92 (95% CI, 0.46 to 1.83).

Conclusion The risk of Type 2 diabetes and LADA is unrelated to the use of snus, despite its high nicotine content. This opens the possibility of the increased risk of Type 2 diabetes seen in smokers may not be attributed to nicotine, but to other substances in tobacco smoke.

Diabet. Med. 34, 514–521 (2017)

Introduction

Cigarette smoking is a known risk factor for Type 2 diabetes [1] and is associated with impaired insulin sensitivity [2], proposedly linked to exposure to nicotine [2]. Swedish moist snuff (snus) is a smokeless tobacco product traditionally used most frequently by men; the prevalence of snus use among women is low [3]. Users place a portion or sachet of snus between the gum and upper lip [4]. The nicotine content of snus is higher than that of cigarettes [5]; it may consequently also promote Type 2 diabetes. Few studies have addressed this question and the results are inconclusive [6–8]. Smoking is common among snus users, and one reason for the conflicting results may be insufficient adjustment for smoking. One way to handle this confounding factor is to restrict the analyses to those who have never smoked. However, studies conducted to date have been too small to allow for such analyses. Also, previous studies have not had the power to analyse the impact of different degrees of snus use on Type 2 diabetes.

Nicotine is known to have anti-inflammatory [9] and immune-modulating [10,11] effects, which, theoretically, might increase or decrease the risk of autoimmune diabetes. In a previous study, smokers displayed a reduced risk of latent autoimmune diabetes of adulthood (LADA) [12]. It could be hypothesized that snus with its higher nicotine

Correspondence to: Bahareh Rasouli. E-mail: bahareh.rasouli@ki.se

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What's new?

- The risk of Type 2 diabetes and latent autoimmune diabetes of adulthood (LADA) is not significantly affected by the use of snus, despite its high nicotine content.
- These results raise the possibility that the excess risk of Type 2 diabetes seen in smokers is due to components of tobacco smoke other than nicotine.

content may have a stronger protective effect than smoking. In line with that notion, snus has been linked to a reduced risk of multiple sclerosis, which is also an autoimmune disease [13]. Whether snus use indeed reduces the risk of autoimmune diabetes remains to be investigated.

The aim of this study was to clarify the role of snus use in the development of Type 2 diabetes and LADA, taking into account dose and duration of use, and co-exposure to smoking. These clarifications are important because 20% of men in Sweden use snus on a daily basis [14] and its use is steadily on the rise in Norway and the USA [15].

Methods

ESTRID study

This study was based on data from the epidemiological study of risk factors for LADA and Type 2 diabetes (ESTRID) [16]. ESTRID is a sub-study of All New Diabetes in Scania (ANDIS; http://andis.ludc.med.lu.se). ANDIS is a large-scale study with the aim of registering all new cases of diabetes in the region of Scania (Southern Sweden; ~ 1 300 000 inhabitants) to improve the classification of diabetes into subgroups on the basis of genetic and phenotypic information. All people with new-onset LADA recorded in ANDIS since 2010, together with a random sample of people with Type 2 diabetes (four per LADA case), were invited to participate in the ESTRID. Controls aged ≥ 35 years were randomly selected from the population of Scania without diabetes. Six controls were selected per LADA case, corresponding to one control per case of diabetes (LADA or Type 2). ESTRID is a density-sampled case–control study [17]; it included incident cases and controls that were matched according to participation date and residential area [17]. In 2012, ESTRID expanded the recruitment to All New Diabetes in Uppsala (ANDIU; http://www.andiu.se/), a sister study to ANDIS conducted in the County of Uppsala (middle of Sweden; ~ 300 000 inhabitants) (Fig. S1).

This study is based on cases and controls collected until July 2015, with a participation rate of 81% (cases) and 66% (controls). The analysis was restricted to men because snus use is very rare in women (4.3% of female participants). Those eligible for the current investigation were thus all men with complete data on snus use and covariates of interest, including 200 LADA cases, 724 Type 2 diabetes cases and 699 controls. Of the participants, 97% came from Scania and 3% from Uppsala. Each participant provided informed consent and ethical approval was obtained from ethical review board in Stockholm.

Tobacco habits and covariates

Participants in ESTRID filled out an extensive questionnaire covering lifestyle, health and sociodemographic factors. People with diabetes received questionnaires as close as possible to the time of diagnosis. Questions regarding lifetime use of snus included number of snus boxes consumed per week (each containing 25–50 g), separate for every period, and we used this to calculate the duration of snus use. One box-year was defined as consuming one box of snus per day for one year (calculated in ever-snus users). In addition, intensity of snus use was assessed in two categories in ever-snus users (light, < 5 boxes/week; and heavy, ≥ 5 boxes/week). Information on smoking was obtained in a similar fashion. The cumulative dose of smoking (pack-year) was assessed in ever-smokers. One pack-year was regarded as smoking 20 cigarettes per day for one year. We defined an index date for each case as the year of diagnosis, and for each control as the year of participation. The exposure to different forms of tobacco was assessed for the period prior to the index year.

Classification of people with diabetes

All people diagnosed with diabetes within the healthcare system of each county provided blood samples for serological analyses upon diagnosis. Glutamic acid decarboxylase antibodies (GAD antibodies) were measured by enzyme-linked immunosorbent assay (RSR Ltd, Cardiff, UK). The cut-off for positivity was 10 IU/ml [18]. At a cut-off level of 10.7 IU/ml, the sensitivity was 84% and the specificity was 98% [18]. C-Peptide status was analysed by IMMULITE 2000 (Siemens Healthcare Diagnostics Product Ltd, Llanberis, UK) or by Cobas e 601 analyser (Roche Diagnostics, Mannheim, Germany) [19]. People with diabetes with age-at-onset ≥ 35 years were classified as having LADA if they were GAD antibody positive (≥ 10 IU/ml) with C-peptide ≥ 0.2 nmol/l (IMMULITE)/or ≥ 0.3 nmol/l (Cobas e 601), and as Type 2 diabetes cases if they were GAD antibody negative (< 10 IU/ml) with C-peptide ≥ 0.6 nmol/l (IMMULITE)/or ≥ 0.72 nmol/l (Cobas e 601). This is in line with generally used criteria for defining LADA [20], except for C-peptide levels, which were used as an indicator of preserved insulin secretion. Fasting plasma glucose and C-peptide measurements were used to calculate the parameters homeostasis model assessment of insulin resistance (HOMA-IR) to estimate insulin resistance and HOMA-%B to assess β-cell function [21].

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Norwegian HUNT study

HUNT is a large population-based study which consists of three consecutive health surveys conducted between 1984 and 2008 in the Nord-Trøndelag County in Norway [22]. Information on snus use was available for the HUNT3 survey, performed between 2006 and 2008. All inhabitants aged ≥ 20 years were invited to participate in the HUNT3 survey. The attendance rate was 54% (n = 50 839). We restricted the analyses to men (n = 21 473) because of the low prevalence of snus use among women (4.4%). People with Type 2 diabetes were diagnosed at ≥ 35 years and were GAD antibody negative (< 0.08 antibody index; n = 829). GAD antibody positivity was used to identify adult-onset autoimmune diabetes (n = 41; including people with LADA and Type 1 diabetes with adult onset), but as the number of affected individuals who used snus was so small (n = 10), these persons were omitted from further analyses.

Information on snus use and other lifestyle factors was collected by questionnaire. Participants were asked whether they had ever used snus (on a daily basis/occasionally) and if so, the number of boxes consumed per month. 'Ever' consumers were classified as light (< 3 boxes/week) and heavy (≥ 3 boxes/week) users. The HUNT surveys were conducted according to regulations from the Norwegian Data Inspectorate and the Regional Committee for Medical Research Ethics, including informed consent from participants.

Statistical methods

Conditional logistic regression (matched for participation date and residential) was used to analyse ESTRID data to estimate the association between snus use and Type 2 diabetes and LADA in terms of the odds ratio (OR) and its 95% confidence interval (CI) (SAS 9.4; SAS Institute, Cary, NC, USA). OR values were interpreted as incidence rate ratios because this case–control study included incident cases and controls sampled by incidence density sampling method (i.e. controls are sampled from the risk set each time a case is diagnosed) [17]. Cross-sectional data was used to calculate ORs by logistic regression models. All analyses were adjusted for age (years, continuous), BMI (calculated as kg/m^2, continuous), family history of diabetes (yes/no) and smoking (pack-year, continuous), unless otherwise stated. Further adjustment for educational level, alcohol consumption and physical activity did not alter the results (change in OR < 10%).

Results

General characteristics of the study populations

In ESTRID, mean age was 63 years in people with Type 2 diabetes, 57 years in people with LADA and 58 years in controls (Table 1). Compared with people with LADA, those with Type 2 diabetes were older and heavier, and had higher prevalence of a family history of diabetes and lower educational level. People with LADA were less insulin resistant, had lower levels of HOMA-β and were more likely to be insulin treated (44% of LADA vs. 6% of Type 2 diabetes). The median duration of diabetes was 5.4 months in people with Type 2 diabetes and 8.0 months in the group with LADA.

Compared with ESTRID participants, those taking part in HUNT were slightly older and leaner, and had lower

Table 1 Characteristics of men with Type 2 diabetes and LADA from ANDIS/ANDIU and controls participating in ESTRID, 2010–2015

	Controls	Type 2 diabetes	LADA	*P*-value*
Characteristics				
No. individuals	699	724	200	–
Age, mean, years (SD)	58 (13)	63 (10)	57 (12)	< 0.0001
Low education level, *N* (%)	176 (25)	257 (36)	49 (24)	0.0038
BMI, mean, kg/m^2 (SD)	26.7 (3.8)	30.6 (5.0)	28.1 (4.9)	< 0.0001
Overweight (25 ≤ BMI), *N* (%)	454 (65)	674 (93)	145 (72)	< 0.0001
Physically inactive, *N* (%)	113 (16)	167 (23)	42 (21)	0.5676
Ever snus user, *N* (%)	222 (32)	209 (29)	64 (32)	0.4308
Never-smoker, *N* (%)	326 (47)	243 (34)	87 (43)	0.0156
Family history of diabetes, *N* (%)	154 (22)	340 (47)	75 (37)	0.0198
Non-drinkers, *N* (%)†	53 (8)	73 (10)	19 (9)	0.8942
Insulin treatment, *N* (%)‡	–	42 (6)	86 (44)	< 0.0001
C-peptide, mean (SD), nmol/l‡	–	1.30 (0.56)	0.83 (0.57)	< 0.0001
HOMA-IR, mean (SD)‡	–	5.9 (13)	5.4 (12)	0.6650
HOMA-β, mean (SD)‡	–	68 (37)	45 (37)	< 0.0001
GAD antibodies, median (interquartile range), IU/ml‡	–	–	128 (20–250)	

ANDIS, all new diabetes in Scania study; ESTRID, epidemiological study of risk factors for LADA and Type 2 diabetes; LADA, latent autoimmune diabetes of adulthood; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of β-cell function; GAD, glutamic acid decarboxylase.
*p for difference between LADA and Type 2 diabetes.
†Non-drinkers including abstainers and former drinkers of alcohol.
‡The information is available only for people with Type 2 diabetes and LADA.

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Table 2 Characteristics of men with and without diabetes in the HUNT3 survey (2006–2008)

	Individuals without diabetes	Type 2 diabetes
Characteristics		
No. individuals	2 0603	829
Age, mean, years (SD)	53 (15)	65 (10)
Low education level, *N* (%)	4 124 (27)	290 (39)
BMI, mean, kg/m^2 (SD)	27.4 (3.7)	29.6 (4.4)
Overweight (25 ≤ BMI), *N* (%)	15 187 (73)	724 (87)
Physically inactive, *N* (%)	5 529 (27)	218 (26)
Ever snus user, *N* (%)	5 777 (28)	157 (19)
Never smoker, *N* (%)	8 798 (43)	211 (25)
Family history of diabetes, *N* (%)	7 570 (37)	597 (72)
Non-drinkers, *N* (%)*	493 (3)	35 (5)
C-peptide, mean (SD), nmol/l†	–	0.86 (0.48)

*Non-drinkers including abstainers and former drinkers of alcohol.
†The information is available only for people with Type 2 diabetes.

prevalence of smoking (Table 2). The prevalence of ever-snus use in HUNT (28%) was as common as in ESTRID (30%).

Comparing the characteristics of participants by tobacco use shows that snus users were younger, less likely to have a low level of education, more physically active, and less likely to be alcohol abstainers compared with smokers and those who do not use tobacco (Table S1). The findings were similar in HUNT and ESTRID, and no other clear differences were seen between the groups.

Snus use and Type 2 diabetes

No association was observed between snus use and Type 2 diabetes in ESTRID (OR, 0.96; 95% CI, 0.67 to 1.37) (Table 3) or in HUNT (OR, 0.91; 95% CI, 0.75 to 1.10) (Table 4). In never-smokers, high snus consumption (ever-users) was not associated with Type 2 diabetes in either ESTRID (Table 3) [OR, 1.01; 95% CI, 0.42 to 2.41 (≥ 5 box/week)] or HUNT [OR, 0.89; 95% CI, 0.21 to 3.78 (≥ 3 boxes/week)] (Table 4). Additional adjustment for educational level, alcohol consumption and physical activity did not change the results [OR, 1.02; 95% CI, 0.46 to 2.26 (≥ 10 box/year) in ESTRID]. Raising the cut-off in ESTRID to ≥ 7 boxes/week indicates an OR of 1.23 (95% CI, 0.34 to 4.47) in ever-users, the results were similar in current heavy snus users (OR, 1.23; 95% CI, 0.30 to 5.12).

Snus use and LADA

In ESTRID, there was no indication of an excess risk of LADA in snus users, but the confidence limits were wide. In never-smokers, OR was estimated at 0.67 (95% CI, 0.24 to 1.86) for ≥ 5 box/week and at 1.01 (95% CI, 0.45 to 2.29) for ≥ 10 box-year (Table 5).

Co-exposure to smoking and snus use

Combining exposure from snus use and cigarettes showed that only smoking was associated with the risk of diabetes (Tables S2 and S3). In ESTRID, those who only smoke cigarettes ('ever') had increased risk (OR, 1.59; 95% CI, 1.16 to 2.18) of Type 2 diabetes (the details are reported

Table 3 Odds ratio of Type 2 diabetes in relation to snus use in men, results from ESTRID, 2010–2015

	Overall				Smoking status					
					Ever-smokers			Never-smokers		
	No. controls	No. cases	Age adjusted OR (95% CI)	Adjustment for age, smoking, BMI and FHD OR (95% CI)	No. controls	No. cases	OR (95% CI)*	No. controls	No. cases	OR (95% CI)
Snus use										
Never	477	515	Reference	Reference	223	310	Reference	223	205	Reference
Former	89	80	0.90 (0.63–1.28)	0.63 (0.41–0.95)	58	58	0.63 (0.39–1.37)	104	11	0.53 (0.20–1.39)
Current	133	129	1.13 (0.84–1.53)	0.96 (0.67–1.37)	92	92	0.91 (0.39–1.01)	36	27	1.17 (0.58–2.37)
Boxes per week (ever snus users)										
Never	477	515	Reference	Reference	223	310	Reference	254	205	Reference
Light snus users (< 5)	161	143	0.90 (0.69–1.20)	0.78 (0.56–1.09)	115	121	0.78 (0.53–1.14)	46	22	0.83 (0.41–1.71)
Heavy snus users (≥ 5)	55	62	1.54 (1.00–2.36)	0.95 (0.57–1.58)	29	46	0.92 (0.49–1.72)	26	16	1.01 (0.42–2.41)
Box-years in ever snus users										
Never	388	390	Reference	Reference	223	310	Reference	254	205	Reference
< 10	123	92	0.89 (0.66–1.20)	0.74 (0.52–1.06)	104	105	0.77 (0.52–1.15)	39	13	0.74 (0.31–1.77)
≥ 10	55	68	1.44 (0.99–2.10)	1.05 (0.67–1.63)	36	60	1.00 (0.57–1.74)	32	22	1.00 (0.47–2.11)

ESTRID, epidemiological study of risk factors for LADA and Type 2 diabetes; OR, odds ratio; FHD, family history of diabetes.
*OR adjusted for age, BMI and family history of diabetes.

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Table 4 Odds ratio of Type 2 diabetes in relation to snus use in men, results from HUNT3 survey, 2006–2008

	Overall				Smoking status					
					Ever-smokers			Never-smokers		
	No. individuals without diabetes	No. cases	Age adjusted OR (95% CI)	Adjustment for age, smoking, BMI and FHD OR (95% CI)	No. individuals without diabetes	No. cases	OR (95% CI)*	No. individuals without diabetes	No. cases	OR (95% CI)*
Snus use										
Never	14 826	672	Reference	Reference	7 807	488	Reference	7 019	184	Reference
Ever	5 777	157	1.00 (0.83–1.20)	0.0.91 (0.75–1.10)	3 998	130	0.86 (0.70–1.07)	1 779	27	1.12 (0.72–1.72)
Boxes per week (ever snus users)										
Never	14 804	669	Reference	Reference	7 791	488	Reference	7 013	181	Reference
Light snus users (< 3)	5 073	130	0.96 (0.79–1.18)	0.88 (0.72–1.08)	3 521	107	0.82 (0.65–1.03)	1 552	23	1.15 (0.72–1.82)
Heavy snus users (≥ 3)	438	9	1.15 (0.58–2.26)	0.92 (0.46–1.83)	262	7	0.90 (0.41–2.00)	176	2	0.89 (0.21–3.78)

OR, odds ratio; FHD, family history of diabetes.
*OR adjusted for age, BMI and family history of diabetes.

elsewhere [23]), a risk that was even more pronounced in heavy smokers (OR, 2.20; 95% CI, 1.40 to 3.45); whereas no increased risk was seen in those who use only snus, or in those who combine snus use and smoking (Table S2). Similar findings were seen in HUNT; in those who only smoke, the OR for Type 2 diabetes was 1.63 (95% CI, 1.36 to 1.96) (Table S3). As for Type 2 diabetes, any combination of tobacco use was unrelated to the risk of LADA, the OR of LADA for individuals combining snus and cigarettes consumption was estimated at 0.97 (95% CI, 0.62 to 1.52) (Table S2).

Discussion

The main finding of this study is the lack of an association between snus use and Type 2 diabetes and LADA, this being in contrast to the effects of smoking documented here and previously [1]. For Type 2 diabetes, our negative findings regarding snus use agree with observations from the MONICA study from Northern Sweden [8], but are in contrast to one cross-sectional [6] and one prospective study [7] based on the Stockholm Diabetes Prevention Program. Confounding from smoking may have contributed to conflicting results. Notably, we did see indications of an increased risk of diabetes in snus users, which disappeared when the analysis was restricted to those who had never smoked. In previous studies, small numbers hampered the analyses of snus users who had never smoked; none of those studies included more than six cases [6–8]. In this context, it is worthy of note that there are no data on the risk of diabetes in relation to the use of other forms of smokeless tobacco such as chewing tobacco. However, US data indicate that use of smokeless tobacco (chewing tobacco) is unrelated to mortality in people with diabetes [24].

We confirm epidemiological and experimental studies linking smoking to an increased risk of Type 2 diabetes [1], proposedly mediated by mechanisms including insulin resistance [2]. Why smoking, but not snus use associates with risk of diabetes is not clear, but this fits with previous findings in cardiovascular diseases such as stroke and myocardial infarction [25–27], indicating that the risk induced by smoking by far exceeds that related to snus use. A lesser degree of toxicity may be due to lower levels of many toxins, e.g. nitrosamine in snus than in cigarettes [28]. In this context, it should be noted that pipe smoking, which provides an exposure to nicotine that is fairly similar to cigarette smoking, is associated with a lower risk of heart disease than cigarette smoking [29], suggesting that substances in cigarette smoke other than nicotine might be important for the risk of Type 2 diabetes.

We previously found a reduced risk of LADA in smokers [12] and based on this we hypothesized that snus may be associated with an even more pronounced risk reduction, due to its high nicotine content. By contrast, data from ESTRID indicate that snus use is unrelated to the risk of LADA and

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Table 5 Odds ratio of LADA in relation to snus use in men, results from ESTRID, 2010–2015

	Overall				Smoking status					
			Age and sex adjusted	Adjustment for age, smoking, BMI and FHD	Ever-smokers			Never-smokers		
	No. controls	No. cases	OR (95% CI)	OR (95% CI)	No. controls	No. cases	OR (95% CI)*	No. controls	No. cases	OR (95% CI)*
Snus use										
Never	477	136	Reference	Reference	223	66	Reference	254	70	Reference
Former	89	19	0.69 (0.40–1.18)	0.60 (0.34-1.04)	58	15	0.70 (0.36–1.35)	31	4	0.46 (0.15–1.43)
Current	133	45	1.11 (0.74–1.66)	1.01 (0.67-1.54)	92	32	1.08 (0.64–1.80)	41	13	0.98 (0.45–2.11)
Boxes per week (ever snus users)										
Never	477	136	Reference	Reference	223	66	Reference	254	70	Reference
Light snus users (< 5)	161	39	0.79 (0.52–1.18)	0.71 (0.46–1.09)	115	29	0.76 (0.46–1.27)	46	10	0.75 (0.34–1.67)
Heavy snus users (≥ 5)	55	22	1.36 (0.78–2.36)	1.18 (0.67–2.10)	29	16	1.64 (0.80–3.35)	26	6	0.67 (0.24–1.86)
Box-year in ever snus users										
Never	477	136	Reference	Reference	223	66	Reference	254	70	Reference
< 10	143	28	0.64 (0.41–1.01)	0.56 (0.35–0.91)	104	23	0.67 (0.39–1.16)	39	5	0.46 (0.16–1.31)
≥ 10	68	33	1.61 (1.00–2.59)	1.45 (0.89–2.37)	36	22	1.82 (0.96–3.46)	32	11	1.01 (0.45–2.29)

LADA, latent autoimmune diabetes of adulthood; ESTRID, epidemiological study of risk factors for LADA and Type 2 diabetes; FHD, family history of diabetes; OR, odds ratio.
*OR adjusted for age, BMI and family history of diabetes.

furthermore, that heavy smoking may increase the risk [23]. These findings are compatible with both negative and beneficial effects of nicotine on the pathogenesis of LADA and the lack of association with overall risk may reflect that a potential beneficial effect on autoimmunity is counterbalanced by increased insulin resistance. However, with regard to other autoimmune diseases such as rheumatoid arthritis [30] and Crohn's disease [31], for which insulin resistance is not part of the pathogenesis, similar null results have been reported in relation to snus use.

One of the strengths of this study is that it was population-based using incident cases that were identified in large well-characterized diabetes registries (ANDIS/ANDIU) with randomly selected population controls. The number of cases was relatively large and we had detailed information on the history of tobacco use and a large number of potential confounders. Recall bias is a potential problem because both studies were based on retrospective data of tobacco use. ESTRID is based on incident cases, which minimizes this problem; furthermore, only information from the years prior to diagnosis was included in our analysis. The HUNT data were based on prevalent cases and hence, recall bias may be more pronounced, e.g. people with diabetes may have quit using tobacco several years ago following diagnosis, thereby underestimating previous use. Importantly, the association between smoking and Type 2 diabetes in both HUNT and ESTRID was similar to reports from prospective studies with tobacco habits assessed several years prior to onset [1,12]. Furthermore, by restricting a major part of our analyses to never-smokers, we avoid bias that may arise from people shifting from smoking to snus use or vice versa following diagnosis. This is the most extensive analysis conducted to date on the risk of Type 2 diabetes in relation to snus use. As a consequence, we could perform separate analysis in never-smokers, thereby minimizing confounding from smoking. However, we cannot exclude the possibility that snus use has a small effect on the risk of diabetes, which we did not have enough power to detect. With our current sample size and snus use prevalence (30%), we have 85% power to detect an OR of 1.5 (or 0.65) for Type 2 diabetes. Further, the number of heavy snus users was limited and thus we cannot exclude an effect in the highest consumption group.

It has been suggested that tobacco use is related to unfavourable social and lifestyle behaviours in general [32,33]. By contrast, the snus users investigated here were younger, more physically active, and had higher educational levels than both smokers and those who had never used tobacco, which may reduce their risk of diabetes. We did find that the results persisted after adjustment for a number of factors, including physical activity and education; however, it is possible that the lack of association between snus and Type 2 diabetes is due to residual confounding.

In conclusion, our findings extend to diabetes previous notions that snus is generally less harmful than other tobacco products [25–27,34]. However, even though this is the largest study on snus use and diabetes to date, further studies with an even larger sample size would be necessary in order to rule out modest effects of snus use on the risk of diabetes and effects pertaining to very high consumption.

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Competing interests

None declared.

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Author contributions

All authors contributed in writing the manuscript, interpretation of the data and critically reviewed the paper, and read and approved the final manuscript. BR was responsible for analysing the data and writing the paper. BR had access to all data in this study and takes responsibility for the integrity of the data and accuracy the data analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. ESTRID study design.
Table S1. Characteristics of individuals according to tobacco consumption in ESTRID (2010–2015) and HUNT3 (2006–2008).
Table S2. OR of LADA and Type 2 diabetes for different combination of smoking and snus use, results from ESTRID, 2010–2015.
Table S3. OR Type 2 diabetes for different combination of smoking and snus use, results from HUNT3 survey, 2006–2008.

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Cigarettes, snus and status: differences in lifestyle of different tobacco user groups in Norway

Gunnar Sæbø

Norwegian Institute of Public Health (FHI), Oslo, Norway

ABSTRACT

During the last decades smoking has been marginalised. Current tobacco policy is characterised by a continuous denormalisation of *all* forms of tobacco consumption. Yet, many people still use tobacco. This article highlights a possible cultural explanation for this: different tobacco products and uses are included in lifestyles of different social status and prestige, and vary in legitimacy. Using nationally representative data of the Norwegian adult population and drawing conceptually and methodologically on Bourdieu's cultural sociology, I show that differences in socio-cultural practices (including tobacco use) are manifested in a structured 'space of lifestyles', homologous to the structure of the objective 'space of social positions'. The contents of the various lifestyles (as identified by multiple correspondence analysis) inform the cultural distinctions associated with tobacco use.

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Introduction

Smoking in the Western world has declined significantly over the past decades (Brandt, 2007). Several health-based initiatives, such as smoking bans, tax hikes and information campaigns, have reinforced a cultural shift towards smoke-free environments. Policies seeking to denormalise all forms of tobacco use have been effective. Smoking in regions where strict tobacco control regimes are in place (e.g. Northern Europe, U.S.A. and Australasia) is now a quite stigmatised social practice (Graham, 2012; Stuber, Galea, & Link, 2008). Many of the remaining smokers in these populations are addicted to nicotine, and while some have tried unsuccessfully to stop, others do not want to. Since tobacco use is linked to an experience of individual autonomy, strict tobacco laws may also provoke reactance and social resistance (Erceg-Hurn & Steed, 2011; Factor, Kawachi, & Williams, 2011).

Even if smoking to an increasing degree occurs concentrated – socially, geographically and economically – among marginal groups, the remaining group of smokers is characterised by a certain socio-demographic heterogeneity (Lund & Lindbak, 2007). Tobacco use is also associated with value ambivalence, especially among adolescents (Plumridge, Fitzgerald, & Abel, 2002; Scheffels, 2009). In addition, there are differences in meaning

CONTACT Gunnar Sæbø gunnar.saebo@fhi.no Norwegian Institute of Public Health (FHI), P.O. Box 4404 Nydalen, N 0403 Oslo, Norway

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between various tobacco products. The historical connotations of pipe and cigar smoking are different from those of cigarette smoking (Klein, 1995). While smoking a pipe has been associated with contemplation and intellectuality, the cigar has been linked to economic power and self-confidence (Gately, 2001). The cigarette has been in a more neutral position (Callison, Karrh, & Zillmann, 2002). Today's young adults, however, consider the use of manufactured cigarettes and rolling tobacco with very different gazes: while the consumption of manufactured cigarettes can still be considered identity construing in a positive way, rolling tobacco is associated with addiction and low status (Scheffels, 2008). The smokeless tobacco product 'snus' (which is legal only in some regions, like the U.S.A. and Scandinavia) differs from other tobacco products in that consumption rates have risen in recent years. It is also surrounded by an aura of 'trendiness' in many youth and sport milieus (Nordby & Wood, 2008; Wiium, Aarø, & Hetland, 2009). Against this background, it is reasonable to assume that status differences between rolling tobacco, cigars, pipes, cigarettes and snus may still exist, and that these differences will be reflected and maintained by the socio-cultural profiles, various lifestyle practices and ways to use tobacco products associated with different user groups.

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Research problem

Basing my analysis on Bourdieu's (1984) theory of lifestyle and taste, I describe in this article status differences associated with consumption of different types of tobacco product. Status differences are not only expressed by income and education, they are also reflected in consumption patterns and everyday activities. Accordingly, I will identify status differences by embedding different uses of tobacco within broadly defined 'collective lifestyles' (Frohlich, Corin, & Potvin, 2001). This analytical approach differs from established perspectives on health behaviour, which often conceive of tobacco use as the health behaviour of individuals, in viewing tobacco use as a social practice embedded in routinised collective lifestyles. This conceptualisation emphasises the importance of social context to explain and understand tobacco use (Poland, Frohlich, & Haines, 2006).

The research was carried out in Norway, a European country known for its strict tobacco laws, low and decreasing smoking prevalence, countered by growth in use of smokeless tobacco among adolescents and young adults (Lund & Lindbak, 2007). As lifestyle differences in themselves can express status differences, it becomes an empirical question whether the differences between tobacco user groups are so large and of such quality (i.e. adequately symbolically charged) to constitute crucial 'distinction differences' in the Norwegian culture. If so, they must not only stand in distant opposition to each other in social space, but also be associated with other, relatively uniform areas of activities and goods, and with different capital structures – in short, *habitus* and class differences.

Using multiple correspondence analysis (MCA), the following questions will be addressed: (a) What activities and points of view characterise the lifestyles that can be identified and how is tobacco use included in the different lifestyles? (b) What capital structures are associated with different lifestyles and tobacco use? First, I analyse the relations between *use of snus*, *combined use of smokeless tobacco and cigarettes*, *daily smoking*, *occasional smoking* and *non-tobacco use* in the population (15 years or older) as a whole. Second, I probe the relations between *cigar smoking*, *pipe smoking*, *rolling tobacco smoking*, *smoking mild cigarettes*, *smoking manufactured cigarettes* and *snus use*

in the social space of tobacco users. Do we find the same lifestyle differences when we analyse tobacco users only? As far as I know, no such systematic comparison of various tobacco users has been conducted previously.

Theoretical assumptions

Distinction differences between various tobacco products and various uses

Historically, smoking in the Western world has developed from being an upper class practice and expression of a distinguished *habitus* in the 1800s to become popular and widespread in the 1950s, but is now seen to be more of a low status phenomenon (Brandt, 2007). Smoking is seen to be an expression of 'elementary' taste, which is also linked to other forms of basic tastes, such as food and drinking habits. When smoking as a social practice has become as sharply devalued as it has in only a few decades, this is not primarily based on the physical flavour of smoking, but rather the development in the aesthetics of smoking, which can best be described as 'de-aestheticisation'. Although it has always been – and still is – possible to find glamorous depictions of smoking, for example, in internationally distributed magazines and on the Internet, the overall gaze on smoking has become increasingly health-oriented. This has happened gradually since the detection of the causal association between smoking and lung cancer in the early 1960s. Aesthetically speaking, daily smoking is today largely an expression of a 'vulgar' taste (Brandt, 2007).

Nevertheless, the gaze on smoking will never be 'purely' aesthetic; as Bourdieu (1984) points out, it will always be socially constituted. When different uses of tobacco in the following are to be understood as expressions of unequal status and taste, this means that tobacco use continues to function as a possible *marker of distinction*. The definition of 'good' and 'bad' lifestyles, 'cool' and 'uncool' tobacco practices and the like is related to which categories gain approval to *designate* reality. Because smoking (compared to non-smoking) or preference for one tobacco product (compared to other products) as social practices will always be an expression of class-associated *habitus*, they may also be categorised in different ways, because they are included in different classes with different dispositions. Habitus can be understood as dispositions: 'generative principles of distinct and distinctive practices' (Bourdieu, 1998, p. 8).

Now that tobacco control policy move in the direction of denormalisation of *all* tobacco consumption, and categorisation of tobacco use and users is steered towards marginalisation (Chapman & Freeman, 2008), the differences in tobacco user status will be even more affected by their overall lifestyle. This relates also to tobacco consumption as potential *signs* of different lifestyles: in the contemporary tobacco culture, it seems 'better' to smoke a cigar than to smoke rolling tobacco, in the sense that social elites may well allow themselves to be photographed with a cigar, but not with a hand rolled cigarette. It also seems 'worse' to smoke rolling tobacco daily than manufactured cigarettes occasionally, because daily smokers often have low levels of education and are seen to not possess the same control over their own smoking habits as the educated social smoker does (Krange & Pedersen, 2001). In this reasoning, traditional beliefs and stereotypes about different tobacco users are expressed: cigar smokers are powerful and dynamic, social smokers are intellectuals exercising self-control, rolling tobacco smokers are nicotine dependent workers, etc. (Gately, 2001).

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Snus use is particularly interesting in this context, as this tobacco product seems to have a different and possibly more positive meaning than the vulgarised combustible smoking products (Nordby & Wood, 2008; Wiium et al., 2009). The logic of distinction suggests that taste-related habits and perceptions will change over time. When practices that have been exclusive become delegitimised (just as cigarette smoking has), the search for exclusivity will find new markers (for example snus) (Bourdieu, 1984). But although there is now, perhaps, a new generation of young people ready to favour snus – a generation without historical associations with snus and chewing tobacco as 'messy' – this does not mean that snus use will automatically become a sign of high status. Awarding high status to a person or activity will also depend on whether the person has a relatively high amount of capital or that the activity is awarded value, based on the socio-cultural context in which it is embedded (or possibly stands in opposition with). Snus use can thus achieve higher status than other tobacco consumption by the lifestyle practices associated with it having a higher status than lifestyle practices that other tobacco uses are embedded within.

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Homologies between the space of lifestyles and the space of social positions

Bourdieu's analytical grip in *Distinction* (and in many other works) consists in constructing multiple and diverse geometric spaces, which are studied both separately and in conjunction, with respect to similarities and differences. While the analysis of the *space of lifestyles* will reveal meaning-based relations, the *space of social positions* shows the differentiation mechanisms that operate in the community under study, and thus also the power relations most prominent in the social space. Studies by both Bourdieu (1984) and others have revealed two main principles of differentiation, that is, a tendency for the total capital amount to be the primary social differentiation mechanism, while the relationship between economic capital (material wealth) and cultural capital (educational knowledge in its embodied state) will be of secondary importance. This latter principle is called 'the capital composition principle'.

The point of placing tobacco practices within a national social space is to analyse the distances between the various collective lifestyle contexts that tobacco use is included in, and simulaneously illustrate health inequalities (Veenstra, 2007). By connecting the analysis of lifestyle with social position, it becomes possible to illuminate power relations associated with the social topography of tobacco consumption, and to illustrate how tobacco use at the same time can be related to both marginalisation and normality, based on the various user groups' position in the social space. The analysis of the collective lifestyles also highlights localisation of tobacco practices in specific environments, and aspects of social identity and symbolic consumption that help to define the nature of lifestyles and the distinction differences between them (Poland et al., 2006).

Methods

Data

The data were obtained from *Norwegian Monitor 2007*, a comprehensive survey conducted under the auspices of IPSOS. A representative sample of the Norwegian

population, aged 15 and older, was asked a series of questions related to social activities and cultural values (see Hellevik (2008) for a presentation of the study's design and procedures). Data were collected in 2007. To improve representativeness, data were weighted by gender, age and region ($N = 3775$).

In *Norwegian Monitor*, a number of lifestyle components are highlighted. In addition to tobacco use, I concentrate on the following: *media use and media interest*, *technology use*, *physical activity*, *eating and drinking habits*, *travel and vacation habits*, *selected shopping habits* and *cultural participation*. In line with Bourdieu's theoretical holism, I include variables from each of these areas and conduct a MCA of the data (Le Roux & Rouanet, 2010).

Selection of lifestyle indicators

In addition to the theoretical ambition of holism, a thematic and balanced spread of questions is essential for methodological considerations. This is to avoid some of the lifestyle areas dominating at the expense of others in the construction of spaces (Le Roux & Rouanet, 2010, p. 38). Even if correspondence analysis is suited to handle several variables at once, the number of variables needs to be limited. First, this is important to keep track in the interpretative work. Second, the explained variance tends to decrease when more variables are included in the model. The model does not necessarily become more appropriate for the underlying reality when more variables are included. It is equally important to identify variables crucial for the *configuration of the underlying structure*.

The selection of lifestyle indicators to the correspondence analysis is thus based on a combination of theoretical and statistical arguments. The main concern was to represent the most common chores and social arenas from each lifestyle area. For example, in relation to media use, measures should include use of the main media channels – public service television (NRK), leading national newspaper (*Aftenposten*) and cinema – while the measures for eating and drinking habits should include vital meals like breakfast and dinner.

Each response alternative constitutes a 'modality'. I have applied 5 modalities for tobacco use, 48 for media use, 14 for media interest, 14 for technology use, 10 for physical activity, 35 for eating and drinking habits (including alcoholic beverages), 20 for travel and vacation habits, 15 for selected shopping habits, 18 for cultural participation and other activities, and 18 for some relevant attitudes and values. In all, 197 modalities based on 84 variables were applied (see online supplementary material, Table A1).

The background variables used to 'explain' the positioning of lifestyle relations in social space are personal income (measure of 'economic capital'), highest completed education (measure of 'cultural capital'), number of books read last year (measure of 'cultural capital') and gender, age, social standing, line of business, public/private employee and parental education. In all, 55 supplementary points were applied (see online supplementary material, Table A2).

Wider questions concerning tobacco use were applied in the analysis of tobacco users only: 'Would you say you smoke daily, occasionally or never?', 'Which of these types of tobacco do you smoke?' (Cigars, pipe, RYO, mild/light cigarettes, regular manufactured cigarettes); 'How many cigarettes do you smoke per day (Don't smoke daily, 1–4, 5–9, 10–14, 15 or more); and 'Would you say you use snus daily, occasionally or never?

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Multiple correspondence analysis

MCA in the statistical program SPAD 7.3 was applied to encircle smoking habits and their connection to other lifestyle practices in Norway.

MCA is descriptive, in that it seeks to adapt the model to the structure of the observed data, not vice-versa, as is the case in causal or inferential statistics (Le Roux & Rouanet, 2010, pp. 1–2). The technique has been described as a geometric variation of factor analysis. MCA presents the overall deviations for all response categories in factorial planes, which are expressed as intersecting axes in a multidimensional geometric space.

The appropriate procedure for determining the number of axes to interpret is based on the variance of the individual axes. *Modified rates in per cent* is an expression of explained variance, and the cumulated modified rates determines the number of axes to be interpreted. The rule of thumb is that about 80% of the total variance is sufficient (Le Roux & Rouanet, 2010, pp. 51–52).

To name the dimensions, one looks to the *absolute contribution of modality to axis variance*. The measure Ctr is an expression of this variance. The rule of thumb is to include every modality 'whose contributions to axis exceed the average contribution' (Le Roux & Rouanet, 2010, p. 52).

In the following, I present analyses based on lifestyle indicators as active categories and social position as supplementary 'explanatory' categories. This is also the strategy Bourdieu (1984, p. 261) used in *Distinction*.

Results

In 2007, over one-third of the Norwegian population reported using some form of tobacco. About 25% smoked daily or occasionally, while 11% used snus, possibly in combination with cigarettes (Table 1). In the population, non-smokers were a clear majority.

Among tobacco users cigarettes are the dominant tobacco product. About 41% of tobacco users smoke manufactured cigarettes, 21% 'mild' cigarettes, while 25% smoke 'roll-your-own', 8% cigars and only 1% a pipe. About 23% of tobacco users use more than one type of product.

Table 1. Different user groups of tobacco 2007, in per cent of total population and the population of tobacco users.

	% of total population	% of the population of tobacco users
Tobacco use		
Do not use tobacco	64	[ ]
Daily smoking	18	51
Occasional smoking	7	20
Snus daily or occasionally	6	16
Combine smoking and snus	5	14
Total	100	100
N	(3775)	(1361)
Product preference (multiple responses)		
Smoke cigar	3	8
Smoke pipe	1	1
Smoke RYO tobacco	9	25
Smoke mild cigarettes	8	21
Smoke manufactured cigarettes	15	41
Use snus	11	29
Combine two or more products	8	23
N	(3775)	(1361)

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Construction of the space of lifestyles

The first MCA was conducted on all respondents. As the data are nationally representative, the space of lifestyles revealed can be understood as applying to the nation as a whole.

Table 2 shows the variance of the axes as identified by the MCA, and the modified rates, which underlies the considerations about the number of axes to include in the further analysis. The cumulated modified rate of Axes 1 and 2 is 77% – that is, close to the thumb rule of 80%.

Modalities providing an above average contribution to the variance of axes are included in the interpretation of these two axes. The average is 100/197 = 0.51. Modalities with a contribution (ctr) higher than 0.51 on Axis 1 or Axis 2 are summarised in Figure 1 (see online supplementary material).

The first (horizontal) dimension is an expression of lifestyle differences in *levels of activity* (including domestic/social orientation) and *technology*. Here, the dimension distinguishes between a passive domestic lifestyle and little use of technology, on the one hand, and an active social style and extensive use of technology on the other hand. To the left of Axis 1, we find a collection of modalities that express a 'passive' stance to the lifestyle practices studied here. To the right on Axis 1, we find a collection of modalities in contrast to those on the left of the axis. In addition to widespread use of digital technology, we find indicators of an active social life, and food and drink consumed at places to go out for food and drinks or 'en route'. Snus use (also in combination with cigarettes) has its place here. The other modalities for tobacco use have little bearing on this axis.

The second (vertical) dimension is an expression of lifestyle differences related to *cultural consumption* and *health*. This is the axis that tobacco use especially contributes to. At the bottom of Axis 2, we find an accumulation of modalities indicating little interest in highbrow culture and health ideals, but all the greater interest in entertainment on commercial television channels, games, 'fast food' – and tobacco. At the top of Axis 2, we find the opposite: participation in cultured activities, use of serious media (*Aftenposten*), health ideals and an active non-tobacco stance.

These structural trends define the main contradictions in the Norwegian space of lifestyles. (As we will see later, they also express social patterns.) In addition, we can identify four relatively distinct lifestyles, and their contents, by taking the four squares in the plot into consideration.The lifestyle encircled in the lower left quadrant can be described as *passive- and entertainment-oriented*. This is a lifestyle where participants have not been to a museum, a theatre or an art exhibition during the last year and where they have not stayed in a hotel for work assignments. If they travel abroad on holiday, they prefer package holidays rather than arranging and booking the trip themselves. They seldom exercise and never eat fruit as a snack. Their lack of interest in sporting activities is

Table 2. MCA: variance of axes, including modified and cumulated rates.

	Axis 1	Axis 2	Axis 3
Eigenvalue (variance of axis)	0.0876	0.0555	0.0387
Per cent	6.40	4.05	2.82
Cumulated per cent	6.40	10.45	13.27
Modified rate	58%	19%	7%
Cumulated modified rate	58%	77%	84%

reflected in never shopping in the sports and wilderness chain store XXL. Their lifestyle is characterised by frequent television viewing, especially the commercial channels TV2 and TVNorge, and reading *Se & Hør* ('See and Hear', a tabloid magazine focussing on celebrities and gossip). This is a way of life where respondents think that foreign aid should be reduced, and that it is wrong to pass more restrictions on where smoking is allowed. Country & Western is a common taste in music. This is the space of lifestyle for daily smoking.

The lifestyle encircled in the upper left quadrant is also characterised by heavy media use, but of a different type than in the previous lifestyle. Here, the non-commercial television channels – NRK – are preferred, in contrast to the commercial channels. Participants do not like rock, but classical music. They use technology infrequently: they do not use search engines on the Internet, and they seldom use credit cards when shopping. The level of physical activity is low, but the health profile of the diet is clear: they eat breakfast every day and avoid both coke and crisps. This is also a tobacco-free segment in which smoking and snus use do not occur. We can call this lifestyle *passive and serious*.

The third lifestyle (top right) is characterised by a high level of physical activity and many resources. This lifestyle is positioned in diametrical opposition to the passive entertainment lifestyle and can be described as *active*. Here we find extensive participation in cultural activities (art exhibitions, the theatre and cinema) and many signs of 'good taste'. Respondents like jazz and read *Aftenposten* daily. They often use train as a means of travel and often stay in hotels in relation to work. They prefer to arrange and book flights and holidays themselves. Several activities take place with others, whether exercise with friends/colleagues or dinner with friends. This lifestyle is characterised by frequent alcohol use, including wine at restaurants, but also by physical activity, which may well be a typical summer holiday activity. This segment reads *Fjell og Vidde* ('Mountains and Plains', a niche magazine focussing on outdoor life in the versatile Norwegian nature) and sometimes shop at the XXL store, but seldom at 7-Eleven. Respondents often think that foreign aid should be maintained at current levels or increased. Tobacco users in this segment smoke occasionally ('social smoking' etc.).[1]

The fourth way of life can be characterised as *youthful* (bottom right). In this segment, participants pay little attention to non-commercial television. Instead, they prefer niche magazines such as *FHM* (for young men), *Cosmopolitan* (for young women) or commercial national radio (P4). Online media are important too, which is reflected in the daily use of search engines on the Internet and that the Internet is the main source of news. They use credit cards in stores on a daily basis. Their diet is not distinctly healthy, and they often eat 'fast food' from snack bars, 7-Eleven or as 'take aways' and drink cola and eat crisps. There is also a tendency that they have not eaten breakfast at all during the last week. But then this is a lifestyle where the view that 'I am not concerned with being healthy and keeping myself in good shape' is more prevalent than in other lifestyles. Yet, football is a sports activity that many participate in. Taste in music is hip-hop/rap or rock. This segment seems willing to take risks, they are open to having tattoos on their body, and they admit that they sometimes drink a lot of alcohol to achieve more intense experiences. This is the snus users' lifestyle, both those who combine snus and cigarettes, and those who use snus only.

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Homologies between lifestyles, tobacco use and social position

Figure 2 (see online supplementary material) shows the plot of social positions that emerge when we project the distribution of socio-demographics, capital indicators and attachments to working life onto the already constructed lifestyle map. Background variables as supplementary points can be considered as structuring of the observed lifestyle structure.

The age variable positions itself along the first horizontal dimension in a systematic way, which means that the differences in levels of activity and use of technology also become an expression of low (right) versus high (left) age. The same applies to personal income, which stations quite systematically along the horizontal axis, as it extends from low to high when we move from left to right on the axis. The education variable too positions itself partially along the horizontal axis and then quite parallel to income. The same goes for parental education, indicating that educational capital is often passed down between the generations. In addition, this first dimension expresses an important contrast between those in work (or about to enter the labour market) to the right, and those who are not working, either because they are retired, insured or married without own paid work to the left.

This indicates that the first lifestyle dimension identified – differences in level of activity and technology use – is also an expression of total amount of capital (i.e. economic plus cultural capital), as we go from left to right. The picture is not clear-cut though, since position variables also lie partially along the vertical dimension. In particular, this applies to the education variables, as lifestyle differences in cultural consumption and health orientation also can be understood as an expression of low (primary or secondary school) versus high (college or university) education in terms of respondents' own education – and between secondary and university levels in terms of parents' education. Thus, the second dimension distinguishes between the amount of cultural capital, as illustrated by number of books read last year being placed from the bottom (do not read books) to the top (more than 10 books).

In the case of Axis 2, we do *not* find a clear contrast between high cultural capital and high economic capital, as Bourdieu has done in France, although Axis 2 brings out a certain opposition between the cultural field and the economic field when looking at respondents' industry or sector affiliation. Axis 2 also expresses differences between the public and private sector. At the top, we find an accumulation of public sector employees (teaching/research, public administration and health/social services), while employees of commodity trade/shops, industry/handicraft and transport, more often employed in the private sector, are located at the bottom. In addition, this dimension expresses a social class-like contrast between officials and managers (top) and workers (bottom).

If we look at the positions prominent in the four squares, the lower left quadrant is characterised by low levels of education, relatively low income and social standing as insured or unemployed. Daily smokers were located here. The upper left quadrant, which was non-smokers' 'passive serious' lifestyle, is characterised by many being retired and thus elderly. The upper right quadrant is characterised by a considerable amount of economic and cultural capital: high education and income and position as officer, manager or self-employed. Parents often have a university education. Occasional 'social' smokers tend to be located here. Young respondents characterise the fourth and final segment. Status-wise, they are either in education (pupils/students) or they are workers/operators. This is the segment of snus users.

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Oppositions in lifestyles in the social space of tobacco users

The following analysis is based on the same empirical model as above, but now with a selection of tobacco users only. In addition, other measures of tobacco consumption were applied, including which tobacco product is used (cf. Table 1).

This analysis shows that the lifestyle differences identified in the total population also structure the space of lifestyles among tobacco users. In addition, use of different tobacco products is placed in the different corners of the two dimensional space (see Figure 3 in online supplementary material; due to communication considerations, the other modalities are not presented here). In the corner of the passive entertainment-oriented lifestyle, we find smoking of rolling tobacco. Here we also find the highest tobacco consumption (measured in number of cigarettes), which may indicate that nicotine addiction is strong. In the corner of the passive serious lifestyle, pipes and mild cigarettes are smoked, while in the space of the active lifestyle, occasional smoking – for example, of cigars – is common. Finally, in the youthful lifestyle, snus prevails.

Looking at social position (not reproduced here), cultural capital (vertically) distinguishes less among the tobacco-using population than in the total population, while overall capital position is located even more clearly along the horizontal axis, which is an expression of relatively little capital (left) and then gradually more capital (to the right). Thus, a dividing line of status lies internally within the social space of tobacco users, where daily smoking (especially of rolling tobacco) on the left is associated with 'low status', and occasional smoking (preferably of cigars) and snus use (right) are more likely to correlate with 'high status'.

Discussion

In adhering to Bourdieu's (1998, p. 2) advice to apply his theory to other special cases of what is possible, this analysis has extracted various lifestyle areas as distinctive of different tobacco user groups. This in itself indicates that different tobacco products are consumed as part of different lifestyles in Norway, and that tobacco products are judged also in relation to the social contexts and lifestyles in which they occur. In other words, how tobacco is used and which products are consumed are defined in relation to each other. People who smoke 'occasionally' are not a variation on the general category of daily smoker, but a *contrast*. Daily smokers differ from the average population and from occasional smokers along other lifestyle and social position variables than snus users do.

A significant proportion of the group of *daily smokers* is currently outside important social arenas and seems to be excluded from several typical tasks. The low income level, and the position as insured or outside the labour force, suggest that this group consists of many marginalised individuals, whose participation in social and cultural life (from this analysis' empirical model) commonly involves commercial TV channels and press. It is among Norwegian daily smokers that we find an accumulation of lifestyle practices often considered problematic in public health (little physical exercise, frequent television viewing, unhealthy dietary habits). This is a lifestyle centred on the home.

Snus users have a lifestyle characterised by many signs of youthfulness, such as technology competence and an active social life. As we have seen, snus users are placed higher on the axis of total capital in the total Norwegian population (which in particular is an

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expression of income and parental education), but lower on the axis of cultural capital and standing. The snus user group is also characterised by a certain polarisation in social position between snus-using pupils/students (who have the potential to acquire more capital over time) and workers who use snus (who cannot be said to have the same potential).

The contemporary group of tobacco users who have the relatively highest status is *occasional smokers*. The lifestyle profile of occasional smokers lies close to the most prestigious lifestyle in Norway, and these tobacco users have more in common with those who do not smoke than daily smokers and snus users. The lifestyle profiles of daily smokers and snus users (especially those who combine snus with cigarettes) differ the most from the lifestyle profile of the average population, and therefore these profiles distinguish and separate daily smokers and snus users in the social space. This can also be seen from the various tobacco user groups' distance to the origo (see online supplementary material, Figure 1).

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Snus as distinctive to cigarettes

Traditionally, tobacco consumption has been a way for teenagers to express style and identity, and thus appear as something other than 'grey and average'. Cigarette smoking may still be filling this function among young people, even in a context of tobacco denormalisation, as many adolescents seem ready to 'consume conspicuously' and take health risks to achieve status and influence among peers (Haines, Poland, & Johnson, 2008; Plumridge et al., 2002). In the international context, however, Norway, Sweden and the U.S.A. are in a unique situation, due to snus being a legal tobacco product. In recent decades, we have observed a surge in the use of snus by Norwegian adolescents. Currently, more young people use snus than smoke on a daily basis (Pedersen & van Soest, 2014). Even if the snus wave has captured many of those who previously would have started smoking, the snus user group has characteristics that indicate that snus may also appeal to other groups than those who typically have been smokers, especially due to associations with sporting milieus (Nordby & Wood, 2008). Snus use can therefore serve as a distinction among young people, as the snus user lifestyle differs from both cigarette smokers and 'neutral' non-smokers. Snus use constitutes a form of symbolic consumption in a youthful lifestyle, and the recent increase in snus use also among girls may indicate that snus is even more identity-defining than cigarettes among young people today. However, the 'youthfulness' associated with snus users' lifestyle is not necessarily a separate form of capital in the national social space, although many of the distinctive signs of young lifestyles certainly may accord high status in certain situations, both in smaller sub-cultural youth groupings and the social space of all tobacco users. The fact that snus especially have been used by young men, with a lifestyle characterised by high technological competence and an active social life, links snus to social groups that may acquire more economic and cultural capital in the future. In a short time, many of these people will occupy more powerful positions in society than they are in today. If current snus users continue to use snus in the future, the group may also become more powerful status-wise. Another possibility is that the group will continue to be located in the middle-of-the-road status-wise, if snus users with the highest capital level stop using snus at an accelerated rate (as we have seen with smoking cessation).

Occasional versus daily smoking

Another distinction revealed in this study is associated with the financially strongest groups and their rejection of tobacco products as part of their lifestyle, which will also be the most prestigious. The affluent middle class has largely stopped smoking, and cigarettes are ill-suited to lifestyles favouring self-control and health focus. Alternatively, the middle class may engage in social smoking or smoking a cigar on special occasions. The exercise of symbolic power may precisely involve approval for a 'beautifying' understanding that this would be a different and more legitimate way to use tobacco, not raising the same critical questions as daily smoking. Even occasional smoking of 'rolling tobacco' now seems a marker of distinction among resourceful young adults in the big city (Tokle, 2010). There may be an element of cultural and political resistance associated with this form of smoking as well, while still being consistent with a self-understanding as autonomous non-smoker, in line with the health authorities' wish to end the tobacco problem (Katainen, 2010). This argument is substantiated by the occasional smokers' location close to non-smokers in the social space, rather than in an intermediate position *between* daily smokers and non-smokers.[2]

While people in the middle class often consider themselves and others in the light of health-oriented behaviour, this is not necessarily the case among people in the working class, who often look at health and the body as private matters and as marked by luck (Lupton, 1995). Among those with the lowest total capital, smoking may have a different meaning than for middle class groups. Perhaps daily smoking of cigarettes for many serves as relaxation and pleasure in a relatively passive life, possibly affected by limited resources? This raises the question of whether daily smoking today may be an expression of what Bourdieu (1984, pp. 372–396) has called 'taste for necessity'. The term refers to the restricted capacity among the least privileged to accumulate capital (including converting various forms of capital), which means that their taste often will be characterised by 'what is necessary'. While the middle and upper class increasingly have been able to accumulate capital, and thus develop distance to the taste of the purely necessary, this is not necessarily the case in the working class or among those outside the workforce (Blasius & Friedrichs, 2008). It is also possible that the dominant *habitus* in these groups means that many daily smokers fail (due to addiction) to quit smoking, or simply do not want to quit smoking, like the middle class and the relatively better-off largely have done. If daily smokers consider the cigarette an expression of relaxation and momentary pleasure, in accordance with the 'taste for necessity', this will be difficult to justify in the current tobacco-hostile culture. This may also be a reason why many smokers perceive the authorities' anti-smoking campaigns as provocative (Factor et al., 2011). From the smokers' point of view, such campaigns can be perceived as promoting a lifestyle that deprives them of joy here and now. Maybe smoking for this group is a symbol of freedom and a way to express opposition to health and government regulation of lifestyle practices (Krange & Skogen, 2007)? As daily smoking overlaps with other lifestyle practices and tastes, such as musical tastes, this indicates that daily smoking not only is an expression of social class and nicotine addiction, but also that the practice works as symbolic distinction (Pampel, 2006).

The analysis presented in this article can thus be read as an illustration of the social and symbolic distance between those who regulate and those who are regulated in the tobacco field, and tensions between marginalisation and normality following in its wake. While the

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daily smoker group seems to move towards the fringes of society, occasional smoking is 'safely' located at the societal centre. Snus use comes, with quite considerable force, from 'below', among the young. If we imagine that tobacco use not only is an expression of addiction, but also of autonomy and possible resistance to the denormalisation of tobacco use, the relationship between resistance and tobacco-related lifestyles is likely to vary. Among daily smokers it may involve rejection of expert health orientation (Krange & Skogen, 2007), among occasional smokers a playful balancing of risk and pleasure is implicated (Krange & Pedersen, 2001; Tokle, 2010), while among snus users it may work in line with the well-documented youthful quest for identity, influence and acceptance of peers through the practice of health risk behaviours.

The analysis of the internal distinctions within the space of tobacco users confirms that the use of different products and the different ways of consuming them vary with social position and cultural capital, largely in line with historical stereotypes of typical pipe, cigar and cigarette smokers known from adverts and popular culture.

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Methodological limitations

In the spirit of Bourdieu, I have looked for principles of differentiation in Norway 2007 – prior to the financial crisis – and used MCA to identify structures and oppositions in the structures. Now the data set is arguably of less current interest, and the technology measures especially may be outmoded. However, we would probably have seen the same overall pattern if we had substituted varieties that are more recent for our technology measures, for instance, possession of smart telephones.

It is a weakness of MCA that the statistical correlations between modalities are not tested for significance in the traditional sense. Nevertheless, if the sample is taken to be representative of the Norwegian population – and we have no evidence of systematic selection effects (Hellevik, 2008, pp. 132–136) – the analyses of the Norwegian social space have revealed what are most likely real and effective underlying structures in lifestyle and social position. However, although Norway, compared to many other countries, is relatively homogeneous in terms of prosperity, the study may have underrepresented the most marginalised smokers (people in prisons, institutions, etc.) who are not easily recruited by this way of sampling data. Caution is also advised before generalising these findings to other social contexts. While Norwegian smoking laws are comparable to those of the U.K., U.S.A. and Australia, I would urge researchers in other countries to heed Bourdieu's advice and conduct similar analyses.

Conclusion

Various smoking practices enable different categories and forms of understanding, which means that different smoking groups are considered different, as expressing different forms of *habitus*. Because different *habitus* function as distinguishing and separating signs, and because these signs also express real social distances, the lifestyle differences identified in the analyses become 'distinction differences' in the social space. Thus, it seems reasonable to conclude that tobacco use is a mechanism of social differentiation, in the sense that tobacco use is a distinguishing sign of more extensive lifestyle differences and status differences in today's Norway.

Notes

1. Admittedly, the point of occasional smoking does not statistically contribute to the axes above average, but is still included here as the only exception because it is of interest to the problem.
2. Even if some occasional smokers may be former daily smokers moving towards a smoke free life, occasional smoking is not a common transitionary step in processes to quit daily smoking, which usually happens 'cold turkey'.

Disclosure statement

No potential conflict of interest was reported by the author.

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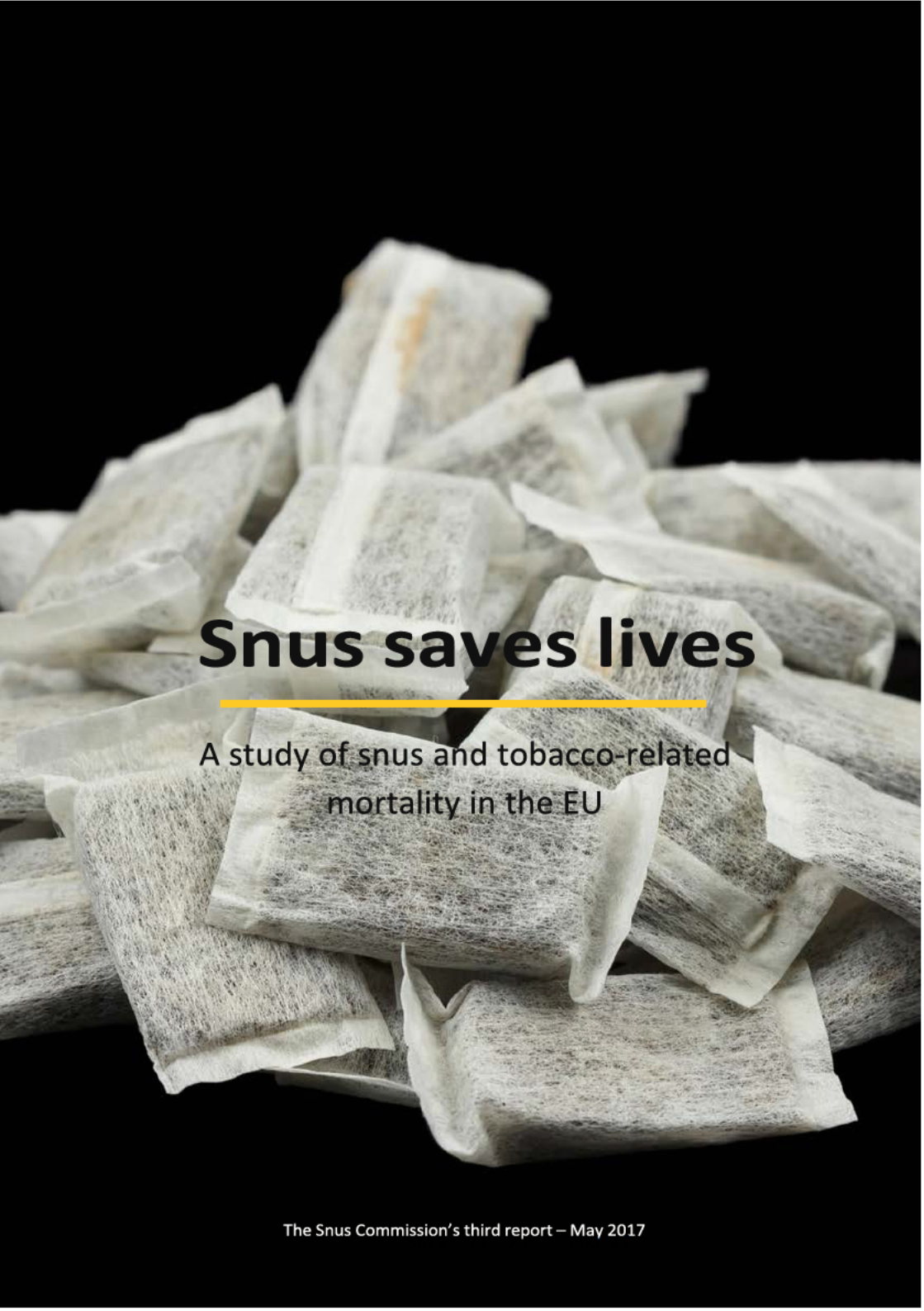
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Snus saves lives

A study of snus and tobacco-related mortality in the EU

The Snus Commission's third report – May 2017

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Summary

This report demonstrates the difference between the current level of tobacco-related mortality in EU countries and the level that would have been achieved had all other EU countries adopted the same tobacco consumption patterns as in Sweden. The basis for the figures in the report consists of data from the World Health Organization (WHO) Global Report: Mortality Attributable to Tobacco (Geneva, 2012). The data processing was carried out by Institutet för Tobaksstudier (Institute for Tobacco Studies) and a compilation of the data was put at our disposal. The figures refer to men over the age of thirty in each individual country and concern several groups of diseases. The report shows that Sweden has the lowest tobacco- related mortality rate of all EU countries relative to its population size. In 24 of the EU's 27 member states, the tobacco-related mortality rate is more than double that of Sweden, relative to their population size.

In total and among men over the age of 30, 355,000 lives per year could have been saved if the other EU countries had matched Sweden's tobacco-related mortality rate. Sweden clearly has the lowest tobacco- related mortality rate within the EU in relation to its population size, despite daily tobacco consumption among men being at the same level as other countries in Europe. Sweden is also the only country in the EU where snus is permitted. There is a clear connection here – snus is a significantly less dangerous product than cigarettes: the difference in terms of health effects corresponds to hundreds of thousands of lives per year in Europe. Snus enables Sweden to have a uniquely low number of smokers, and it is difficult to ignore the connection between the low level of smokers and the uniquely low tobacco-related mortality rate. In other words, the tobacco-related mortality rate would have been lower across the EU today had snus been permitted within the union over the past few decades.

Introduction

Cigarette smoking is the single greatest threat to health in the developed world; up to 6,000 hazardous substances and particles are released when a cigarette is lit. Similarly, a reduction in cigarette smoking is the single most important factor in terms of improving health in major parts of the world. According to the World Health Organization (WHO), 5 million people die each year from smoking cigarettes[1]. In one of our previous reports, "The health

[1] WHO Global Report Mortality Attributable to Tobacco (2012), p 8 http://apps who int/iris/bitstre am/10665/44815/1/9789241564434_eng pdf

effects of snus", we highlighted a major difference in the impact on health depending on the type of tobacco consumed. We noted in that report that snus does not cause cancer and that the overall negative health effects of using snus are minor. This is in sharp contrast to the extensive impact on health caused by smoking cigarettes.

This report provides an answer to an important question: **How many lives would have been saved if the consumption of both snus and cigarettes had been the same in the rest of the EU as in Sweden?** If public health is to be improved and the number of deaths reduced by replacing cigarette smoking with snus, the ban on sales of snus within the EU needs to be lifted. If different political decisions were taken, the mortality rate from tobacco throughout the EU could fall to the levels seen in Sweden. Our belief is that the EU banned the wrong type of tobacco product when it focused on snus.

What is The Snus Commission?

The Snus Commission is an independent commission that produces reports on issues related to Swedish snus. The Commission is financed by the Swedish Association of Snus Manufacturers – a coalition of companies in Sweden that manufacture, market and sell snus. However, since the Commission's reports, analyses and conclusions are independent of its financiers, those financiers have not been able to read the report in advance nor provide feedback on the contents of the report.

In May 2016, The Snus Commission released its first report: "The health effects of snus". The report assessed current research into the alleged health effects of snus, and noted that using snus does not raise the risk of either cancer or heart and cardiovascular diseases. The Snus Commission also produced a number of recommendations for politicians in the report. A second report was released in December 2016: "The State's issue with snus – the link between information and health". This report described the political proposals to limit commercial freedom of expression, such as exposure bans and neutral tobacco packaging, and how these proposals would negatively affect snus consumption and consumers' ability to obtain correct information.



Snuskommissionens ledamöter:

Photo: P-O Roséen, Strångnäs



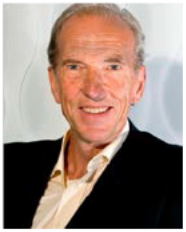
Anders Milton, Chairman of The Snus Commission. Milton is a physician whose former roles include being President and Chairman of the Swedish Medical Association, President of the Swedish Confederation of Professional Associations from 1993 to 2001 and President of the Swedish Red Cross from 2002 to 2005. Milton has also served as the government's investigator into various different issues.
anders.milton@snuskommissionen.se, +46 70 526 46 02



Christina Bellander, is a journalist who was previously Executive Vice President (EVP) of MTG AB and Head of business development at TV4. She is a Board Member of New Wave Group AB, Mittmedia AB and the Swedish Educational Broadcasting Company.
kinna.bellander@snuskommissionen.se



Göran Johnsson's positions include Chairman of the IF Metall labour union from 1993 to 2005, as well as being a former member of the Social Democratic Party's Executive Committee and a Board Member of Volvo AB. He was the Chairman of Swedish national television broadcaster SVT from 2011 to 2014.
goran.johnsson@snuskommissionen.se



Karl Olov Fagerström, Associate Professor and Researcher into tobacco and nicotine. Fagerström was decorated by the WHO for his initiatives against tobacco and has 150 articles published in peer reviewed journal in the nicotine and tobacco area. He also founded a company that sells smoking cessation products and has held positions at Pharmacia.
karl.fagerstrom@snuskommissionen.se

Approach

The basis used for this report consists of the WHO's own data, which is compiled in the WHO Global Report: Mortality Attributable to Tobacco (2012).

WHO's report as source material

The WHO is the only organisation to provide a compilation of statistics surrounding tobacco-related mortality at EU level. Other source material, for example from EUCAN (http://eco.iarc.fr/eucan), provides the number of deaths from cancer but not the proportion specifically attributable to tobacco.

The statistics we refer to here are limited to men. As our intention has been to report the tobacco-related mortality rate and to compare Sweden, where snus is used, to countries where snus is not available as an alternative to cigarettes, it is most relevant to focus on men since it is principally men who use smoke-free tobacco products such as snus. Eighteen percent of the adult male population used snus in Sweden in 2016[2]. Consumption of smoke-free tobacco such as snus among women has generally been a more recent phenomenon. The tobacco habits established several decades ago are those now having an impact on today's statistics into causes of death, and for this reason the male group is more relevant as snus underwent a renaissance in the 1970s and 80s, while use of snus among women initially increased much later. Furthermore, the female group of snus users in Sweden still constitutes just 4 percent of the population in 2016[3].

For our study, we were obliged to adhere to the restriction to people over the age of 30 as used by the WHO. The reason for this restriction is that from a tobacco-related mortality perspective, there is no reason to study people younger than this. Although use of tobacco typically begins in earlier years, it is only after the age of 30 that tobacco-related diseases such as lung cancer begin to occur.

The basis for the material is data from 2004, which represents the WHO's latest statistics in this area and which were published in 2012 in the WHO Global Report: Mortality Attributable to Tobacco[4]. Snus consumption began to increase during the 1970s, reaching a level of 22 percent among men in 2004[5]. A minor reduction has since occurred (from 22 to 18 percent), while the proportion of female snus users in Sweden has remained stable at around 3-4 percent. It is true that smoking has generally decreased in all EU countries, but Sweden – where snus has been available as a substitute – is one of the countries where this reduction has been most evident. Across the EU,

[2] Tobaksvanor – nationella resultat och tidsserier 2016, (Tobacco habits – national results and time series, 2016) (in Swedish), The Public Health Agency of Sweden, https://www folkhalsomyndigheten se/folkhalsorapportering-statistik/ statistikdatabaser-och-visualisering/nationella-folkhalsoenkaten/levnadsvanor/tobaksvanor/

[3] Ibid

[4] WHO Global Report: Mortality Attributable to Tobacco (2012)

[5] Tobaksvanor i Sverige, (Tobacco habits in Sweden), The Swedish Council for Information on Alcohol and Other Drugs, CAN, 2014, (In Swedish), http://www can se/contentassets/d8abb62cc1964de8ba9270862dab25a9/rapport151---tobaksvanor-i-sverige pdf

smoking decreased by 12 percent among adults between 2002 and 2012, while in Sweden smoking decreased by 25 percent during the same period[6].

In our first report, "The health effects of snus" (May 2016), we carried out a major review of current research into snus and its alleged connections with various diseases. This review did not show any support for the conclusion that snus causes cancer or that snus causes heart and cardiovascular diseases. Pregnant women should avoid snus, however, and the effects of a heart attack could be worse for a snus user, for example, even if snus consumption did not in itself increase the risk of the attack. In addition to a review of "all causes of death", in this report we have chosen to focus on two major categories of disease: cancer and heart and cardiovascular diseases. There are generally three major categories of disease with which smoking is typically associated, with respiratory diseases (mainly chronic obstructive pulmonary disease, or COPD) joining the two categories mentioned above. The notion that respiratory diseases are particularly associated with smoking barely needs supporting with evidence. At some points we report cancer of the lungs, bronchi and trachea separately within the category of cancer. It has at times been alleged that using snus could be associated with cancer of the pancreas,[7] but in a review by Bertuccio et al. no such connection could be found[8]. Neither was new research in 2017 by Araghi et al. able to establish that consuming snus increased the risk of pancreatic cancer[9]. If such a risk does exist, it must necessarily be minor given that Sweden has the EU's second lowest occurrence of this type of cancer among men[9]. The WHO estimates the tobacco-related mortality rate based on facts regarding the difference in mortality between smokers and non-smokers. Consideration is also taken of the prevalence of smoking in the relevant country and the country's statistics concerning the number of deaths. For more detailed descriptions of the approach taken in the WHO's calculations, please refer to the WHO Global Report: Mortality attributable to tobacco[10].

Results

This country-based comparison within the EU, based on the WHO's figures, provides very clear results. The tobaccorelated mortality rate in the majority of EU member states is more than twice as high as in Sweden, relative to population size. The tobacco-related mortality rate in three countries – Hungary, Estonia and Latvia –

[6] OECD: Health at a Glance: Europe 2016, p 93, and Health at a Glance, Europe 2014, p 49

[7] Including by the think-tank Tobaksfakta in this text (in Swedish): http://www tobaksfakta se/halsoeffekter-av-snus/ (read 9 April 2017)

[8] Bertuccio P et al (2011): Cigar and pipe smoking, smokeless tobacco use and pancreatic cancer: an analysis from the international Pancreatic Cancer Case-Control Consortium, Annals of Oncology, Volume 22, Issue 6, pp 1420-1426, 2011

[9] Araghi M, et al (2017): Use of moist oral snuff (snus) and pancreatic cancer: Pooled analysis of nine prospective observational studies Int J Cancer

[9] International Agency for Research on Cancer, Estimated incidence, mortality & prevalence in men, 2012 http://eco iarc fr/eucan/Cancer aspx?Cancer=15

[10] WHO Global Report: mortality attributable to tobacco (2012), p 7ff

is almost four times higher than Sweden relative to population size. In absolute terms, eighteen of the EU's members states have a higher tobacco-related mortality rate than Sweden, including countries with smaller populations than Sweden, such as Denmark and Bulgaria.

We would like to stress that there is a significant delay between the start of consumption and the effects of disease. Even if the levels of both smokers and snus users were to match Sweden's level as of tomorrow, the effects of tobacco-related mortality would only be noted several decades from now. This can sadly act as a disincentive among our political leaders in Europe in terms of bringing about change, as not they but their successors will be the ones who reap the political rewards of a new snus policy.

Table 1. Number of deaths attributable to tobacco-related heart and cardiovascular diseases per year, men over 30 in the EU + Norway (2004)

1. Poland 25 658
2. Germany 24 441
3. Romania 15 780
4. Italy 15 264
5. The UK 14 531
6. France 12 527
7. Spain 9 574
8. Hungary 8 576
9. Bulgaria 7 729
10. Czech Republic 5 566

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22. Sweden 1 122

Tobacco-related heart and cardiovascular diseases Every member state in the EU has a higher mortality rate due to tobacco-related heart and cardiovascular diseases than Sweden, relative to population size. In 25 of 27 EU countries, the mortality rate is more than double that of Sweden relative to population size. In Spain and France, the mortality rate due to tobacco-related heart and cardiovascular diseases is "only" 94 and 83 percent higher than Sweden respectively.

No other country is therefore even close to Sweden's level in terms of tobacco-related heart and cardiovascular diseases. In ten of the EU's member states, the mortality rate (the number of deaths per 100,000 inhabitants) is more than five times higher than in Sweden. These ten countries with considerably higher mortality rates due to

Table 2. Number of deaths attributable to tobacco-related cancer per year, men over 30 in the EU + Norway (2004)

1. Germany 41 361
2. France 38 118
3. Italy 37 015
4. The UK 29 241
5. Poland 26 075
6. Spain 25 194
7. Romania 10 991
8. The Netherlands 9 343
9. Hungary 9 181
10. Belgium 7 734

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18. Sweden 2 077

tobacco-related heart and cardiovascular diseases are Estonia, Latvia, Lithuania, Poland, Hungary, Czech Republic, Slovakia, Croatia, Romania and Bulgaria. These EU countries in eastern and central Europe have had a high proportion of smokers throughout the 1900s.

Even in absolute terms, most EU countries have a higher number of deaths attributable to tobacco-related heart and cardiovascular diseases than Sweden. The highest number of deaths annually occur in Poland, Germany and Romania (see table 1).

If the EU had the same mortality rate due to tobacco-related heart and cardiovascular diseases as Sweden, around 115,000 lives would be saved in the union each year. The highest number of lives would be saved in Poland, where over 22,000 people would be saved each year if the tobacco-related mortality rate linked to heart and cardiovascular diseases was at the same level as in Sweden.

Tobacco-related cancer

Every other member state in the EU has a higher mortality rate due to tobacco-related cancer than Sweden, relative to population size. In 25 of 27 EU countries, the annual mortality rate is more than double that of Sweden. In Cyprus and Finland, the tobacco-related mortality rate for cancer relative to population size is higher than in Sweden, but not quite twice.

In absolute terms, the number of deaths attributable to tobacco-related cancer is highest in Germany, France and Italy, as shown in Table 2. Only countries with considerably smaller populations than Sweden have fewer deaths due to tobacco-related cancer in absolute terms.

If the mortality rate for tobacco-related cancer were equivalent to the rate in Sweden in 2004, around 178,000 lives would be saved annually. The highest number of lives would be saved in France (around 26,000 lives annually), Germany (around 23,000 lives annually) and Poland (around 20,000 lives annually) if the mortality rate in these member states matched the rate in Sweden. We have chosen to report the most commonly occurring types of tobacco-related cancer separately, i.e. tobacco-related cancer of the lungs, bronchi and trachea. Every other member state in the EU has a higher mortality rate due to tobacco-related cancer of the lungs, bronchi and trachea than Sweden, relative to population size. In 23 of 27 EU countries, the mortality rate for these types of cancer is more than double that of Sweden relative to population size. Cyprus, Finland, Portugal and Austria are the only countries where the tobacco-related mortality rate for cancer of the lungs and trachea are not over double that of Sweden in relation to their population size. If the mortality rate for tobacco-related cancer of the lungs, bronchi and trachea were equivalent to the rate in Sweden, around 111,000 lives would be saved annually. The highest number of lives would be saved in Italy, where over 15,000 lives would be saved each year if the mortality rate due to tobacco-related cancer of the lungs, bronchi and trachea matched the Swedish mortality rate.

Total tobacco-related mortality

We have reported the tobacco-related mortality rate for heart and cardiovascular diseases and cancer separately. The total mortality rate for both of these groups is higher in all other EU member states than in Sweden, relative to population size. Sweden also has the lowest proportion of tobacco-related deaths when taking into account all causes of death. Every other member state in the EU has a higher tobacco-related mortality rate than Sweden, relative to population size. In 24 of 27 EU countries, the mortality rate is more than double that of Sweden. In Estonia, Latvia and Hungary, the mortality rate is more than four times that of Sweden. The countries topping the list generally coincide with the countries that continue to have the highest proportion of smokers in Europe, with Hungary, Latvia and Estonia all reporting over 30 percent of men smoking in 2014 according to the OECD, compared to the EU average of 26 percent[11]. In this context, it should be pointed out that the

Table 3. Number of deaths attributable to tobacco per 100,000 inhabitants per year, men over 30, all causes of death in the EU + Norway (2004)

1. Hungary 699
2. Latvia 657
3. Estonia 636
4. Lithuania 573
5. Poland 566
6. Bulgaria 530
7. Croatia 530
8. Romania 510
9. Belgium 490
10. Slovakia 469

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18. Luxembourg 310
19. Ireland 301
20. Germany 301
21. Malta 292
22. Portugal 288
23. Austria 247
24. Norway 245
25. Finland 237 26. Cyprus 191

27. Sweden 152

number of male smokers has been even higher in these countries in the past. If we go back to 2004, which is the year on which the statistics concerning tobacco-related mortality rates in this report are based, the number of smokers in these countries was even higher[12]. This means that fewer people would be saved by a new snus policy introduced by the EU today

[11] OECD: Health at a Glance: Europe 2016, p 93, http://www keepeek com/Digital-Asset-Management/oecd/social-issues-migration-health/health-at-a-glance-europe-2016_9789264265592-en# WQCswfmGOM8#page93

[12] OECD: Health at a Glance: Europe 2014: p 49

because fewer people are smoking at the start of the period, although the relationship between countries would however generally remain, and the gains in terms of saved lives would still be considerable, to say the least.

The lives that have now been lost due to the EU's snus ban in the 1990s cannot be brought back. However, the EU can do a lot to ensure that fewer people today lay the foundations for a premature death by ensuring that more people make the transition from cigarettes to snus. Sweden also has a low tobacco-related mortality rate in absolute terms. According to the WHO's statistics from 2004, 4,267 people in Sweden died from tobacco-related causes that year – a figure that can be compared to Denmark's 6,160, despite Denmark having a lower population. The highest number of tobacco-related deaths per year was in Germany (80,843), Italy (65,444) and the UK (61,890).

If every EU member state had had the same tobacco-related mortality rate as Sweden, many lives would have been saved. Around 355,000 lives would have been saved each year, if the rate of the rest of the EU had been the same as in Sweden.

The highest number of lives would be saved in Poland (around 46,000 lives annually), Germany (around 44,000 lives annually),
Italy (around 37,000 lives annually), the UK (around 36,000 lives annually) and France (around 34,000 lives annually) if the mortality rate in these member states matched the rate in Sweden. The aspect of Sweden's tobacco policy that differs in relation to other EU countries is precisely the consumption of snus. We have also compared the tobacco-related mortality rate with Norway, which is another country where snus is consumed, and this provides support for the existence of a positive connection with snus, as the tobacco-related mortality rate is also very low in Norway. Out of all the countries in the comparison, only Sweden, Finland and Cyprus have a lower tobacco-related mortality rate than Norway. If snus had been permitted thirty years ago, the mortality rate today would have been much lower as it would have resulted in a lower proportion of smokers and therefore better public health with fewer tobacco-related deaths.

The consumption of snus in Norway could also likely explain the low tobacco-related

Table 4. Number of deaths attributable to tobacco (men over 30) per year that could have been spared had tobacco habits been the same as in Sweden, all causes of death (2004)

1. Poland 46 730
2. Germany44 473
3. Italy 37 160
4. The UK 36 452
5. France 33 693
6. Spain 29 081
7. Romania 23 432
8. Hungary 16 463
9. The Netherlands 11 961
10. Belgium 11 149
11. Czech Republic 9 754

mortality rate there – see page 8. In Norway, the proportion of snus users among men over 30 is 14 percent[13]. We put a question mark over the figures for Cyprus, which consistently ranks low in statistics for tobacco-related deaths despite a high proportion of smokers in both the OECD's and WHO's statistics[14].

What do other researchers say?

In our first report, "The health effects of snus" (May 2016), we noted that "Sweden has the highest percentage of snus users in Europe, while also having the lowest percentage of smokers" and that we in Sweden have replaced one kind of tobacco with another more harmless kind.

The connection we highlight in this report, which shows that the proportion of smokers is low in Sweden compared to the rest of the EU and that the number of snus users is high compared to the rest of the EU, is no coincidence. The notion that the high proportion of snus users is due both to the fact that snus is used as a smoking cessation product and as an alternative to cigarettes can be considered to have scientific support, including in a report from the Royal College of Physicians published in 2016. In that report, it was noted that "particularly among men, availability of snus as a substitute to cigarettes has helped to reduce the prevalence of smoking in Sweden"[15]. In our first report, we were able to report that Australian researchers, in their study by using Sweden as an example, established that there is a correlation between the increased use of snus and the decreased use of cigarettes – and thus a reduction in mortality caused by tobacco-related ill health. The same researchers also conclud-

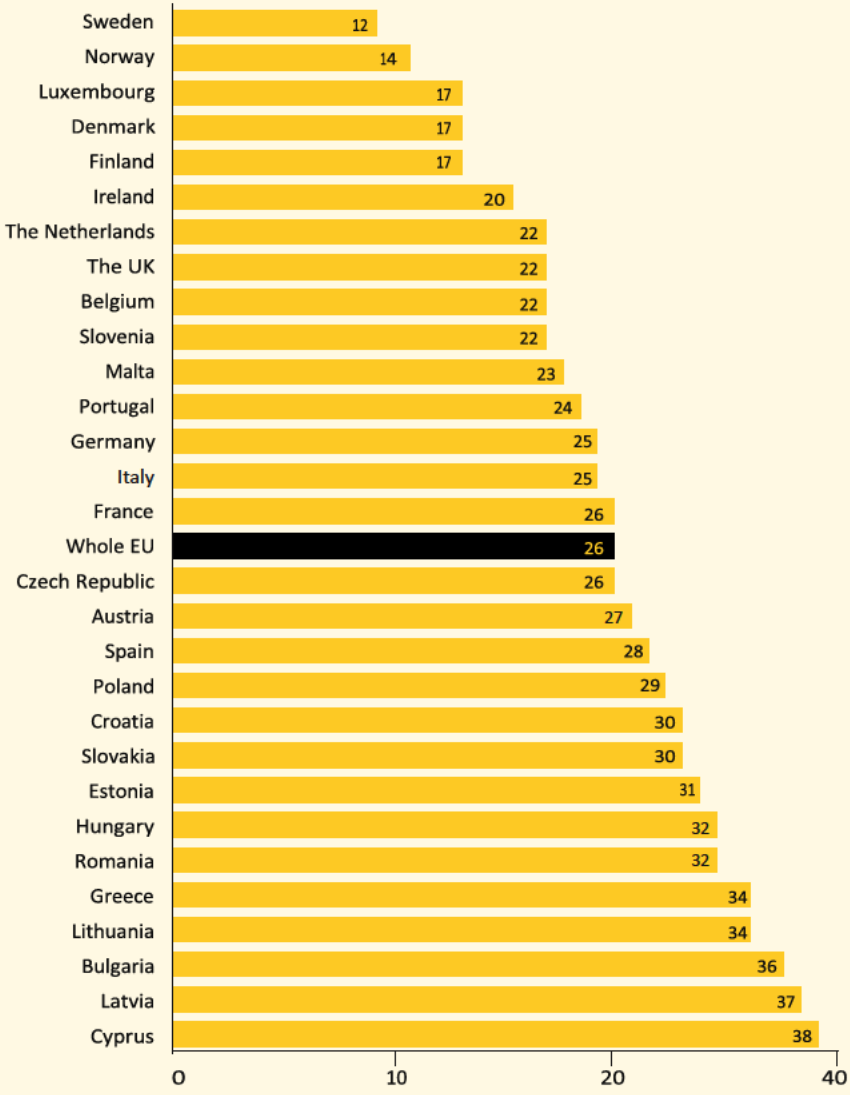
[13] Helserisiko ved bruk av snus 2014 (Health risks linked to use of snus) (in Norwegian), Norwegian Institute of Public Health, https://www fhi no/globalassets/migrering/dokumenter/pdf/ helserisiko-ved-bruk-av-snus-pdf pdf

[14] OECD: Health at a Glance: Europe 2016, p 93, http://www keepeek com/Digital-Asset-Management/oecd/social-issues-migration-health/health-at-a-glance-europe-2016_9789264265592-en# WQCswfmGOM8#page93

[15] Royal College of Physicians (2016): Nicotine without smoke: Tobacco harm reduction, s 6

Table 5. Number of people who smoke on a daily basis among men (%)

Proportion in percent among men over 30 in 2014 in the EU + Norway



Source: OECD: Health at a Glance: Europe 2016, p. 93, http://www.keepeek.com/Digital-Asset-Management/oecd/social-issues-migration-health/health-at-a-glance-europe-2016_9789264265592-en#.WQCswfnGOM8#page93

ed that the health benefits when cigarette smokers switch to smokeless tobacco with a low level of nitrosamines (such as Swedish snus) are almost as great as when these smokers completely abstain from tobacco consumption[16]. There are also several other studies that show how low tobacco-related mortality figures in Sweden are consistent with Sweden's high proportion of snus users[18]. We can also note that several different studies support the conclusion that snus replaced the cigarettes, thereby yielding positive results for public health in Sweden. Other researchers have also explicitly calculated the health effects in terms of their influence on the number of premature deaths. In one study, calculations were made concerning how many lives would be saved if snus consumption increased in conjunction with an equivalent fall in the number of cigarette smokers.

Their conclusion was that 200,000 fewer men and 1,100 fewer women would die from tobacco-related diseases each year within the EU if the proportion of smokers and snus users reached the levels registered in Sweden, i.e. if the number of smokers exceeding the Swedish level made the transition to using snus to a greater extent instead[17]. These researchers therefore came to slightly lower estimations than the WHO's calculations in this report, but their conclusion points in exactly the same direction: the number of lives that would be saved if more people used snus and fewer people smoked amount to six-figure levels.

Similar estimates regarding how many lives would be saved if more people switched from cigarettes to snus have also been made by researchers in the US. Modelling conducted by US and Canadian researchers showed how much smoking could be estimated to be reduced in the US if smokeless tobacco with a low level of nitrosamines – as in Swedish snus – were introduced in the US market and marketed using the correct information about its harmfulness. They concluded that a new policy under which smokeless tobacco with a low level of nitrosamines was permitted would result in an accelerating trend towards fewer smokers in the US. The current decline in the number of smokers would accelerate over a five-year period, from 1.3 percent to 3.1. There are thus several studies that point in the direction as our study[18].

[16] Gartner C, Hall W (2009): Harm reduction policies for tobacco users Int J Drug Policy 2010 Mar; 21(2):129-30

[17] (Rodu B , Cole P (2004): The burden of mortality from smoking: comparing Sweden with other countries in the European Union Eur J Epidemiol 2004;19(2):129-31)

[18] Ramström, Wikmans (2013): Mortality attributable to tobacco among men in Sweden and other European countries: an analysis of data in a WHO report https://tobaccoinduceddiseases biomedcentral com/articles/10 1186/1617-9625-12-14

[18] Levy D, Mumford E, Cummings KM, Gilpin EA, Giovino GA, Hyland A, Sweanor D, Warner KE, Compton C (2005): The potential impact of a low-nitrosamine smokeless tobacco product on cigarette

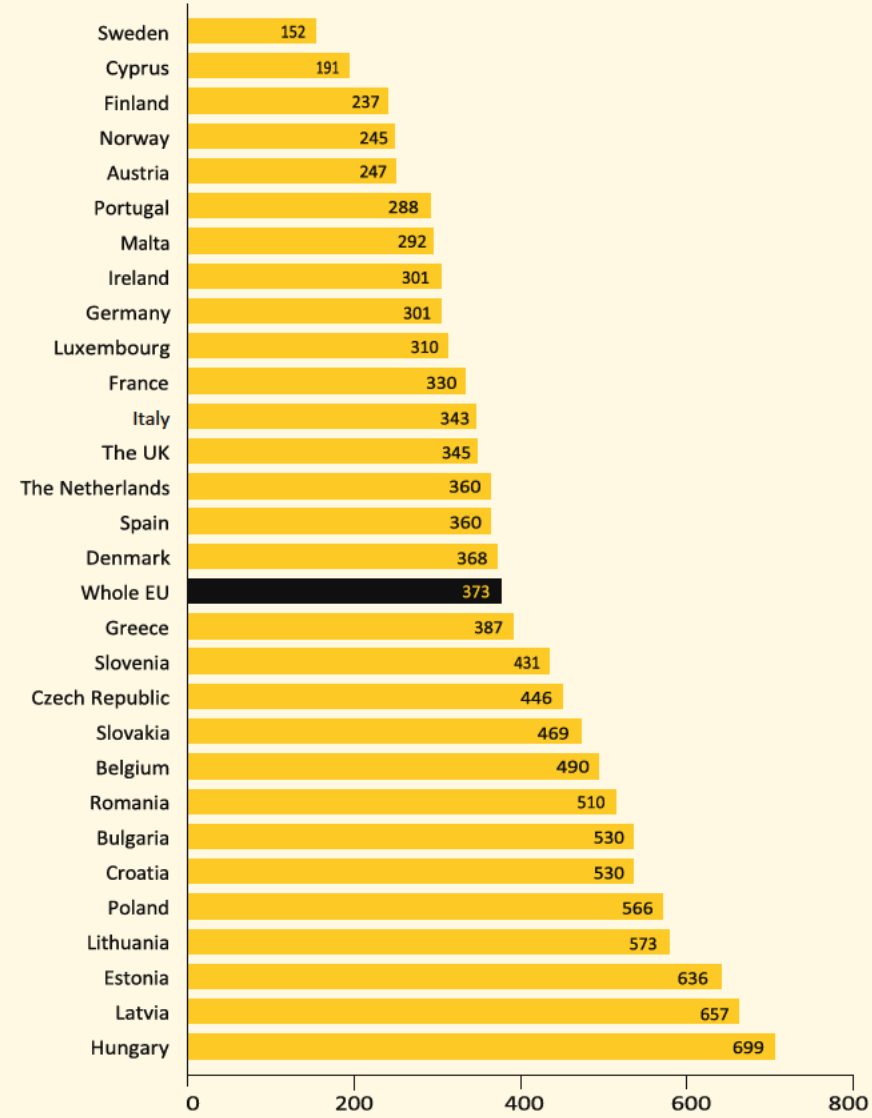
The Commission's conclusions

Important to highlight the differences in tobacco-related mortality between Sweden and other EU countries

Sweden has both the EU's lowest tobacco-related mortality rate and the EU's lowest proportion of smokers among its adult population. Sweden also distinguishes itself by way of its high proportion of snus users. The notion that the tobacco-related mortality rate is consistent with the number

smoking in the United States: Estimates of a panel of experts Addict Behav 2006 Jul;31(7):1190-200 Epub 2005 Oct 26

Table 6. Mortality attributable to tobacco per 100,000 inhabitants (men over 30) in the EU + Norway, all causes of death



Source: The calculations are based on data in the WHO Global Report Mortality Attributable to Tobacco (2012)

of smokers is indisputable. In this report, we show that there is clear support for the theory that Sweden's low number of smokers is a result of its high number of snus users. The role of snus as a means of stopping smoking and as an alternative to cigarettes has made a positive contribution to the health of people in Sweden. We have been able to show in this report that the low tobacco-related mortality rate applies both generally and to both the heart and cardiovascular disease and cancer groups respectively.

Time for European politicians to reconsider the ban on snus in the EU In light of the fact that Sweden clearly has a lower tobacco-related mortality rate than other countries, the question arises as to what it is that distinguishes us. The only real difference with respect to tobacco is that Sweden permits the use of snus. This report shows that snus has played an important role in public health as an alternative to cigarettes and as a means to stop smoking, which is why there are very strong grounds for the EU to consider a review of the ban on snus in the EU.

Lifting the ban would, according to the calculations that we report here, eventually save hundreds of thousands of lives per year within the EU.

The Swedish government should promote snus within the EU

In light of Sweden's low tobacco-related mortality rate, it is surprising that the Swedish government does not promote snus within the EU more often and more purposefully. Sweden fought to keep snus available when joining the EU, despite the fact that we did not know everything we know now about the health effects of replacing cigarettes with snus. There are grounds for the Swedish government to bring this to the EU's attention, not least because if Sweden is to maintain its snus exemption in the long term, its case may need to be highlighted regularly at EU level.

The EU has shown in various policy areas that it does not always have a thorough understanding of the circumstances in Sweden, so it is up to Swedish politicians to inform them and explain how it works – that snus has contributed to Sweden's low levels of tobacco-related mortality.

EU citizens should be offered information about the health effects of snus

Snus has a significantly lower impact on a person's health than cigarettes. The EU has banned the wrong tobacco product. The
difference is so large that it is, in fact, problematic to talk about snus and cigarettes as part of the same category – tobacco. The focus would be better placed on combatting consumption of smoking tobacco within the EU. Instead, the ban has been placed on snus which could serve as an effective smoking cessation product for EU citizens. Let EU citizens choose for themselves whether they want to use snus or not, and let EU citizens access correct information about the impact on health of both snus and cigarettes. More information would be a positive thing for both consumers and public health, not a danger.

Different nicotine products are not treated in a fair manner There is

epidemiological research into snus – a product that is not permitted within the EU. There is, however, no equivalent research into e-cigarettes, for example, despite them being permitted within the EU and released as a new product. Nor are the regulations proportional with respect to the impact on health of different nicotine products, with cigarettes – that have a major impact on health – permitted in every EU member state while snus – that has a very limited impact – only permitted in Sweden.

Arguments against the release of new tobacco products have collapsed

During Sweden's negotiations for EU membership, the EU wanted to maintain its general ban on snus and offer Sweden an exemption from that rule, instead of removing the general ban. This was due to the fact that the EU did not want to establish new tobacco products in EU countries. This argument has now collapsed as the EU recently permitted e-cigarettes to enter the market, and the issue of permitting snus within the EU has thereby been reawakened.

Statistical basis: Tobacco-related mortality among men over 30 in the EU, 2004

The calculations are based on data in the WHO Global Report: Mortality Attributable to Tobacco (2012)

Explanation of the tables: In the tables on the following pages, the number of deaths attributable to tobacco in different cause of death categories are presented for men aged 30 or above in EU countries and Norway.

The numbers have been calculated based on data presented in the WHO report for five different age groups in terms of population size and number of deaths attributable to tobacco (number per 100,000 people) in different cause of death



categories. The number of deaths attributable to tobacco has been calculated as the total of the numbers in the individual age groups. For each country, calculations have been made for two different scenarios. The first refers to the relevant number of deaths according to the country's reported mortality rate. The second scenario refers to the expected number of deaths if the equivalent Swedish mortality rate had applied in that country.

In the "All causes of death" category, the number of deaths per 100,000 people is also stated, i.e. that country's mortality rate. This enables us to make broad comparisons between countries in terms of the extent of mortality attributable to tobacco, regardless of differences in population.

Appendix A. Table 7. Number of tobacco-related deaths, heart and cardiovascular diseases among men over the age of 30 in the EU + Norway, 2004

Country	Population (number of men over 30, millions)	Tobacco-related deaths per year, heart and cardiovascular diseases	If in line with Swedish mortality rate
Belgium	3,196	3.739	1.238
Bulgaria	2,371	7.729	959
Cyprus	0,221	183	79
Denmark	1,674	1.841	623
Estonia	0,358	1.178	132
Finland	1,589	1.255	608
France	17,896	12.527	6.834
Greece	3,484	4.564	1.411
Ireland	1,092	863	369
Italy	19,080	15 264	7.549
Croatia	1,349	2.779	526
Latvia	0,624	2.346	228
Lithuania	0,916	2.574	322
Luxembourg	0,138	120	48
Malta	0,116	89	42
The Netherlands	5,050	4.293	1.773
Poland	10,430	25.658	3.673

Portugal	3,107	2.392	1.209
Romania	6,140	15.780	2.296
Slovakia	1,459	3.137	479
Slovenia	0,609	834	220
Spain	13,260	9.574	4.966
The UK	17,939	14.531	6.833
Sweden	2,807	1.122	1.122
Czech Republic	3,025	5.566	1.100
Germany	26,858	24.441	10.378
Hungary	2,878	8.576	1.060
Austria	2,597	1.584	929
Whole EU	**150,263**	**174.539**	**57.006**
Norway	1,386	790	503

Appendix B. Table 8. Tobacco-related deaths, cancer among men over the age of 30 in the EU + Norway, 2004

Country	Population (number of men over 30, millions)	Tobacco-related deaths per year, cancer of the lungs, bronchi and trachea	If mortality rate were in line with Swedish rate	Tobacco-related deaths per year, cancer total	If mortality rate were in line with Swedish rate
Belgium	3,196	5.465	1.493	7.734	2.226
Bulgaria	2,371	2.442	1.139	3.770	1.693
Cyprus	0,221	122	93	170	140
Denmark	1,674	1 841	743	2.829	1.110
Estonia	0,358	494	150	816	283
Finland	1,589	1.224	699	1.764	1.041
France	17,896	22.012	8.299	38.118	12.421
Greece	3,484	4.947	1.738	7.107	2.586
Ireland	1,092	928	427	1.441	638
Italy	19,080	24 232	9.366	37.015	13.958
Croatia	1,349	2.077	601	3.292	891
Latvia	0,624	842	260	1.404	385

Lithuania	0,916	1.099	372	1.878	551
Luxembourg	0,138	141	55	218	82
Malta	0,116	106	47	150	71
The Netherlands	5,050	6.313	2.056	9.343	3.068
Poland	10,430	16.792	4.073	26.075	6.079
Portugal	3,107	2.454	1.467	4.319	2.184
Romania	6,140	6.447	2 651	10.991	3.934
Slovakia	1,459	1.576	529	2.889	790
Slovenia	0,609	816	246	1.285	366
Spain	13,260	16.045	6.152	25.194	9.181
The UK	17,939	18 298	8.370	29.241	12.502
Sweden	2,807	1.404	1.404	2.077	2.077
Czech Republic	3,025	4.084	1 245	6.443	1.854
Germany	26,858	26.589	12.312	41 361	18.225
Hungary	2,878	5.555	1.215	9.181	1.810
Austria	2,597	2.078	1.104	3.350	1.644
Whole EU	**150,263**	**176.423**	**65.674**	**279.455**	**101.790**
Norway	1,386	1.137	611	1 677	917

Appendix C. Table 9. Total tobacco-related deaths among men over the age of 30 in the EU + Norway, 2004

Country	Population (number of men over 30, millions)	Tobacco-related deaths per year	If mortality rate were in line with Swedish rate	Different in no. of deaths
Belgium	3,196	15.660	4.511	-11.149
Bulgaria	2,371	12.566	3.406	-9.160
Cyprus	0,221	422	284	-138
Denmark	1,674	6.160	2.254	-3.906
Estonia	0,358	2.277	445	-1.832
Finland	1,589	3.766	2.105	-1.661
France	17,896	59.057	25.364	-33.693
Greece	3,484	13.483	5.223	-8.260

Ireland	1,092	3.286	1.292	-1.994
Italy	19,080	65.444	28.284	-37.160
Croatia	1,349	7.150	1.781	-5.369
Latvia	0,624	4.100	767	-3.333
Lithuania	0,916	5.249	1.105	-4144
Luxembourg	0,138	428	164	-264
Malta	0,116	339	144	-195
The Netherlands	5,050	18.180	6.219	-11 961
Poland	10,430	59.034	12.304	-46.730
Portugal	3,107	8.948	4.410	-4.538
Romania	6,140	31 314	7.882	-23.432
Slovakia	1,459	6.843	1.600	-5.243
Slovenia	0,609	2.625	734	-1.891
Spain	13,260	47.736	18.655	-29.081
The UK	17,939	61.890	25.438	-36.452
Sweden	2,807	4.267	4.267	0
Czech Republic	3,025	13.492	3.738	-9.754
Germany	26,858	80.843	36.370	-44.473
Hungary	2,878	20.117	3.654	-16.463
Austria	2,597	6.415	3.319	-3.096
Whole EU	**150,263**	**561.091**	**205.719**	**-355.372**
Norway	1,386	3.396	1.880	-1.516

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info@snuskommissionen.se

Anders Milton, anders.milton@snuskommissionen.se, +46 (0)70 526 46 02
Christina Bellander, kinna.bellander@snuskommissionen.se
Göran Johnsson, goran.johnsson@snuskommissionen.se
Karl Olov Fagerström, karl.fagerstrom@snuskommissionen.se

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Cute as candy: a qualitative study of perceptions of snus branding and package design among youth in Norway

Janne Scheffels, Ingeborg Lund

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Department of Drug Policy, Norwegian Institute of Public Health, Oslo, Norway

Correspondence to
Dr Janne Scheffels;
janne.scheffels@fhi.no

ABSTRACT

Objectives: Snus use has increased among youth in Norway in recent years and is now more prevalent than smoking. Concurrently, a range of new products and package designs have been introduced to the market. The aim of this study was to explore how youth perceive snus branding and package design, and the role, if any, of snus packaging on perceptions of appeal and harm of snus among youth.

Participants: Adolescent tobacco users and non-users (N=35) ages 15–17 years.

Design: We conducted interviews among 6 focus groups (each with 4–7 participants). Participants were shown snus packages with a variety of designs and with different product qualities (flavour additives, slim, regular, white and brown sachets) and group discussions focused on how they perceived packages and products. The focus group discussions were semistructured using a standard guide, and analysed thematically.

Results: The participants in the focus groups narrated distinct images of snus brands and associated user identities. Package design elements such as shapes, colours, images and fonts were described as guiding these perceptions. Packaging elements and flavour additives were associated with perceptions of product harm. The appeal of flavoured snus products and new types of snus sachets seemed to blend in with these processes, reinforcing positive attitudes and contributing to the creation of particular identities for products and their users.

Conclusions: The findings indicate that packaging is vital to consumer's identification with, and differentiation between, snus brands. In view of this, snus branding and packaging can be seen as fulfilling a similar promotional role as advertising messages: generating preferences and appeal.

Strengths and limitations of this study

- The first study to explore perceptions of snus branding and packaging in Scandinavia, where snus use is most common.
- Exploratory design provides insight into what the study group perceives as important regarding snus branding and packaging.
- Small sample size does not provide representative data, although given the exploratory nature of the study, this was not our aim.

INTRODUCTION

Snus (a traditional Swedish-type oral moist snuff) has a long product history and use tradition among men in Scandinavia. In Norway, snus sales were low until around 1990, when the traditional moist snuff was reintroduced as a new, drier product packed in small sachets. Since then, snus use has increased dramatically, particularly among youth. In 2016, 25% of 16-24 year olds were daily or occasional snus users. Daily use was slightly more prevalent among males (21%) than females (17%). In comparison, the prevalence of daily and occasional smoking in this age segment was 3% and 13% respectively.[1] The only country with a similar level of snus use is Sweden, that was granted an exemption from the ban on sale of snus in the European Union (EU) as a condition of their membership treaty. Norway is not a member of the EU, and snus is legally sold and used. In other countries where snus is marketed, like the USA, the prevalence of snus use is low.[2 3] Massive marketing of snus in the USA, including new products from companies also producing cigarettes, such as Camel and Marlboro snus,[4 5] has however given rise to some concern that snus use may be escalating also outside Scandinavia. While the majority of adult snus users in Norway are former smokers, the majority of young users started without prior smoking.[6] Consequently, the public health effects from adolescent use could be quite different from that of adult use, as switching to snus implies a substantial harm reduction for individual smokers while initiation of snus use implies an increased risk of harm for non-tobacco users.[7 8] Similar to cigarettes, the minimum age for purchasing snus in Norway is 18 and warning labels are mandatory, although for snus a smaller sized, non-graphic version, with a more moderated textual content, is

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used. Both products are sold in regular shops, and no form of advertising is allowed, including point of sale advertising ban.[9] During the period of increase in snus use in Norway, a range of new snus brands and products have been introduced to the market, including a wide variety of products with flavour additives such as mint, fruit or chocolate and with different shapes and colours of snus sachets. For example, Swedish Match, the market-leading snus-producing company in Scandinavia, increased its brand portfolio from 22 variants in 2002 to 180 variants 6 years later.[10] Snus packages, traditionally dark-coloured and round boxes that resemble an ice hockey puck, are today available in a wide variety of colours and designs. In countries with comprehensive restrictions on advertising, tobacco packaging is the most prominent form of marketing. Through the use of colour, fonts, images and trademarks, packages can project a brand image that says something about the user of the product and about product qualities.[11] Research has demonstrated how light-coloured or white packages are perceived as less harmful than darker packages,[12 13] and how notions of relative harm can be strengthened by variant descriptor names denoting colour.[14 15] A systematic review of research on cigarette packaging has indicated that such processes can be counteracted by standardising cigarette pack appearance.[16] This conclusion has been largely supported by recent evaluation results from Australia,[17 18] where legislation mandating standardised packaging of tobacco was implemented in 2012. It has also been indicated by experimental research that cigarette packaging developed to appeal particularly to young women, for example, packages looking like lipstick boxes, are perceived as appealing and as less harmful than more standard cigarette packages, because of their smaller size and likeness to cosmetics.[19] Similarly, studies of flavoured cigarettes has indicated that these products often are perceived as less harmful and as more attractive than regular cigarettes, in particular among young women.[20 21]

While research about cigarette packaging has increased over the latest decade, only a few studies have explored how consumers perceive snus packaging and product design. In the USA, Choi *et al*[22] found that youth perceived snus products as attractive, thought the packages were more modern than cigarette packages and found them cleverly designed to attract attention. Liu *et al*[23] similarly found that the colour, design and size of smokeless tobacco packaging appealed to adults and adolescents and influenced purchase decisions. Adkison *et al*[24] analysed the impact of snus packaging on perceptions of product appeal and beliefs about health risk in a US sample, and concluded that branded packages were seen as more appealing than packages without corporate branding, and that youth and young adults were more likely than adults to indicate that package elements affected their perceptions of appeal and risk associated with the use of snus. To date, there have been no published studies exploring the perceptions of snus branding and packaging in Scandinavia, where snus use is most common and has been on the market for some time.

In order to provide insight into youth's perceptions of snus branding and package design in a mature snus market, we recruited young tobacco users and non-users of both products to participate in a series of focus group discussions. We addressed the following research question: How do young tobacco users and non-users perceive snus branding, packaging and product design? More specifically focusing on the upswing in adolescent snus use, we also sought to explore the role, if any, of snus packaging on perceptions of appeal and harm of snus among youth.

METHODS

Six focus group interviews (4–7 participants in each group) of male and female tobacco users and non-users (N=35) aged 15–17 years were undertaken. We recruited the participants into the different groups based on their self-definition as smokers, snus users or non-users of tobacco (table 1). In four of the focus groups, all participants defined themselves as snus users or smokers, while in two, they defined themselves as non-users of both products. In the focus group discussions, it was revealed that many of the smokers had experience with using both products, ranging from having tried snus once or twice, to using snus when cigarettes were not available to them, to regular dual use. Also, some of the snus users smoked occasionally, in particular at parties, and most had tried smoking at least once. These patterns were not unexpected, given that dual use of cigarettes and snus is common among youth in Norway.[25 26] A recent survey

Table 1 Overview of groups and participants

Group number	Number of participants	Self-defined tobacco use status	Age range (years)	Gender
1	7	Non-users	16–17	6 girls, 1 boy
2	5	Tobacco users	16–17	3 girls, 2 boys
3	4	Tobacco users	16–17	4 boys
4	6	Tobacco users	16–17	6 girls
5	6	Non-users	15–16	6 girls
6	7	Tobacco users	16–17	4 girls, 3 boys
Total	35	22 tobacco users, 13 non-users	15–17	25 girls, 10 boys

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Table 2 Overview of packages

Brand	Brand variant	Package design	Flavour additive
General	Classic Loose	Dark colour, gold decor	None
General	Classic Portion	Dark colour, red decor	None
The Lab	06 Extra Strong slim portion	Light colour, red decor	None
The Lab	Fresh Lemon slim white portion	Light colour, yellow and white decor	Lemon
Skruf	Fresh White slim portion	Light colour, light blue decor	Mint
Skruf	Nord 66 Strong portion	Dark colour, green decor	None
Nick & Johnny	Radical Red Xtra strong	Dark colour, purple decor	Chili
Nick & Johnny	Captain Yankee Xtra strong	Dark colour, red and blue decor	None
Catch	White Spearmint Slim	Light colour, light blue decor	Spearmint
Mocca	Mint White miniportion	Light colour, pink decor, smaller size package	Mint

among 15-year-old adolescents showed that almost half of all tobacco users were dual users of snus and cigarettes.[27] As this means that also self-defined smokers were likely to be familiar with snus and snus branding, 'we decided to treat both groups of tobacco users as one in the reporting of results. In the groups that self-defined as non-users of tobacco, we learnt during the discussions that some had tried snus or cigarettes. However, as the group discussions indicated that the distinction between use and non-use of tobacco was important to their self-understanding, we decided to retain the label non-users in our reporting of results.

Non-tobacco users were included in the sample in order to compare their opinions and reflections with those of tobacco users, and to explore more directly the appeal of snus packages to potential new users. All participants were recruited through three initial contacts who were aged 15, 16 and 17 years, respectively, and who were members of the two researchers' social networks. These contacts attended three different schools and recruited participants from their social networks, who in turn recruited additional participants from their circles of friends and acquaintances. The total sample included individuals from nine different junior and senior high schools in Oslo. The three initial contacts received remuneration of 50 Norwegian kroner for each participant they recruited. The focus group participants received a gift voucher of 300 Norwegian kroner as compensation for their study participation.

The focus group interviews were semistructured using a standard guide. The groups were conducted in Norwegian, and only the quotes used in the paper were translated into English, with validation from both authors. All participants were asked about experience with tobacco use and general perceptions of snus use and smoking, including ideas about typical users. Ten snus packages were then introduced as stimuli for the discussions. The participants were asked how they perceived the products before them, with regard to appeal and potential for harm to health. They were also asked to associate typical users of the different packages. The packages were chosen to represent a variety of designs, colours and shapes, including light-coloured and dark-coloured packages and packages in different sizes. We included also packages with and without flavour additives and packages with white and brown sachets (see table 2 for an overview of packages). Summing up, the moderator asked the groups to discuss the role of branding and packaging for the appeal of snus.

The group sessions lasted an average of 60 min. Each was audio recorded, transcribed and analysed using the HyperResearch software package for qualitative analysis. Analyses was performed systematically,[28] beginning with several rounds of transcript reading followed by thematic coding in HyperResearch. Both authors read all transcripts, the first author did the initial coding and the coding was then discussed between the authors before they were organised into larger themes. The codes were reworked both by setting up charts of patterns of occurrence, as well as by close reading of coded text for themes and possible connections between themes. Informed participant consent, and parental consent for those under 16 years, was obtained from all participants. Quotations are marked with focus group number and the speaker's tobacco use status and gender.

RESULTS

Snus package design communicates identity

The participants generally described positive associations with snus use, including user typologies like young, social and popular. When presented with the stimulus snus packages, distinct user identities were narrated in relation to each of them. Gender, age and social class were important associations, and package design elements such as colours and fonts stood out as guiding these perceptions; for example, they perceived darker packages as masculine and lighter coloured packages as feminine. In gender mixed groups as well as groups with boys or girls only, packaging and gender identity was discussed in an engaged way. The participants generally preferred packages they associated with their own gender, with some exceptions made for the most stereotypical 'feminine packages', such as Mocca. The pink colour, ornamented font, small and rounded shape and metallic look was described as 'too much', in the sense that it was too 'girly', by some female participants;

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however, among others—in particular females who did not use snus themselves—this design was valued. They liked the colour and that the package did not look like snus to them, but like candy or make-up. The Lab 06 snus was the most popular brand among the boys and the brand identity described by non-users and females confirmed that this was understood to be a typical brand for young males. Some brands, such as General snus, were perceived as brands for older people while others, such as Lab 06, for youth. Associations between packaging and lifestyle or social class were frequent in most groups and the user identities linked to these were most often positively loaded. For example, regarding the black and gold-coloured General Portion snus package, "that one is the snus for the guy who drives his Lexus to his posh office" (group 6, male tobacco user), or Lab Lemon Fresh snus, "this is the snus for those very energetic people. Sporty, happy people!" (group 5, female non-user). In the discussion following the latter statement, the other girls in the group agreed with this interpretation and commented that it was the light colour, the brand name (Fresh) and the flavour additive that generated this association.

The participants differentiated clearly between snus brands with reference to package designs: "Skruf looks like it's good for your health, the Lab Fresh looks like sport or fitness snus and those Nick & Johnny look like they make you a good skater. The loose General, it looks like it will destroy you" (group 5, female non-user). In one group of tobacco users, some of the participants talked about how they interpreted snus packages as carrying more meaning than cigarette packages: "The brand of snus that you use kind of define what kind of person you are, more than cigarette brands even, because the selection of brands and products is bigger" (group 2, female tobacco user). The same view was stated in both the groups of self-defined non-users. Snus packages were seen as more diverse because of the package design, but also because of descriptors stating product strength and flavour additives that cigarettes do not have in Norway: "I can't see from cigarette packages which ones are stronger, and there are no flavours like lemon or strawberry" (group 5, female non-user).

The tobacco users described also how using particular brands of snus or cigarettes was an element in defining what social group they were part of or wanted to be associated with. In this, tobacco brand associations and distinctions between them stood out as serving as a way of signalling social identity. One of the participants stated her view on the social meaning of snus branding in this way:

> It seems also like those, The Lab 06 and those products, are more like, trendy. That you are not a real snus user if you do not use those. Just like, you go to Starbucks, you don't go to like, Waynes Coffee or Kaffebrenneriet, if you want a frappe, you buy it at Starbucks because it is a brand. (Group 6, female tobacco user)

Across the focus groups with tobacco users, snus branding was described as contributing to increasing the competitive position of some products, and as constituting the core of the snus user's identity. Differentiation between snus brands, and identification with some more than others, stood out as the dynamics of this process. While most of the non-users expressed confidence in that they themselves would not start, they believed that many other non-users could be tempted to try snus because of the wide variety in brands and package designs on the market: "You see the package first, and it becomes an advertisement, kind of" (group 1, female non-user).

Package design and perceptions of product qualities

Package design elements were frequently associated with product qualities, with the most common being product strength. Smaller, lighter coloured packages and packages with rounded edges were perceived as less strong than products packed in darker, sharp-edged boxes. The colour white, particularly when combined with green or blue décor or letters, was seen as signalling a milder product. In some cases, packages were differentiated by potential for harm directly, such as when a group of boys described the dark General snus packages as looking rougher, more masculine and more harmful, in contrast to lighter coloured packages.

Associations with products other than tobacco, such as candy, cosmetics or chewing gum were common across interviews, and the interviewees described pack colours, fonts in brand names and flavour additives as generating these associations: "Because it says 'spearmint', you could think; oh, it's candy"(group 4, female tobacco user). In one of the non-user groups, the colourful Nick & Johnny snus package was described as looking like "that chewing gum, Hubba Bubba, that we used to like when we were younger" (group 1, female non-user). In many cases, such associations with other products seemed intertwined with perceptions of harm. For example, the light grey Skruf White box was often interpreted as fresh, mild and "looking sterile, or like skin cream" (group 6, female tobacco user). The white Catch Spearmint package was associated with medicines: "Looks like the dentist gave it to you. It would fit well in a chemist shop" (group 6, male tobacco user). In some cases, the linkage between associations to products other than tobacco and perceptions of product harm were explicit: "The candy snus looks not to be dangerous at all, I mean, it's not like it's really candy, but it is as dangerous as candy" (group 3, male tobacco user). These associations with products other than tobacco thus seemed to add to more general diversification between products with regard to potential for harm to health. In addition, associations with candy and other products that are appealing to youth appeared to contribute to the appeal of snus among the study participants.

Flavour additives and snus sachet colour and shape

Flavour additives and sachet colour and shape was not a theme in the interview guide, but in all the focus

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groups, one or more participants spontaneously brought this up as important to how they perceived different snus brands and products. Flavoured snus was perceived as particularly appealing to youth and to new users: "Bright colours appeal to the young, and taste, not the least. Old people are not that interested in, kind of, tastes like lemon and that" (group 2, male tobacco user). They explained their opinion by stating that flavour additives made the tobacco taste less prominent and in this way more appealing to inexperienced users. In addition, some perceived that youth could be attracted by the notion of a milder product created by flavour additives such as mint and fruit.

In the groups of self-defined non-users, all participants stated that they were determined not to use tobacco, and that nice package design would not influence their stance. Nevertheless, they would sometimes express more ambiguity, and statements indicating curiosity to try snus usually related to flavour:

> I would think that one was really mild, so I could try it, because it's not dangerous at all. In particular when it's written on it, like, taste, and it says lemon. Lemon, that sounds good. (Group 1, female non user)

In the tobacco user groups, many also talked about sachet colour and shape as important for the appeal of snus to young users. Slim snus sachets were described as preferable for youth in particular ('snus for starters'), since they are less visible during use:

> It doesn't show so much that you are using snus. It's for those people who, kind of, just want snus, but don't want to take the consequences of it, then. (Group 6, female tobacco user)

Slim sachets were perceived as practical, since most youths use snus without permission from parents and go to schools where snus use is not permitted. Some participants also said that slim sachets, particularly if they were also white (as opposed to brown), were less visible, and gave the impression that they contained a product that was less harmful to health. The statement above may indicate a similar view; not wanting to 'take the consequences' of snus use could relate to health consequences and social consequences.

DISCUSSION

All focus group participants described distinct snus brand images and packaging elements such as colours, letter fonts or shape or size of the package appeared to be essential in the shaping of these images. Differentiation between snus brands, and the identification with some more than others, stood out as essential for the processes in which snus use became important for these young study participants. The findings showed also how perceptions of some products as less harmful than others and the appeal of flavour additives and new types of snus sachets seemed to blend in with these processes, reinforcing positive attitudes and contributing to the creation of particular identities for products and their users. While all non-users expressed that they had no intention to start using tobacco, and that snus packaging was not very likely to affect their decision, they did think that *other* non-users could be attracted to snus by the variety of brands and packages available. This could be related to how people generally tend to believe that others are more likely to be affected by persuasive communication than they themselves are.[29]

A recent qualitative study among young snus users from Sweden showed that identity development was of major importance when adolescents started using snus. These snus users felt that males could confirm their masculinity by using snus and associated snus use with craftsman professions such as carpenters and car mechanics. The snus package was seen as an attribute of this identity, which created a feeling of belonging.[30] In the current study, perceptions of a typical snus user were much more diverse and included urban, middle class and feminine typologies. Studies of tobacco industry strategies from the USA have shown how smokeless tobacco manufacturers such as R.J. Reynolds and Phillip Morris have invested in research, product development and marketing for decades, aiming to attract new consumer groups. While the main target group was initially low-income males (portrayed in advertising as 'hard working'), they also targeted more urban, female, smokeless tobacco users with their marketing strategies after 1990.[31] In Scandinavia, tobacco advertising has been banned for more than 40 years. Our findings indicate, however, that branding and package design to some extent can fulfil a similar promotional role as advertising messages, creating preferences and differentiation between brands and product types.[32] In addition to gender, age and social status appeared as important distinctions in the focus group participants' conceptions of and identification with snus brand images.

Our analyses indicate also that snus package design was seen as communicating information about product strength, and that packages with light colours were perceived as less harmful than darker packages. Flavoured products in candy-like boxes were perceived positively, in particular among non-users, and flavour additives (eg, mint or fruit) seemed also to contribute to ideas of product strength and harm. Similarly, product qualities and appeal were attributed to the colour and shape of snus sachets. This is similar to studies of combustible tobacco, where flavour additives have been shown to influence cigarette appeal and make the initial experience of smoking less aversive and more appealing to youth.[33 34] Experimental studies of cigarette packaging have shown how standardising colours can reduce false beliefs about some cigarette products being less harmful to health than others,[16] and that removing flavour descriptors from cigarette packages can make them less appealing to young women.[35] Similar conclusions have

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been drawn from studies exploring the potential of dissuasive cigarette sticks.[36]

This study has several limitations. Given the small sample size, the findings are not generalisable to wider youth populations, although given the exploratory nature of the study, this was not our aim. The sampling of participants through peer networks may have limited the variety of beliefs expressed in the focus groups. While young people's perceptions of snus packaging were influenced by design features such as colour, fonts and pack shape, the study gives no direct insight into whether this would impact on brand choice or snus use behaviour. Future studies could consider evaluating how snus branding and package design affects perceptions of appeal and health risk by using experimental survey study design. The exploration of how young people themselves talk about how they perceive snus branding and package design presented in this study can, however, provide useful qualitative information to understand the processes snus branding works through.

Since standardised tobacco packaging was introduced in Australia in 2012, bills mandating this regulation have been passed in several European countries, including Norway. The results from this study indicate that snus branding and packaging play a vital role in the appeal of snus among young people and, consequently, points towards the utility of including snus in regulations of tobacco packaging in markets where this product is sold.

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Snus use, smoking and survival among prostate cancer patients

Kathryn M. Wilson[1,2], Sarah C. Markt[1], Fang Fang[3], Caroline Nordenvall[4], Jennifer R. Rider[1,5], Weimin Ye[3], Hans-Olov Adami[1,3], Pär Stattin[6], Olof Nyrén[3] and Lorelei A. Mucci[1,2]

[1] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA
[2] Channing Division of Network Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA
[3] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[4] Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
[5] Department of Epidemiology, Boston University School of Public Health, Boston, MA
[6] Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University, Umeå, Sweden

Smoking is associated with prostate cancer mortality. The Scandinavian smokeless tobacco product snus is a source of nicotine but not the combustion products of smoke and has not been studied with respect to prostate cancer survival. The study is nested among 9,582 men with incident prostate cancer within a prospective cohort of 336,381 Swedish construction workers. Information on tobacco use was collected at study entry between 1971 and 1992, and categorized into (*i*) never users of any tobacco, (*ii*) exclusive snus: ever users of snus only, (*iii*) exclusive smokers: ever smokers (cigarette, cigar and/or pipe) only and (*iv*) ever users of both snus and smoking. Hazard ratios for prostate cancer-specific and total mortality for smoking and snus use based on Cox proportional hazards models adjusted for age, calendar period at diagnosis and body mass index at baseline. During 36 years of follow-up, 4,758 patients died—2,489 due to prostate cancer. Compared to never users of tobacco, exclusive smokers were at increased risk of prostate cancer mortality (HR 1.15, 95% CI: 1.05–1.27) and total mortality (HR 1.17, 95% CI: 1.09–1.26). Exclusive snus users also had increased risks for prostate cancer mortality (HR 1.24, 95% CI: 1.03–1.49) and total mortality (HR 1.19, 95% CI: 1.04–1.37). Among men diagnosed with nonmetastatic disease, the HR for prostate cancer death among exclusive snus users was 3.17 (95% CI: 1.66–6.06). The study is limited by a single assessment of tobacco use prior to diagnosis. Snus use was associated with increased risks of prostate cancer and total mortality among prostate cancer patients. This suggests that tobacco-related components such as nicotine or tobacco-specific carcinogens may promote cancer progression independent of tobacco's combustion products.

The medical community and the general public generally accept that cigarette smoking is a major determinant of mortality. Perhaps less appreciated, a pooled analysis of nearly one million people found that 17% of this excess mortality was due to causes of death not typically associated with smoking, including prostate cancer.[1] The 2014 Surgeon General's report found current or recent smoking was associated with an increased risk of advanced stage prostate cancer and death from prostate cancer, although not associated with overall incidence of the disease.[2–7] Because the specific compounds in cigarettes that most strongly influence prostate cancer outcomes have not been identified, it is not clear the extent to which other tobacco products, some of which are increasing in popularity, also pose risks.

Key words: cancer survival, prostate cancer, smoking, snus, tobacco use
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Correspondence to: Kathryn M. Wilson, Department of Epidemiology, Harvard T. H. Chan School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA, Tel.: +1 617 432 2305, E mail: kwilson@hsph.harvard.edu

Snus, a moist smokeless tobacco product common in Scandinavia, is of interest in the study of the biological effects of tobacco, as users are exposed to high levels of nicotine and other compounds over long periods of time, but without exposure to tobacco combustion products known to influence cancer risk and outcomes.[8,9] The potential effect of snus on cancer progression is also of policy interest, as snus is generally suggested as a risk reducing alternative to smoking by authorities including the World Health Organization.[10–13] In fact, tobacco companies have introduced and promoted the use of snus outside of Sweden as a healthier alternative to smoking over the past decade and have sold it in the United States since 2006.

No study to date has investigated whether snus use is, in fact, a less detrimental alternative to smoking among men with prostate cancer. We used data from a cohort of male Swedish construction workers to study the associations of both smoking and snus use with prostate cancer mortality among 9,582 men diagnosed with prostate cancer between 1971 and 2007.

Patients and Methods

Study population and follow-up

The Swedish construction industry's Organization for Working Environment, Safety and Health (Bygghälsan) offered

What's new?

Snus, a smokeless tobacco product, is often promoted as a safe alternative to cigarettes as it lacks the combustion products of smoking that are generally associated with cancer risk. Here the authors show that snus use is independently associated with risks of overall and prostate cancer-specific mortality that are comparable to cigarette smoking. These findings may lead to a re-evaluation of the public health impact of smokeless tobacco products as they suggest that nicotine or possibly other tobacco ingredients play a role in the progression of prostate cancer.

annual preventive health check ups to all construction work ers in the industry between 1969 and 1992. A total of 343,811 male workers had at least one visit between 1971 and 1992. Each visit included detailed questions about tobac co use.

The nationwide Swedish Cancer Register, established in 1958 and 96 98% complete,[14] was used to identify incident cases of prostate cancer to form the study population for this analysis. Men with previous history of any cancer at study entry were excluded. We included 9,582 workers with a diagnosis of pros tate cancer after the time of the first study visit and between 1971 and 2007. Mortality and cause of death through 2007, the most recent date for which mortality data was available, were ascertained through linkage to the nationwide Swedish Death Register, which has been shown to have high reliability for cause of death among prostate cancer patients.[15–17] Follow up for mortality in Sweden is essentially complete.

Information on tumor characteristics for men with newly diagnosed prostate cancer was available from the National Prostate Cancer Register (NPCR) of Sweden, which has been nation wide since 1998 with a capture rate of 98% compared to the Swedish Cancer Register. Thus tumor characteristics were available for 5,346 men (56% of total). In this subset of cases, we defined risk categories using a modified version of the National Comprehensive Cancer Network Practice Guide lines in Oncology, Version.1.2010, based on stage, Gleason score and serum prostate specific antigen (PSA) at diagnosis.[18] Low risk was defined as clinical stage T1 or T2, Gleason score 2 6 and PSA <10 ng/ml. Intermediate risk was stage T1 T2, Gleason score 7 and/or PSA 10 to <20 ng/ml. High risk local ized disease was stage T3 T4 and/or Gleason 8 10, and/or PSA 20 <50 ng/ml. Regionally metastatic disease was defined as N1 and/or PSA 50 to <100 ng/ml and M0/MX. Distant metastases was defined as M1 and/or PSA > 100 ng/ml.

Assessment of tobacco use

Between 1971 and 1975, participating workers filled out a 200 item questionnaire with detailed questions about smok ing and snus use. Collection of information on tobacco use was not done in 1976 1977 but resumed in 1978 with a questionnaire completed by the examining nurse. All data were compiled in a central computerized register. The num ber of total visits per person ranged from 1 to 13. However, we limited our analysis to exposure information collected in the first visit because the number and timing of visits may be linked with mortality. Based on the first visit, never, past and current tobacco use was obtained and past and current users were combined. Therefore, men were classified as (*i*) never users of any tobacco, (*ii*) ever users of snus only ("exclusive snus"), (*iii*) ever smokers only: cigarette, cigar and/or pipe ("exclusive smoking") and (*iv*) ever users of both snus and smoking (either concurrently or sequentially).

Statistical analysis

Each worker contributed person time from the date of pros tate cancer diagnosis to the date of death, emigration, or end of study, December 31, 2007, whichever came first. The asso ciation between tobacco use and either prostate cancer specific or total mortality was studied using Cox proportional hazards models with time since cancer diagnosis as the underlying time scale. Models were stratified by age at diag nosis (<60 years, 60 64, 65 69, 70 74, 75+ years) and time period of diagnosis (1971 1984, 1985 1994, 1995 2007) and were adjusted for body mass index (BMI) at baseline (contin uous) and time between baseline examination and cancer diagnosis (continuous). In the subset of patients with clinical data, additional models were adjusted for stage, grade and PSA at diagnosis. We also studied the association between tobacco use and death among men diagnosed with nonmeta static disease, *i.e.*, excluding those with evidence of regionally spread disease and distant metastases, under the hypothesis that lifestyle factors may play a greater role in survival among men with less advanced disease at diagnosis.

SAS statistical software (release 9.2) was used for the anal ysis. The study was approved by the Stockholm Regional Ethics Vetting Board.

Results

We identified 9,582 new cases of prostate cancer among workers with at least one clinic visit prior to diagnosis. Mean age at diagnosis was 70.2 years. During the study period 4,758 (50%) men died. Of those, 52% (*n* 2,489) died of prostate cancer. The mean time from study entry/baseline examination to prostate cancer diagnosis was 20.6 years (range: 1 week 33.2 years), and the mean follow up time after diagnosis was 4.5 years. 869 cases (9%) were diagnosed between 1972 and 1984, 2,674 (28%) between 1985 and 1994 and 6,039 (63%) from 1995 to 2004. Among the 5,346 cases included in The National Prostate Cancer Register (NPCR) of Sweden, the median PSA at diagnosis was 15.0 ng/ml, and

Table 1. Characteristics of the 9,582 prostate cancer cases by ever use of tobacco at study entry, Swedish Construction Workers cohort 1971 2007

	Never-users	Exclusive snus	Exclusive smoking	Used snus and smoked
N (%)	2,762 (29)	460 (5)	4,833 (50)	1,527 (16)
No. prostate cancer deaths (%)	640 (23)	141 (31)	1,336 (28)	372 (24)
No. deaths (%)	1,207 (44)	261 (57)	2,539 (53)	751 (49)
Mean age at diagnosis (years)	70.4	71.3	70.3	69.3
Time period of diagnosis				
1972 1984	197 (7%)	46 (10%)	486 (10%)	140 (9%)
1985 1994	701 (25%)	143 (31%)	1,412 (29%)	418 (27%)
1995 2004	1,864 (67%)	271 (59%)	2,935 (61%)	969 (63%)
Mean time between study entry and dx (years)	20.8	20.2	20.3	20.9
Mean follow up time (from diagnosis, years)	4.6	4.4	4.6	4.4
Tobacco use at study entry				
Mean cigarettes/day			9.3	7.9
Mean pipes/week			20.7	14.6
Mean cigars/day			0.3	0.3
Mean duration of smoking (at examination)			24.7	23.0
Mean duration of snus use (at examination)		24.3		15.2
Mean BMI (kg/m^2)	25.5	26.3	25.1	25.4
Tumor characteristics				
N in NPCR[1]	1,668 (60%)	243 (53%)	2,580 (53%)	866 (56%)
Risk category (%)[2]				
Low risk	21	19	19	24
Intermediate risk	21	21	21	20
High risk	28	30	30	29
Regional spread	11	9	9	10
Distant metastases	20	22	21	17%
Gleason score 8 10 (%)[3]	20	20	20	18
Median PSA at dx (ng/ml)	15.0	16.0	16.0	13.6

[1]Data for 5,346 men diagnosed after 1995 from the National Prostate Cancer Register of Sweden. [2]Risk category classification: low risk: T1 2, Gleason 2 6 and PSA <10; intermediate risk: T1 2, Gleason 7 and/or PSA 10 to <20; high risk: T3 4 and/or Gleason 8 10 and/or PSA 20 to <50; regionally metastatic: N1 and/or PSA 50 to <100 and M0 or MX; distant metastases: M1 and/or PSA 100+. [3]Among those in prostate cancer registry with nonmissing Gleason data.

20% had Gleason 8 10 disease. 20% of cases were diagnosed with distant metastases and another 10% with regionally spread disease.

Table 1 describes the study population according to tobacco use at study entry. Never users accounted for 29% of the population, while 5% were exclusive snus users, 50% were exclusive smokers (cigarettes, pipe and/or cigars) and 16% both used snus and smoked. Mean age at diagnosis was slightly higher among exclusive snus users and slightly lower among users of both. Mean BMI at study entry was slightly higher among exclusive snus users than in other groups. Mean duration of snus use at baseline among exclusive snus users was 24.3 years and 15.2 years among users of both. Mean duration of smoking was 24.7 years among exclusive smokers and 23.0 years among users of both. The frequency of Gleason score 8 10 at diagnosis was similar across groups. PSA at diagnosis was 15.0 ng/mL among never users, 16.0 ng/mL among exclusive smokers, 16.0 ng/mL among exclusive snus users and 13.6 ng/mL among users of both. Users of both snus and smoking were slightly more likely to be in lower risk groups at diagnosis, while distribution of risk groups was similar in the other three groups.

Compared to never users, men in all three groups of tobacco use were at increased risk of overall mortality (Table 2; HR 1.19, 95% CI: 1.04 1.37 for exclusive snus; HR 1.17, 95% CI: 1.09 1.26 for exclusive smoking; HR 1.17, 95% CI: 1.06 1.28 for both). Exclusive snus users and exclusive smokers were at increased risk of prostate cancer specific mortality, with a hazard ratio of 1.24 (95% CI: 1.03 1.49) for exclusive snus users and 1.15, (95% CI: 1.05 1.27) for

Table 2. Hazard ratios and 95% confidence intervals of the association of overall and prostate cancer specific mortality according to category of ever use of tobacco at study entry, Swedish Construction Workers cohort 1971 2007

	Tobacco exposure			
	Never-users *N*=2,762	Exclusive snus *N*=460	Exclusive smoking *N*=4,833	Used snus and smoked *N*=1,527
Overall mortality				
No. deaths	1,207	261	2,539	751
HR (95% CI) full cohort[1]	1.00 (ref)	1.19 (1.04 1.37)	1.17 (1.09 1.26)	1.17 (1.06 1.28)
HR (95% CI) with clinical data[2]	1.00 (ref)	1.15 (0.88 1.51)	1.15 (1.01 1.31)	1.15 (0.96 1.39)
Cancer specific mortality				
No. prostate cancer deaths	640	141	1,336	372
HR (95% CI) full cohort[1]	1.00 (ref)	1.24 (1.03 1.49)	1.15 (1.05 1.27)	1.08 (0.95 1.23)
HR (95% CI) with clinical data[2]	1.00 (ref)	1.28 (0.88 1.88)	1.06 (0.87 1.30)	1.11 (0.84 1.45)
Among nonmetastatic risk groups[3]				
Total no.	1,087	160	1,691	586
Overall mortality				
No. deaths	107	25	272	80
HR (95% CI) with clinical data[2]	1.00 (ref)	1.36 (0.88 2.11)	1.53 (1.22 1.91)	1.65 (1.23 2.21)
Cancer specific mortality				
No. prostate cancer deaths	28	14	60	23
HR (95% CI) with clinical data[2]	1.00 (ref)	3.17 (1.66 6.06)	1.33 (0.85 2.08)	1.93 (1.11 3.35)

[1]"Full cohort" includes all 9,582 men diagnosed with prostate cancer during follow up. Models are stratified by age group at dx and time period of dx, and adjusted for BMI (continuous) and time between examination and dx. [2]Subcohort "with clinical data" includes 5,346 men diagnosed after 1995 with available tumor characteristics from the National Prostate Cancer Register. Models are stratified by age group at dx and adjusted for BMI (continuous), time between examination and dx and clinical risk category. [3]Includes men in "low," "intermediate" and "high" categories, excludes "regionally metastatic" and "distant metastases."

exclusive smokers. Users of both were at nonsignificantly increased risk of prostate cancer death (HR 1.08, 95% CI: 0.95 1.23).

When the analysis was restricted to men with available tumor characteristics, results were largely similar, though some associations were no longer statistically significant, like ly due to smaller numbers of events (Table 2).

In the subgroup of men diagnosed with nonmetastatic dis ease, men in all three groups of tobacco use were at increased risk of total mortality, with somewhat greater hazard ratios than observed for the total patient cohort (Table 2). The association between tobacco use and prostate cancer specific death was also more pronounced. Exclusive snus users had a hazard ratio of 3.17, 95% CI: 1.66 6.06, exclusive smokers had a hazard ratio of 1.33, 95% CI: 0.85 2.08, and users of both had a hazard ratio of 1.93, 95% CI: 1.11 3.35.

Discussion

Data from this large cohort study provides further support that tobacco use increases the risk of total mortality as well as prostate cancer specific mortality among men newly diag nosed with prostate cancer.[2–4] For the first time, we also show an excess risk of overall and cancer specific mortality among snus users, a noteworthy observation as snus is generally considered and has been promoted, as a healthier and less carcinogenic alternative to smoking.[10–13] Indeed, among men who never smoked, snus use was positively asso ciated with overall and prostate cancer specific mortality.

A previous study in this cohort[19] among all cancer cases found an excess risk of cancer specific death among exclusive snus users, exclusive smokers and combined users (snus and smoking), with a 24% increased risk among exclusive snus users. A study in Uppsala county, Sweden among men with out cancer at baseline found an increased risk of all cause mortality for those who reported any snus use and a sugges tion of an increased risk of cancer mortality for exclusive snus use (HR: 1.28, 95% CI: 0.96 1.69).[20]

There are many hypothesized mechanisms behind the association between snus use and prostate cancer progression, although the exact mechanism is unclear. While snus users are not exposed to the combustion products of tobacco, they are exposed to nicotine and to multiple carcinogens present in smokeless tobacco, such as tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), volatile aldehydes and *N* nitrosamino acids (NNAs), which have been shown to form DNA adducts.[21–25] Furthermore, they also contain aldehydes and metals, which have been associat ed with inflammation and increased cell proliferation.[25]

Snus users have blood levels of nicotine metabolites similar to those seen in cigarette smokers.[26] Experimental evidence from *in vitro* and *in vivo* studies suggests that nicotine, while not carcinogenic, may promote cancer progression at concentrations relevant to tobacco use.[27] Nicotine binds to nicotinic acetylcholine receptors (nAChR), which are present in various nonneuronal tissues.[28] A broad range of laboratory findings show that nicotine promotes cell proliferation and angiogenesis through its effects on nAChR in breast and lung cancer cell lines and in animal models of breast and colon cancer.[7,27,29–35]

Nicotine and nAChR expression has not been studied in prostate cancer models. However, a variety of signaling cascades downstream of nAChR play important roles in prostate cancer, including the c Src, PI3K/AKT/mTOR, MAPK and HIF 1alpha.[36–41] These effects of nicotine could explain, in part, the association of smoking and snus with prostate cancer progression, but lack of association with cancer incidence.

Beyond nicotine, snus users are exposed to multiple tobacco derived carcinogens. The TSNA content of snus is comparable to cigarette smoke.[9,42–44] TSNAs, including 4 (methylnitrosamino) 1 (3 pyridyl) 1 butanone (NNK) and *N*′ nitrosonornicotine (NNN), are carcinogenic compounds formed during the processing of tobacco and have been shown to cause lung, nasal and esophageal tumors in various rodent models and to induce DNA adducts.[22,45] In addition, these compounds, like their precursor nicotine, bind to nAChR to promote tumor progression through multiple pathways.[46,47]

While our results are suggestive, particularly in light of previous cell line studies, animal models and epidemiological studies, it is important to note several important limitations of this work. First, we have only a single assessment of tobacco use, recorded an average of 20 years before cancer diagnosis. This leaves room for substantial misclassification of tobacco use closer to and after the cancer diagnosis. It is likely that some users in all three tobacco use categories quit after the examination or after their diagnosis, given general trends over time. Such measurement error would result in an underestimation of the true effect of snus use and smoking on mortality. In addition, if smokers were more likely to quit than snus users, due to the perception that snus is less harmful, then the extent of the underestimation might be greater for smoking groups than for the exclusive snus group. The lack of detailed smoking history prevents us from evaluating a dose response relationship or examining whether quitting is eventually associated with lower prostate cancer mortality, which is an important public health question. However, despite these limitations of the data, this unique cohort adds to our understanding of tobacco and prostate cancer survival.

Our study is also limited by the lack of covariate data, such as leisure time physical activity. However, because few lifestyle factors have been strongly related to prostate cancer mortality, the lack of lifestyle information is less likely to substantially influence the results than for other causes of mortality. We did adjust for BMI at study entry, as exclusive snus users had slightly higher mean BMI at this point; however, we lack information on BMI at later time points, which may be important given evidence that obesity plays a role in prostate cancer progression.[48] Unfortunately, we did not have information on the use of PSA screening among the men in this study, preventing us from ruling out differences in screening according to tobacco use at study entry. However, median PSA at diagnosis was similar between tobacco use groups, and the associations remained when we adjusted for stage and grade, and when we restricted the analysis to patients in lower risk categories at diagnosis. With that said, the number of prostate cancer deaths among exclusive snus users is low when restricted to lower risk categories.

In addition to adding to our understanding of the biology of prostate cancer, our findings have important implications for public health policy. Snus has been marketed as a less harmful alternative to smoking,[10–13,49,50] and is sometimes promoted as a smoking cessation aid, despite several studies finding no benefit.[51] Indeed, the US Food and Drug Administration (FDA) received and rejected an application from Swedish manufacturers seeking approval to modify language on snus warning labeling to state: "No tobacco product is safe but this product presents substantially lower risks to health than cigarettes."[52] However, the content of TSNAs and other carcinogens in snus is quite variable and is not labeled or routinely monitored.[24] Other smokeless tobacco products are also increasing in popularity. Particularly, e cigarettes are increasingly common, with recent surveys reporting 12.6% of adults ever tried an e cigarette and e cigarette use tripling among adolescents in just one year.[53–58] In addition, a recent survey of high school students found those who had ever used e cigarettes were more likely to report use of combustible tobacco products.[59] E cigarettes are marketed as safer than cigarette smoking, with companies claiming they are healthier, cheaper and cleaner than cigarettes.[60,61] However, the concentration of nicotine in e cigarette liquid varies widely across and even within products,[62] and some e cigarettes also contain TSNAs and other toxicants.[63]

Conclusions

We found that a history of both smoking and snus use was associated with increased risk of prostate cancer specific mortality and total mortality among men with prostate cancer in a large cohort in Sweden. Our results suggest that nicotine or other carcinogens in smokeless tobacco products may promote cancer progression independent of the combustion products of tobacco smoke and may have implications for the regulation of smokeless tobacco products.

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Moist smokeless tobacco (Snus) use and risk of Parkinson's disease

Fei Yang[1]*, Nancy L. Pedersen[1,2], Weimin Ye[1], Zhiwei Liu[1], Margareta Norberg[3], Lars Forsgren[4], Ylva Trolle Lagerros[5,6], Rino Bellocco[1,7], Lars Alfredsson[8], Anders Knutsson[9], Jan-Håkan Jansson[10], Patrik Wennberg[11], Maria Rosaria Galanti[12,13], Anton C.J. Lager[12,13], Marzieh Araghi[12], Michael Lundberg[12], Cecilia Magnusson[12,13†] and Karin Wirdefeldt[1,14†]

[1]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, [2]Department of Psychology, University of Southern California, Los Angeles, CA, USA, [3]Epidemiology and Global Health, Department of Public Health and Clinical Medicine, [4]Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden, [5]Department of Medicine, Clinical Epidemiology Unit, [6]Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden, [7]Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan, Italy, [8]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [9]Department of Health Sciences, Mid Sweden University, Sundsvall, Sweden, [10]Department of Public Health and Clinical Medicine, Skellefteå Research Unit, [11]Department of Public Health and Clinical Medicine, Family Medicine, Umeå University, Umeå, Sweden, [12]Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden, [13]Centre for Epidemiology and Community Medicine, Stockholm County Council, Stockholm, Sweden and [14]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

[†]Karin Wirdefeldt and Cecilia Magnusson contributed equally to this article.*Corresponding author. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, SE-17 177 Stockholm, Sweden. E-mail: fei.yang@ki.se

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Abstract

Background: Cigarette smoking is associated with a lower risk of Parkinson's disease. It is unclear what constituent of tobacco smoke may lower the risk. Use of Swedish moist smokeless tobacco (snus) can serve as a model to disentangle what constituent of tobacco smoke may lower the risk. The aim of this study was to determine whether snus use was associated with a lower risk of Parkinson's disease.

Methods: Individual participant data were collected from seven prospective cohort studies, including 348 601 men. We used survival analysis with multivariable Cox regression to estimate study-specific relative risk of Parkinson's disease due to snus use, and random-effects models to pool estimates in a meta-analysis. The primary analyses were restricted to never-smokers to eliminate the potential confounding effect of tobacco smoking.

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Results: During a mean follow-up time of 16.1 years, 1199 incident Parkinson's disease cases were identified. Among men who never smoked, ever-snus users had about 60% lower Parkinson's disease risk compared with never-snus users [pooled hazard ratio (HR) 0.41, 95% confidence interval (CI) 0.28-0.61]. The inverse association between snus use and Parkinson's disease risk was more pronounced in current (pooled HR 0.38, 95% CI 0.23-0.63), moderate-heavy amount (pooled HR 0.41, 95% CI 0.19-0.90) and long-term snus users (pooled HR 0.44, 95% CI 0.24-0.83).
Conclusions: Non-smoking men who used snus had a substantially lower risk of Parkinson's disease. Results also indicated an inverse dose-response relationship between snus use and Parkinson's disease risk. Our findings suggest that nicotine or other components of tobacco leaves may influence the development of Parkinson's disease.

Key words: Parkinson's disease, individual participant data, risk factors, epidemiology, nicotine

Key Messages

- Non smoking men who used snus had a 60% lower risk of Parkinson's disease compared with never snus users.
- An inverse dose response relationship between snus use and subsequent risk of Parkinson's disease was indicated.
- Nicotine or other components of tobacco leaves may influence the development of Parkinson's disease and explain the inverse relationship between cigarette smoking and risk of Parkinson's disease.
- This is the first pooled analysis of individual participant data from prospective cohort studies that addresses the issue of a potential protective effect of nicotine or other components of tobacco leaves from smokeless tobacco on Parkinson's disease risk.

Introduction

The aetiology of Parkinson's disease remains poorly understood, whereas epidemiological evidence has consistently demonstrated an inverse association between cigarette smoking and risk of Parkinson's disease.[1] Despite the well-known adverse health consequences of smoking, the risk of Parkinson's disease is about 36% lower in ever-smokers compared with non-smokers,[2] and the risk is lower with heavier and longer duration of smoking.[3] The exact mechanism explaining the reduced risk of Parkinson's disease with smoking is unclear, but nicotine may be implicated, as shown to have a neuroprotective effect in parkinsonian animal models.[4] Contrasting the relationship with Parkinson's disease between use of different tobacco products, and between smokeless and smoked tobacco in particular, may shed further light on what constituent of tobacco smoke may be responsible for the reduced Parkinson's disease risk, if causal.

A strong inverse association between smokeless tobacco use, including chewing tobacco or snuff, and Parkinson's disease risk was reported in two small studies hitherto comprising the science base.[5,6] Snus is a Swedish oral moist smokeless tobacco product that delivers a dose of nicotine into blood equivalent to cigarette smoking, but without toxic combustion yields from cigarettes.[7] Snus is used by Swedish men more frequently than cigarettes, with a prevalence at about 18%,[8] and the sales of snus are increasing in the USA.[9,10]

To evaluate the association between snus use and Parkinson's disease risk, we collected individual participant data from the Swedish Collaboration on Health Effects of Snus Use, which brought together Swedish prospective cohort studies with detailed information on tobacco smoking and snus use. Here we present results from this first-ever pooled analysis of the association between snus use and Parkinson's disease risk, based on the largest sample to date.

Methods

Study design, setting and participants

The Swedish Collaboration on Health Effects of Snus Use is a national pooling project, including individual participant data from already established prospective Swedish

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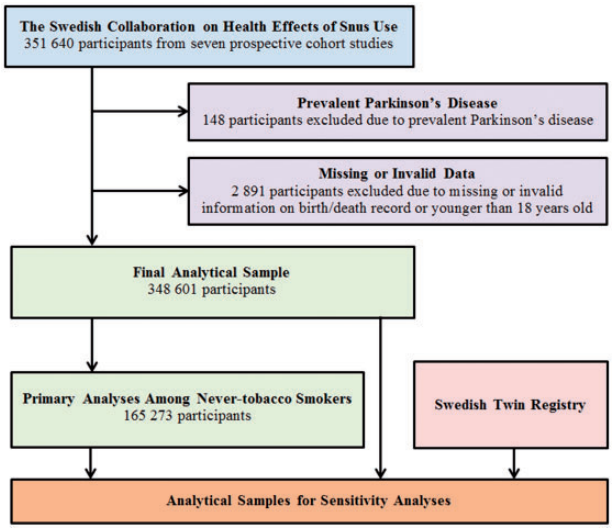


Figure 1. Flowchart deriving the analytical sample.

Additional sensitivity analyses using data from the Swedish Twin Registry were conducted to assess whether potential misclassifications or changes of exposure over time attenuated the association, and whether there was a dose response relationship of years of lifetime snus use with Parkinson's disease risk (full details are provided in Supplementary Methods).

cohort studies with information on both snus use and tobacco smoking. In total, seven cohorts were included in present study. Participants were excluded if they had a Parkinson's disease diagnosis prior to study enrolment or were younger than 18 years old. The present study was restricted to men, due to low prevalence of snus use in women. Figure 1 illustrates the process of derivation of the analytical sample. Details on study design and measures regarding the collaborative pooling project, as well as the individual studies, have been reported elsewhere.[11–18]

Principal investigators of each cohort study provided raw individual participant data to the collaborative pooling project. Data handling and analyses were performed centrally.

Ascertainment of outcome

Parkinson's disease cases were identified from the Swedish National Patient Register[19] and the Cause of Death Register.[20] The Swedish National Patient Register, which started in 1964 and became nationwide in 1987, includes records on hospital discharge diagnoses as well as outpatient visits since 2001. Diagnoses in the national patient register were coded according to the Swedish Revisions of the *International Classification of Diseases (ICD)*. The Cause of Death Register covers all death records coded in Sweden since 1961. ICD codes used for Parkinson's disease were: 350 (ICD-7, 1964–68), 342 (ICD-8, 1969–86), 332.0 (ICD-9, 1987–96) and G20 (ICD-10, 1997–2013). Index date of Parkinson's disease was defined as the date of first-ever primary or secondary Parkinson's disease diagnoses in the national patient register, or the date of underlying cause of death in the death register. A previous validation study showed a positive predictive value of 70.8% and a sensitivity of 72.7%, comparing hospital discharge diagnoses of Parkinson's disease against clinical diagnoses.[21]

Data collection

Information on tobacco use was collected at baseline using self-reported questionnaires in six studies[12–15,17,18] and by a structured telephone interview in one study.[16] All seven studies contributed information on regular use of snus and tobacco smoking (never, former or current use). Tobacco smoking was classified as lifetime regular use of any type of smoked tobacco products (e.g. cigarettes, pipes and cigars). Information about average amount (cans per week) and duration (years) of snus use for current-snus users at baseline was available in all studies.

Covariates collected at baseline included coffee drinking, alcohol intake, educational level and physical activity level.[1,22] Information on coffee drinking (number of cups per day) was available in two studies (Screening Across the Lifespan Twins, SALT, and Vasterbotten Intervention Programme, VIP),[16,18] and categorized into three groups: 'never', '1 to 3 cups per day' and 'more than 3 cups per day'. Information on alcohol intake was available in all studies except one (Construction Workers Cohort, CWC).[13] Total amount of alcohol intake from all types of alcoholic beverages was calculated (grams per week) and then categorized as 'never', 'low', 'medium' and 'high' intake (in tertiles). Highest achieved level of education, categorized as 'compulsory school', 'secondary or high school' and 'university or above', was available from all studies except the CWC.[13] Information about physical activity level was available in five studies[12,14–17] and categorized into four groups, approximately equivalent to 'less than 2 h light activity per week', 'more than 2 h light activity per week', '1–2 h of exercise per week' and 'more than 2 h exercise per week'. Full details about the covariates and information sources in each cohort are provided in Table 1.

Statistical analysis

Use of snus and smoking were primarily categorized into 'never' and 'ever' use, of which 'ever' use was further categorized into 'current' and 'former' use. For current-snus users, amount was classified as 'light' (less than 2 cans per week) or 'moderate-heavy' (equal to or more than 2 cans per week), except in the National March Cohort (NMC)[15] where 'light' was classified as 'less than or equal to 2 cans

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Table 1. Baseline characteristics of participants in seven prospective cohort studies in the Swedish Collaboration of Health Effects of Snus Use (total sample)

Characteristics	CWC	SPHC	MONICA	NMC	SALT	VIP	WOLF
Male participants (*n*)	214 381	39 212	4 553	15 012	18 316	49 940	7187
Period of recruitment (years)	1978 93	2002 10	1986 2004	1997	1998 2003	1992 2013	1992 1997
Age at recruitment (years, mean ± SD)	34.2 ± 12.5	50.2 ± 16.9	49.0 ± 13.4	53.4 ± 17.1	56.6 ± 8.0	47.2±9.2	42.9±10.8
Follow up time (years, mean ± SD)	20.0 ± 5.1	4.8 ± 3.1	12.5 ± 6.3	12.6 ± 2.5	10.0 ± 1.6	12.4±6.3	13.9±2.1
Age at PD diagnosis (years, mean ± SD)	74.4 ± 9.2	73.9 ± 9.0	73.0 ± 5.7	75.0 ± 8.4	69.4 ± 7.4	66.4±7.3	60.5±9.0
Incident PD cases (*n*)	474	89	31	165	117	291	32
Snus use status (%)							
Never	70.3	70.1	59.8	70.1	74.8	53.6	64.6
Former	3.4	10.2	15.1	14.0	8.5	16.1	11.3
Current	26.4	17.6	23.5	9.1	15.4	26.1	23.0
Missing	0.0	2.1	1.6	6.7	1.4	4.2	1.1
Amount (%)[a]							
Light	30.5	12.3	40.0	51.6	19.4	32.4	15.9
Moderate heavy	69.5	86.9	60.0	48.3	80.1	67.6	83.1
Missing	0.0	0.8	0.0	0.2	0.5	0.0	1.0
Duration (*n*)[b]	55,799	2,140	895	1,258	2,770	12,238	1,448
Years (mean ± SD)	9.4 ± 7.7	18.0 ± 11.9	17.9 ± 11.3	15.0 ± 12.0	25.3 ± 12.0	19.7±9.8	15.9±8.7
Tobacco smoking (%)							
Never	45.8	55.3	43.4	50.2	45.5	48.2	49.3
Former	17.6	31.3	34.7	41.3	36.0	30.9	30.0
Current	36.6	12.8	21.9	7.1	17.1	19.4	20.4
Missing	0.0	0.6	0.1	1.5	1.4	1.5	0.3
Alcohol intake (%)[c]							
Never		9.7	4.9	9.6	10.4	5.2	4.2
Low		28.0	24.8	29.3	28.1	31.5	27.7
Medium	N/A	28.0	24.7	29.5	30.1	31.7	27.6
High		28.1	24.7	29.4	30.0	31.6	27.7
Missing		6.3	20.9	2.2	1.5	0.0	12.9
Coffee drinking (%)							
Never					6.1	3.5	
1 3 cups per day	N/A	N/A	N/A	N/A	36.5	94.9	N/A
3+ cups per day					55.8	1.6	
Missing					1.6	0.0	
Physical activity level (%)							
0 2 h light activity per week		14.1	18.1	4.6	11.6		26.5
2+ h light activity per week		42.8	33.5	10.1	41.1		40.0
1 2 h exercise per week	N/A	25.8	21.0	70.4	35.0	N/A	16.3
2+ h exercise per week		15.8	5.4	13.0	10.6		16.6
Missing		1.4	22.0	1.9	1.7		0.6
Educational level (%)							
Compulsory		16.9	37.0	38.0	34.8	21.9	20.9
High school	N/A	42.6	46.2	36.1	35.7	53.9	56.7
University or above		39.5	15.5	23.1	23.8	23.5	22.1
Missing		1.0	1.3	2.8	5.8	0.7	0.3

N/A, not applicable; PD, Parkinson's disease; CWC, Construction Workers Cohort; SPHC, Stockholm Public Health Cohort; MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; NMC, National March Cohort; SALT, Screening Across the Lifespan Twin Study; VIP, Västerbotten Intervention Program; WOLF, Work, Lipids and Fibrinogen Study.

Percentages may not add to 100% due to rounding.

[a]Amount, cans per week of snus use among current-users (light: < 2 cans per week, moderate-heavy: ≥ 2 cans per week).

[b]Duration, years of using snus among current-users at baseline.

[c]Alcohol intake (grams per week) was calculated from all types of alcoholic beverage, and categorized into no alcohol intake and tertiles.

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per week'. Duration of using snus at baseline among current-snus users was categorized into '1 to 20 years' or 'more than 20 years' in studies where that information was available.[12,15–18]

First, we performed analyses within each cohort. Participants were followed from baseline until index date of Parkinson's disease, date of death or end of follow-up, whichever came first. To assess associations between snus use and incident Parkinson's disease in an age-adjusted model, we used Cox proportional hazards regression with attained age as the underlying time scale, estimating hazard ratios (HRs) with 95% confidence intervals (CIs). Then, we examined the associations in a multivariable-adjusted model (referred to as fully-adjusted model). Covariates included in the fully-adjusted model varied slightly between studies, based on availability of relevant information in each study. We calculated pooled estimates and 95% CIs by meta-analysing the study-specific estimates of relative risk (RR) using a random-effects model.[23] We tested for heterogeneity among the included studies, using a Q test and I^2 statistics.[24] To eliminate the potential confounding effect of tobacco smoking on the association between snus use and Parkinson's disease, we restricted the primary analyses to never-tobacco smokers, but analyses of the total sample, regardless of smoking status, were also performed. In all analyses, we used 'never-snus users' as the reference group. Further, to investigate the combined effect of snus use and tobacco smoking on Parkinson's disease risk, we cross-classified snus use and tobacco smoking according to timing of use. For this analysis, we used 'never-snus use and never-tobacco smoking' as the reference group. Last, since confounding structures for snus use and smoking might be different, we directly compared the associations for snus use versus smoking with Parkinson's disease risk. For this analysis, we categorized the snus and smoking variables into ever/never use and cross-classified the variables again, but used 'never-snus use and ever-tobacco smoking' category as the reference.

To assess the robustness of the main analyses, we conducted several sensitivity analyses. First, we excluded CWC in the meta-analyses since this cohort constituted 61.5% of the total sample size. Second, to assess the impact of missing values for potential confounders on the estimates, we ran the fully-adjusted model in each cohort by estimating missing values on all covariates with multiple imputation chain equation assuming missingness at random,[25] and then pooled study-specific estimates. Third, to examine the effect of a possible preclinical disease phase, we performed lag-time analyses by excluding the first 8 years of follow-up.[26] Fourth, to examine the extent of a possible confounding effect for covariates other than tobacco smoking (e.g. coffee and alcohol drinking), we compared the pooled HRs of SALT and VIP age-adjusted and fully-adjusted models with the primary analyses. Last, we investigated whether potential misclassifications or changes of exposure during the follow-up attenuated the association and whether there was a dose-response relationship of years of lifetime snus use with Parkinson's disease risk in the Swedish Twin Registry, in which longitudinal exposure information was available in 1974 and 1998–2002 (full details are provided in Supplementary Methods, available as Supplementary data at *IJE* online). In these analyses, exposures were treated as time-varying variables. We tested the proportional hazards assumption in the Cox model using scaled Schoenfeld residuals,[27] and no evidence of non-proportionality was found. All statistical analyses were performed with Stata, version 12 (StataCorp LP, College Station, TX).

Ethical approval

The present study was approved by the Regional Ethics Review Board in Stockholm. As the study does not include any patient's personal information, written patient consent is not required.

Results

A total of 348 601 men without a history of Parkinson's disease diagnosis at baseline were included in the study, of whom 107 838 (30.9%) reported ever-use of snus and 165 273 (47.4%) were never-tobacco smokers (Table 1). Ever-snus users were younger than never-snus users at baseline (mean ages of 37.2 and 41.4 years, respectively). There were 1199 incident Parkinson's disease cases identified (1063 only from the Patient Register, 121 from both the Patient Register and the Cause of Death Register and 15 only from the Cause of Death Register) during a mean follow-up time of 16.1 years [standard deviation (SD) 7.2]. Of the 1199 cases, 598 (49.9%) never smoked tobacco products. Mean age at Parkinson's disease diagnosis was 68.1 years for ever-snus users and 70.9 years for never-snus users.

Among never-tobacco smokers, Parkinson's disease risk in ever-snus users was lower than in never-users (pooled HR 0.41, 95% CI 0.28-0.61, $P < 0.001$ for the fully-adjusted model; Table 2). Current-snus use was associated with a lower Parkinson's disease risk than former use (Figure 2). In addition, there was evidence of dose-response relationships such that moderate-heavy amount (pooled HR 0.41, 95% CI 0.19-0.90, $P = 0.03$) and long-term current-snus users (pooled HR 0.44, 95% CI 0.24-0.83, $P = 0.01$) had the lowest Parkinson's disease risks. There was no evidence of heterogeneity between studies for the results mentioned above

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Table 2. Associations between snus use and risk of Parkinson's disease among never tobacco smokers

Snus use status	Never tobacco smokers									
	Age adjusted model[a]					Fully adjusted model[b]				
				Heterogeneity					Heterogeneity	
	n	HR	95% CI	I^2	*P* value	*n*	HR	95% CI	I^2	*P* value
Never users	550	1	reference			531	1	reference		
Ever users	27	0.40	0.27 0.59	0%	0.95	27	0.41	0.28 0.61	0%	0.93
Former users	10	0.65	0.35 1.23	0%	0.95	10	0.68	0.36 1.28	0%	0.91
Current users	17	0.37	0.22 0.60	0%	0.89	17	0.38	0.23 0.63	0%	0.73
Amount[c]										
Light	8	0.65	0.32 1.32	0%	0.81	7	0.71	0.35 1.43	0%	0.52
Moderate heavy	9	0.40	0.21 0.79	0%	0.38	9	0.41	0.19 0.90	18%	0.29
Per year of using snus	16	0.96	0.94 0.98	0%	0.82	16	0.96	0.94 0.98	0%	0.85
1 ~ 20 years	6	0.54	0.19 1.52	30%	0.23	6	0.56	0.19 1.68	35%	0.20
21 ~	10	0.44	0.24 0.83	0%	0.95	10	0.44	0.24 0.83	0%	0.94

n, number of Parkinson's disease cases; HR, hazard ratio; CI, confidence interval; I^2, heterogeneity statistics; *P*-value, heterogeneity Q-test.

[a]Analyses were first performed separately in each cohort among never-smokers, using Cox regression with attained age as time scale, and then pooled together using meta-analyses with a random-effects model.

[b]Adjusted for age in CWC; adjusted for age, education, alcohol and physical activity in SPHC; adjusted for age, alcohol and physical activity in MONICA; adjusted for age, alcohol and physical activity in NMC; adjusted for age, alcohol, education, physical activity and coffee intake in SALT; adjusted for age, education, alcohol and coffee intake in VIP; adjusted for age, alcohol, education and physical activity in WOLF. All analyses were conducted in never-tobacco smokers.

[c]Amount, cans per week of snus use among current-users (light: < 2 cans/week; moderate-heavy: ≥ 2 cans/week).

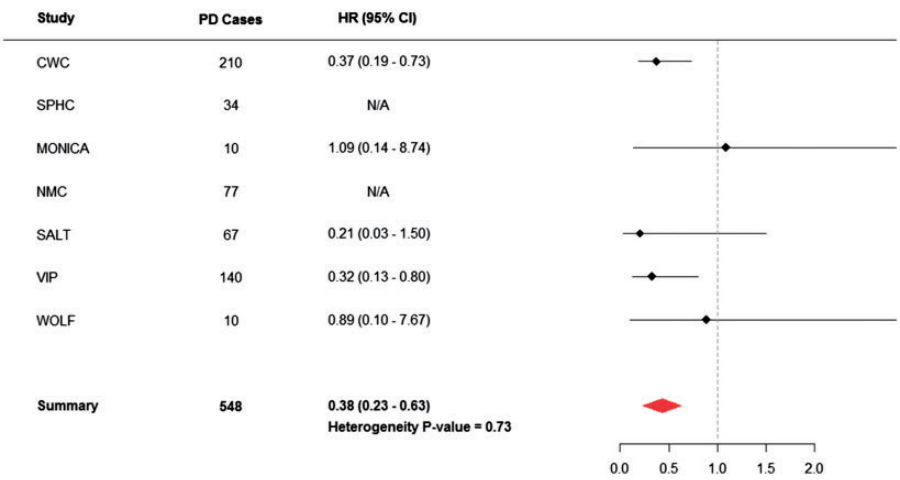


Figure 2. Study specific and pooled estimates for the relative risk of Parkinson's disease among never tobacco smokers comparing current snus users versus never snus users.

HR=hazard ratio, CI=confidence interval, PD=Parkinson's disease, N/A=Not applicable (no exposed PD cases). Analyses were first performed separately in each cohort using Cox regression with the fully adjusted model. Study specific estimates were then pooled together using meta analyses with a random effects model.

(I^2 statistics 0–35%). Analyses in the total sample regardless of smoking status showed similar results compared with the primary analyses, but with weaker associations (Supplementary Table 1, available as Supplementary data at *IJE* online). When snus use and smoking were combined, Parkinson's disease risks were lower among tobacco users, with more prominent risk reductions with current use regardless of tobacco type (Figure 3). Further, ever-snus users and never-smokers seemed to have a lower risk of Parkinson's disease, compared with never-snus users and ever-smokers (pooled HR 0.74, 95% CI 0.50-1.11, $P = 0.15$).

Sensitivity analyses left the main findings largely unchanged. In the analyses restricted to never-tobacco smokers, excluding the CWC altered the pooled HR in ever-snus users by 0.03 (Supplementary Table 2, left panel, available as Supplementary data at *IJE* online); imputation of

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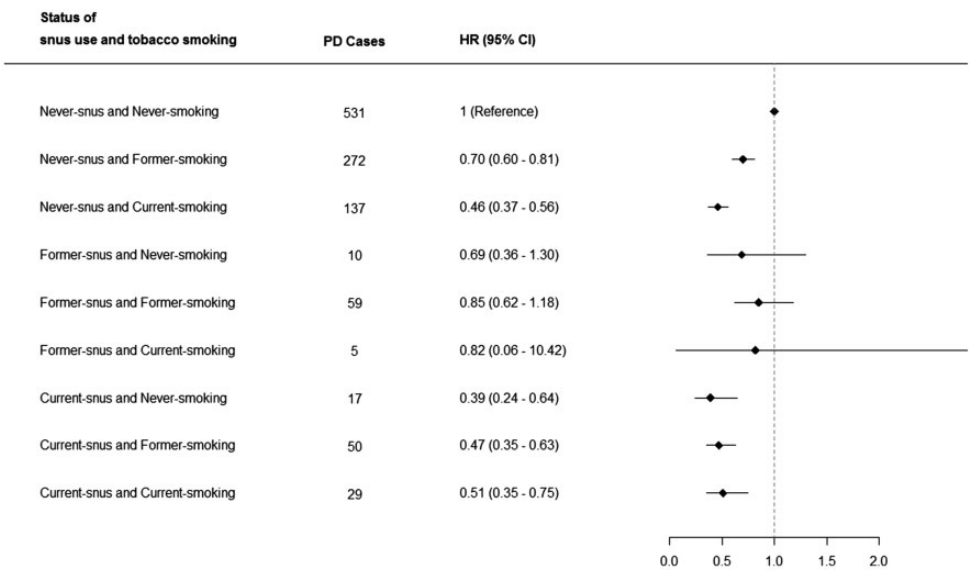


Figure 3. Combined effect of snus use and tobacco smoking on Parkinson's disease risk in seven Swedish prospective cohort studies.

HR=hazard ratio, CI=confidence interval, PD=Parkinson's disease. Analyses were first performed separately in each cohort using Cox regression with the fully adjusted model. Study specific estimates were then pooled together using meta analyses with a random effects model. Heterogeneity (P value) for different combinations of snus use and tobacco smoking are 0.51, 0.53, 0.91, 0.26, 0.01 (former snus users and current tobacco smokers), 0.74, 0.74, and 0.65, respectively.

missing values on covariates altered the pooled HR in ever-snus users by 0.01 (Supplementary Table 2, middle panel); and results from lag-time analyses were similar to those of the primary analyses (Supplementary Table 2, right panel). When analyses were performed within studies with information on more covariates, differences on pooled HRs between age-adjusted and fully-adjusted models were small (Supplementary Table 3, available as Supplementary data at *IJE* online); and the fully-adjusted HR did not differ from results in the primary analyses in Table 2. When considering snus use, smoking and alcohol drinking as time-varying exposures, the associations of snus use status and longer duration of lifetime snus use with Parkinson's disease risk in the Swedish Twin Registry all had the same direction compared with the primary analyses (Supplementary Tables 4 and 5, available as Supplementary data at *IJE* online).

Discussion

This is the first pooled analysis of the relationship between smokeless tobacco use and risk of Parkinson's disease. It brought together and analysed individual participant data from seven Swedish prospective cohorts, thus compiling the largest study on Parkinson's disease and smokeless tobacco to date. We found that non-smoking men who used snus had a substantially reduced risk of Parkinson's disease. In addition, there were indications of dose-response relationships, such that risk reduction appeared more pronounced in current, moderate-heavy amount and long-term snus users. Similarly, tobacco smoking was associated with a decreased risk of Parkinson's disease, most pronounced in current smokers. Further, we examined the combined effect of snus use and tobacco smoking on Parkinson's disease risk; as expected, the reduced Parkinson's disease risks were more apparent among current-users of both types of tobacco.

To date, there is no direct evidence that nicotine, rather than other compounds from smoking, confers the protective effect on Parkinson's disease in humans. The present study addressed this issue by evaluating snus use in never-tobacco smokers for Parkinson's disease risk. The levels of nicotine concentration in blood from snus use are similar compared with smoking, but without combustion and yields of toxicants in tobacco smoke.[7] Together with the neuroprotective effect of nicotine shown in animal and *in vitro* studies,[4] our findings support the notion that nicotine can help prevent Parkinson's disease or slow the presymtomatic disease progression.

There are several lines of evidence for the possible neuroprotective properties of nicotine. Findings from *in vitro* studies suggest that nicotine inhibits α-synuclein fibrillation,[28] and that stimulation of subunits of the nicotinic-acetylcholine-receptor (nAChR) modulates inflammatory pathways.[29] In addition, there is a close anatomical relationship between nicotinic cholinergic and dopaminergic neurotransmitter systems in the striatum.[30] Extensive evidence from rodent models suggest that nicotine modulates

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dopamine release by acting at nAChRs on dopaminergic nerve terminals.[31,32] Further, in non-human primates with Parkinson's disease-like motor symptoms induced by neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), chronic nicotine administration reduces striatal damage,[33] and nicotine decreases L-dopa-induced dyskinesia.[34] Last, nicotine can have a symptomatic effect in Parkinson's disease patients.[35]

An important strength of this study is that we meta-analysed data from prospective studies, thus minimizing the influence of recall bias and reverse causation. The relationship between snus use and Parkinson's disease was stable in various sensitivity analyses, and apparently not due to confounding by established Parkinson's disease risk factors. Our findings were also homogeneous among the studies included in the meta-analyses, thus suggesting high external validity. An advantage of examining the association in snus users compared with cigarette smokers is that survival bias is less likely to occur for snus use than for cigarette smoking.

Nevertheless, there are some limitations. First, although our Parkinson's disease diagnoses in the national health registers have been validated showing good accuracy,[21] this is not perfect with regard to differentiation between Parkinson's disease and other parkinsonian disorders. We believe, however, that any such misclassification is likely to be non-differential by exposure, resulting in a bias towards the null. Second, although snus use is relatively stable over time,[36] a shift to other tobacco products (e.g. cigarettes) during follow-up may have occurred. However, our sensitivity analyses with time-varying tobacco use information in the Swedish Twin Registry provided similar results compared with the primary analyses of non-smokers. Of note, it has recently been suggested that Parkinson's disease patients may experience less nicotine-mediated 'reward' from tobacco use, and therefore smoking (and snus use) cessation may be a consequence rather than a cause of Parkinson's disease.[37,38] It is also conceivable that cessation of snus use may be due to loss of dexterity in handling snus in Parkinson's disease patients not yet diagnosed. These hypotheses, unfortunately, could not be tested in the present study. Nevertheless, results from a recent study investigating the relationship between Parkinson's disease risk and time-since-cessation of smoking,[39] and our results from lag-time analyses of non-smokers, all showed significant lower Parkinson's disease risks for tobacco use, advocating a truly inverse association. Other limitations include restriction of the study population to men, and lack of possibility to adjust for all known confounders and possible unknown confounders for every study included in the meta-analyses. However, our sensitivity analyses in studies with more complete covariate information showed pooled RRs similar to the primary analyses. Although the present study pooled data from several large prospective cohorts in Sweden and results were statistically stable, larger sample size including studies from places other than Sweden would be needed to examine amount and duration of snus use, and use of other forms of smokeless tobacco, in relation to Parkinson's disease risk. Finally, other than nicotine in itself, the inverse association with Parkinson's disease could be due to a combination of nicotine and other compounds in tobacco leaf, such as monoamine oxidase inhibitors.[40]

In conclusion, data from this large pooling project showed that non-smoking men who used snus had a substantially reduced risk of Parkinson's disease. Results also indicated an inverse dose-response relationship between use of snus and subsequent risk of Parkinson's disease. Our findings hence suggest that nicotine or other components of tobacco leaves may influence the development of Parkinson's disease and explain the inverse association between cigarette smoking and Parkinson's disease risk. Further research into the role of nicotine in the prevention of Parkinson's disease is warranted.

Supplementary Data

Supplementary data are available at *IJE* online.

Funding

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Author Contributions

F.Y. performed statistical analyses, wrote the first draft and finalized the paper based on the feedback from all other co-authors. F.Y., N.L.P., K.W. and C.M. contributed to the writing of more than one draft. C.M. coordinated the collaborative pooling project. M.L. and F.Y. prepared the data. K.W., C.M., N.L.P. and F.Y. designed the study and contributed to data interpretation. All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and critically reviewed and commented on the paper.

Conflict of interest: All authors report no conflict of interest.

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Summary of Consumer Complaints (Adverse Experiences)

Product	**General Portion Original Large**
SKU Number	**4880**
FDA Tracking number	**PM0000012**
Reporting Period	**October 1, 2016 to September 30, 2017**

Item#	Complaint Description	Count	Importance
1	(b) (4)	1	Not serious and unexpected adverse experience.
2		3	None serious and unexpected adverse experience.
3		1	Not serious and unexpected adverse experience.
4		1	Not serious and unexpected adverse experience.
5		2	None serious and unexpected adverse experience.
6		1	Not serious and unexpected adverse experience.

Detail for Consumer Complaints (Adverse Experiences)

Product	**General Portion Original Large**
SKU Number	**4880**
FDA Tracking number	**PM0000012**
Reporting Period	**October 1, 2016 to September 30, 2017**

Item #	Consumer No.	Complaint Received	Code Date	Time Stamp	Mach No.	Complaint Description	SASE[1] Sent	Complaint Confirmed	Additional Information
1	177832	10/21/2016	11/7/16	---	---	(b) (4)			
2	205738	3/29/2017	22MAY2017	60000-4	34216 411-1				
3	177832	4/6/2017	---	---	---				
4	206092	4/11/2017	---	---	---				
5	207174	5/26/2017	07 Sep 2017	61026 571	---				
6	208162	7/10/2017	---	---	---				

Detail for Consumer Complaints (Adverse Experiences)

Product	**General Portion Original Large**
SKU Number	**4880**
FDA Tracking number	**PM0000012**
Reporting Period	**October 1, 2016 to September 30, 2017**

Item #	Consumer No.	Complaint Received	Code Date	Time Stamp	Mach No.	Complaint Description	SASE[1] Sent	Complaint Confirmed	Additional Information
7	208548	7/24/2017	23oct2017	52073411-2	60000-4	(b) (4)			
8	174184	8/14/2017	---	---	---				
9	210038	9/15/2017	---	---	---				

[1] SASE = The consumer is provided with a postage paid, pre-address envelope for use in returning product to Swedish Match for confirmation of complaint.
--- = No data available.
N/A = Not Applicable

Product	**General Portion Original Large**
SKU Number	**4880**
FDA Tracking number	**PM0000012**
Reporting Period	**October 1, 2016 to September 30, 2017**

Summary of Total US Distribution (Cans) by US Census Region and Retail Markets and Channels (Units)

Item #	US Census Region	Total - All Classes of	Convenience/ Distributors	Premium Cigar Retailer	Internet Retailers	Grocery/Food	Smoke Shops	Duty Free	All Other	Employee Shiptos
(b) (4)										

Item #	US Census Region	Total - All Classes of	Convenience/ Distributors	Premium Cigar Retailer	Internet Retailers	Grocery/Food	Smoke Shops	Duty Free	All Other	Employee Shiptos
(b) (4)										

Summary of Total US Sales by US Census Region and Retail Markets and Channels (US Dollars)

Item #	US Census Region	Total - All Classes of	Convenience/ Distributors	Premium Cigar Retailer	Internet Retailers	Grocery/Food	Smoke Shops	Duty Free	All Other	Employee Shiptos
(b) (4)										

Post Marketing Annual Report
Section III. Periodic Reporting
Requirement 3

Certification Statement

General Portion Original Large (PM0000012)

I, Gerard J. Roerty, Jr., on behalf of Swedish Match USA, Inc., certify that General Portion Original Large, has a reported change made to the manufacturing, facilities or controls during the reporting period (10/1/2016 - 9/30/2017), but is otherwise identical to General Portion Original Large that was described in the PMTA. As directed in FDA's Marketing Order, dated November 10, 2015: section III. Periodic Reporting requirement 3, this submission describes the change, compares the reported change to what was described in the PMTA and provides a rational for making the change. I certify that Swedish Match USA, Inc. understands this means the reported change did not result in any modification (including a change in design, any component, any part, or any constituent, including a smoke constituent, or in the content, delivery or form of nicotine, or any other additive or ingredient) of the tobacco product. I certify that this information and the accompanying submission are true and correct, and that I am authorized to submit this on the company's behalf. I understand that under section 1001 of title 18 of the United States Code, anyone who makes a materially false, fictitious, or fraudulent statement to the Government of the United States is subject to criminal penalties.

(b) (6)

Gerard J. Roerty, Jr.
Vice President, General Counsel & Secretary

Summary of All Manufacuring Deviations

Product:	General Portion Original Large
SKU Number:	4880
FDA Tracking number:	PM0000012
Reporting Period:	October 1, 2016 to September 30, 2017

Deviation Number	Type of Manufacturing Deviation	Production Date (YYYY-MM-DD)	Description of Deviation	Design Feature	Deviation May Affect the Characteristics of the Final Product (Yes/No)	Product With Deviation Distributed at Retail Level (Yes/No)
---	---	---	---	---	---	---

Justification; why product that reached retail would not affect public health

	No manufacturing deviations to report. As discussed in section III.3 of the periodic report, a change in manufacturing occurred the 9th of December 2016.
---	---

--- = Not applicable.

Brand Consumer Communications

CATEGORY: SNUS

BRAND: GENERAL

October 1, 2016 – September 30, 2017

Email

Monthly Emails

October Emails



Email 1



Email 2

Monthly Emails

November Emails



Email 1



Email 2



Email 3

Monthly Emails

December Emails



WARNING: This product is not a safe alternative to cigarettes.

Email 1



WARNING: This product is not a safe alternative to cigarettes.

Email 2

Monthly Emails (deployed 1/10 and 1/24)

January Emails



Email 1



Email 2

Monthly Emails (deploys 2/14 and 2/28)

February Emails



Email 1



Email 2

Monthly Emails (deployed 3/7 and 3/21)

March Emails



Email 1

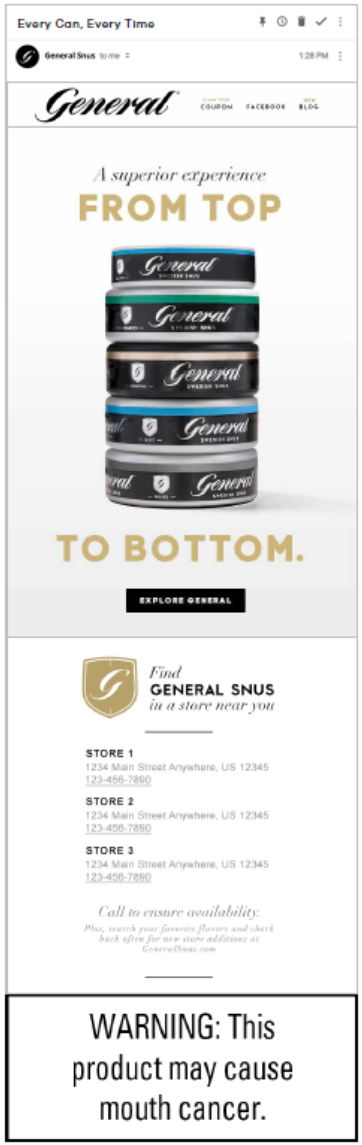


Email 2

SWEDISH MATCH

Monthly Emails (deployed 4/11 and 4/25)

April Emails



Email 1

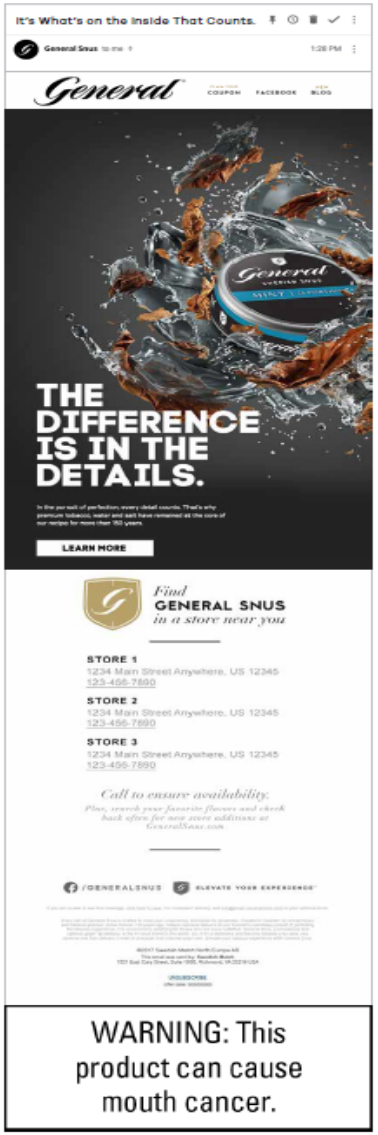


Email 2

SWEDISH MATCH

Monthly Emails (deployed 5/9 and 5/23)

May Emails



Email 1



Email 2

SWEDISH MATCH

Monthly Emails (deployed 6/6 and 6/20)

June Emails



Email 1

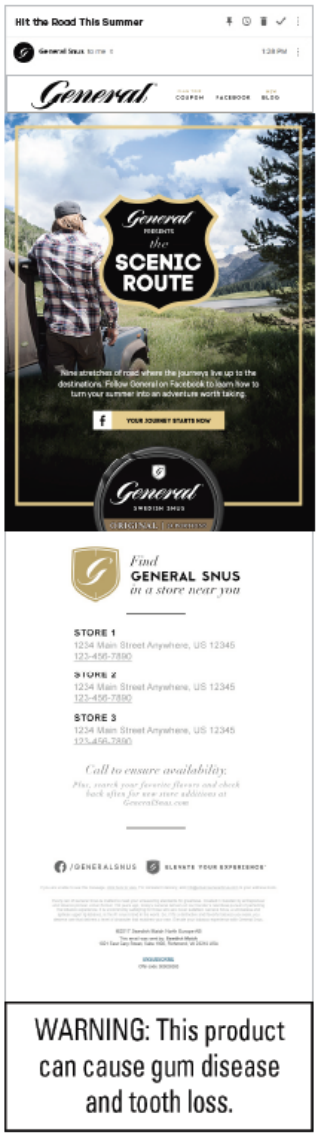


Email 2

SWEDISH MATCH

Monthly Emails (deployed 7/11 and 7/25)

July Emails



Email 1



Email 2

Monthly Emails (deployed 8/8 and 8/22)

August Emails



Email 1

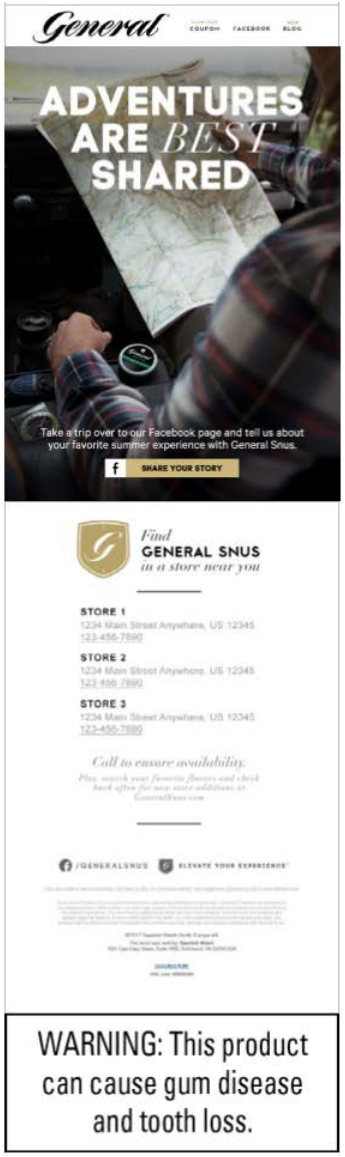


Email 2

SWEDISH MATCH

Monthly Emails (deployed 9/5 and 9/19)

September Emails



Email 1



Email 2

Ongoing Emails (Feb)

Welcome Email



Birthday Email



SWEDISH MATCH

Ongoing Emails (Feb)

3 Month Coupon Reminder



1 Year Coupon Reminder Email



SWEDISH MATCH

Ongoing Emails (Feb)

Store Near You Email



3 Star Retailer Email



SWEDISH MATCH

Ongoing Emails (deployment TBD)

Survey Email



Experiential Follow Up Emails (Feb)

Green Email 1



Yellow and Red Email

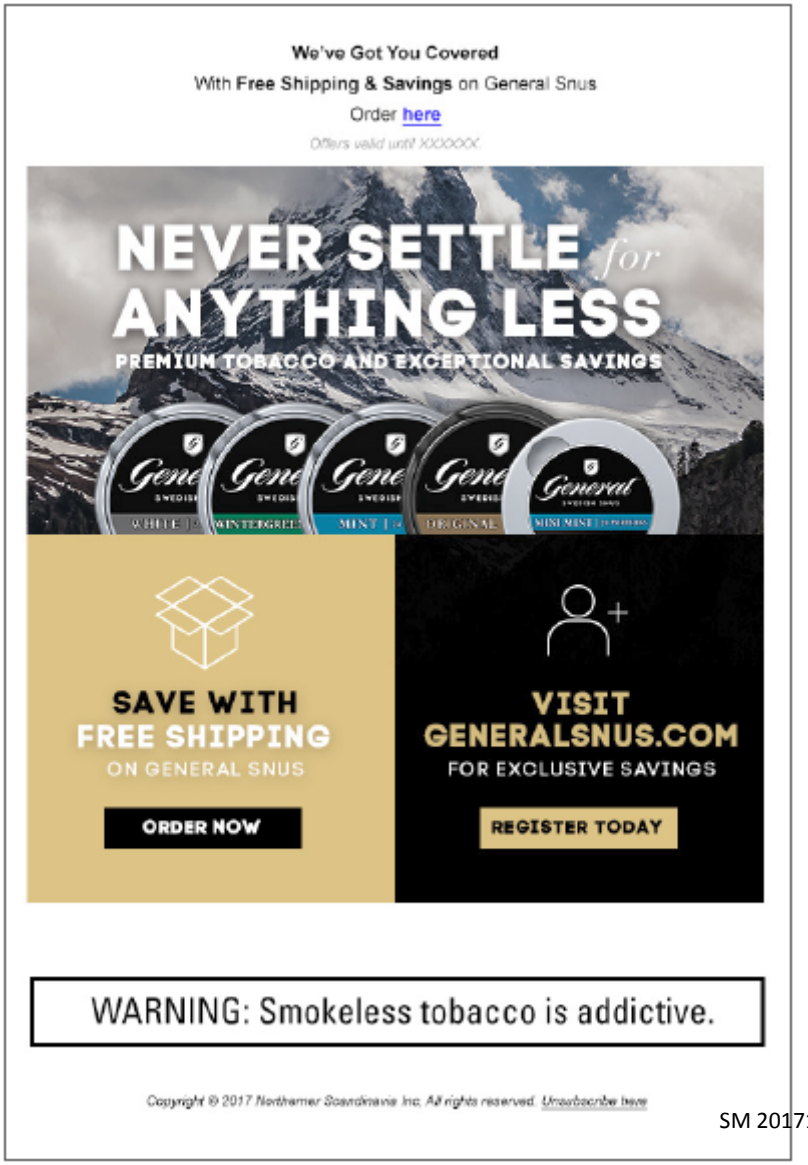


Green Email 2



SWEDISH MATCH

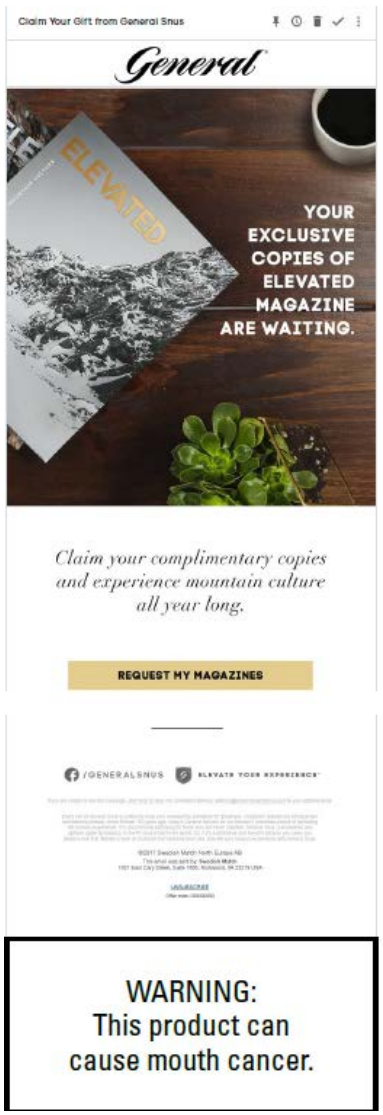
Northerner Newsletter (deployed *by Northerner* on 2/21)



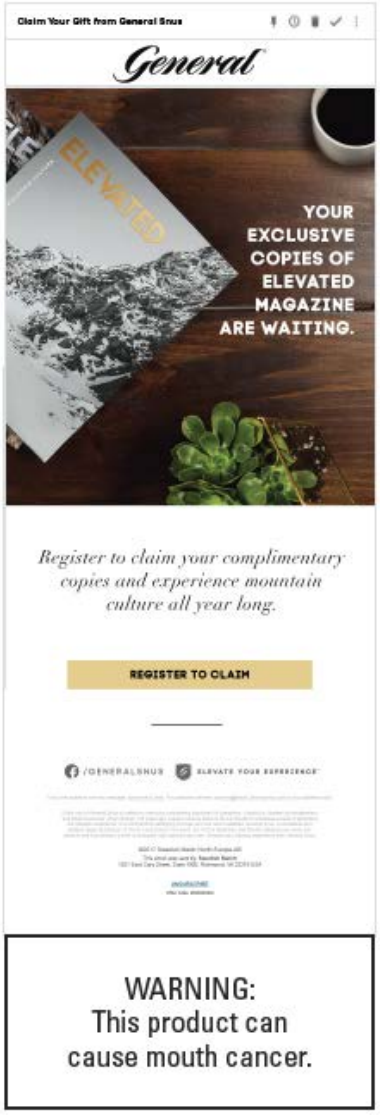
Chicago Store Near You (deployed 5/24)



Elevated Email (deploys mid-June)



Email 1

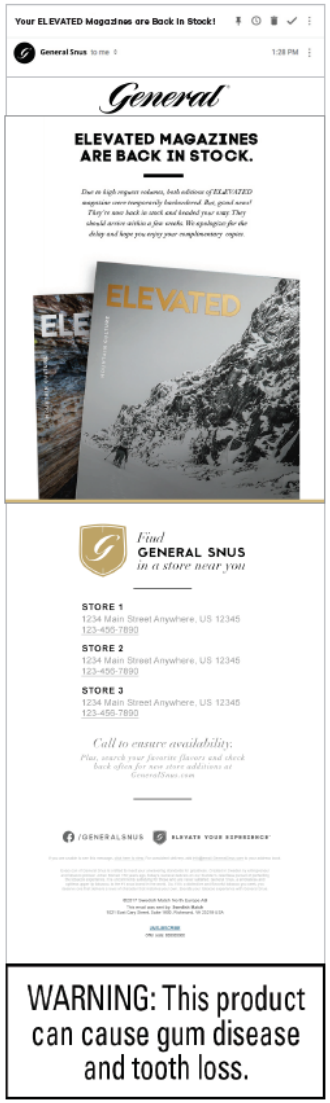


Email 2

SWEDISH MATCH

Elevated Email (deployed 9/25)

Elevated Email

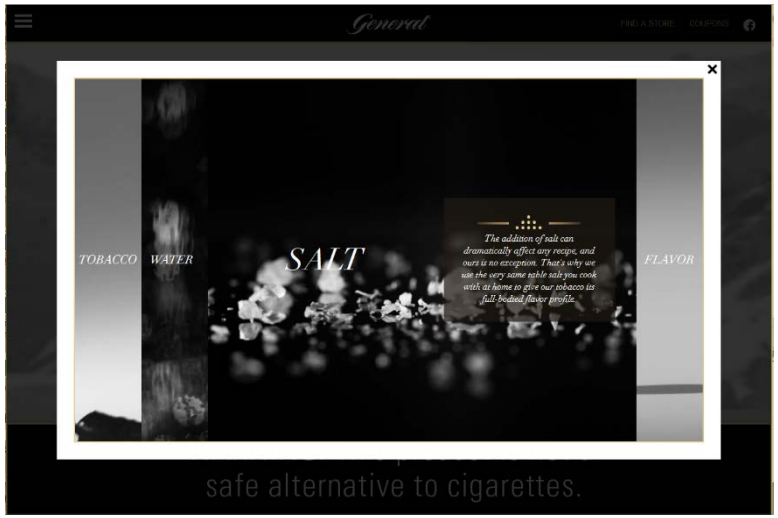


Website

Ingredients Page









Dashboard


General
FIND A STORE
COUPONS
ELEVATING YOUR TOBACCO EXPERIENCE FOR 150 Years EXPLORE THE HISTORY OF GENERAL SNUS
MINI MINT
WINTERGREEN
MINT
WHITE
ORIGINAL
LOOSE
ON THE MOUNTAIN BLOG
WHO WE ARE
WHY GENERAL
SIMPLE INGREDIENTS
CAN YOU SNUS THERE?
FAQ | Privacy Policy | Contact Us | Snus & Science | See Winners | Official Rules | ©2016 Swedish Match North Europe AB
WARNING: This product is not a safe alternative to cigarettes.

On The Mountain Blog



Experiential Follow-Up Webpages (Feb)

Green Segment



Experiential Follow-Up Webpages (Cont.)

Yellow & Red Segment



Experiential Follow-Up Webpages (Cont.)

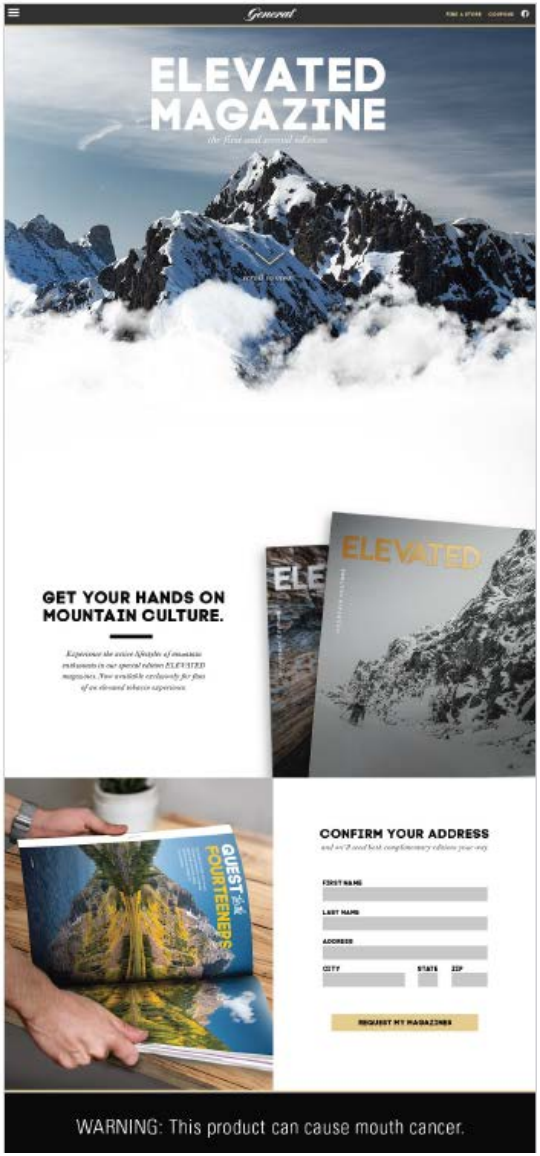


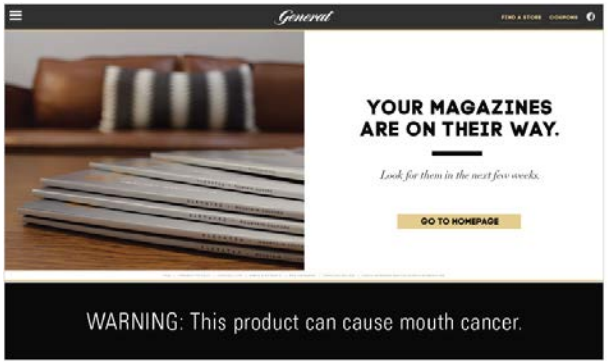
Rules Webpage

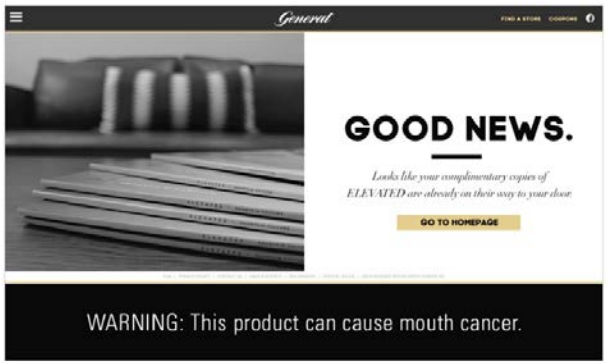


Winners Webpage

Elevated Magazine Request Webpages (May)





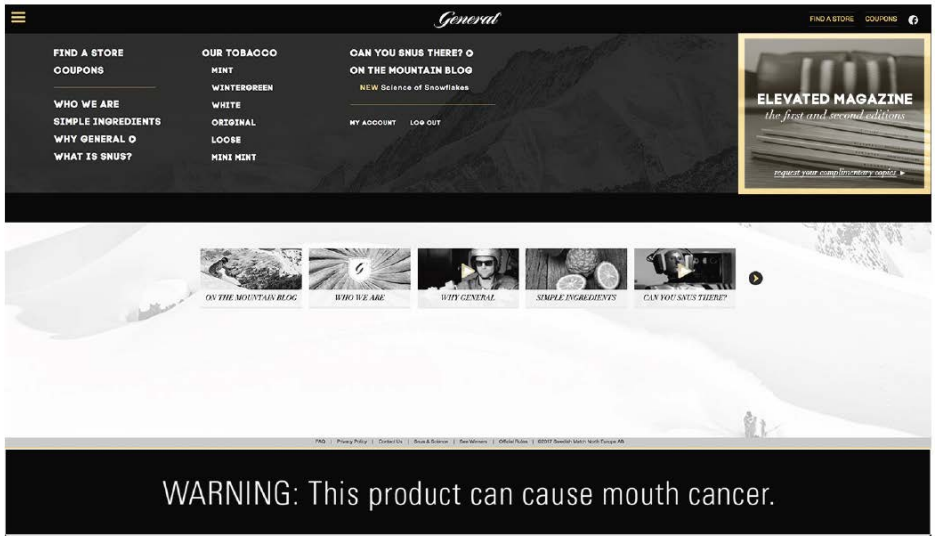




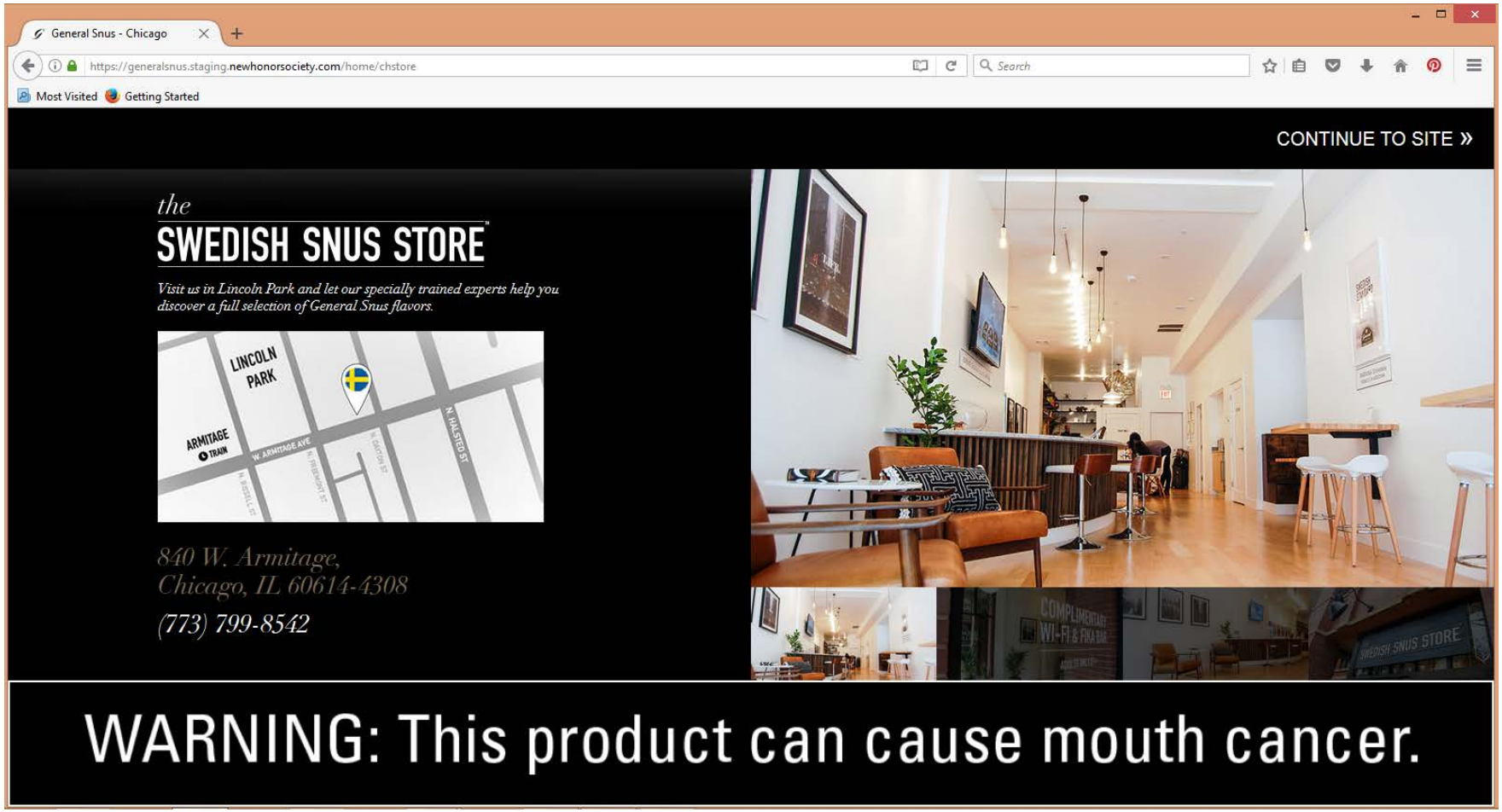
SWEDISH MATCH

Elevated Magazine Request Webpages (Cont.)

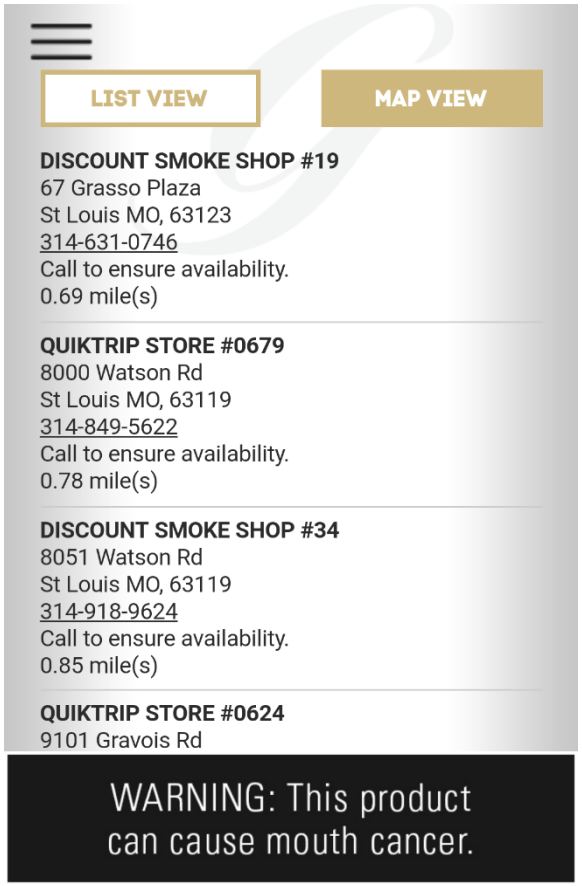


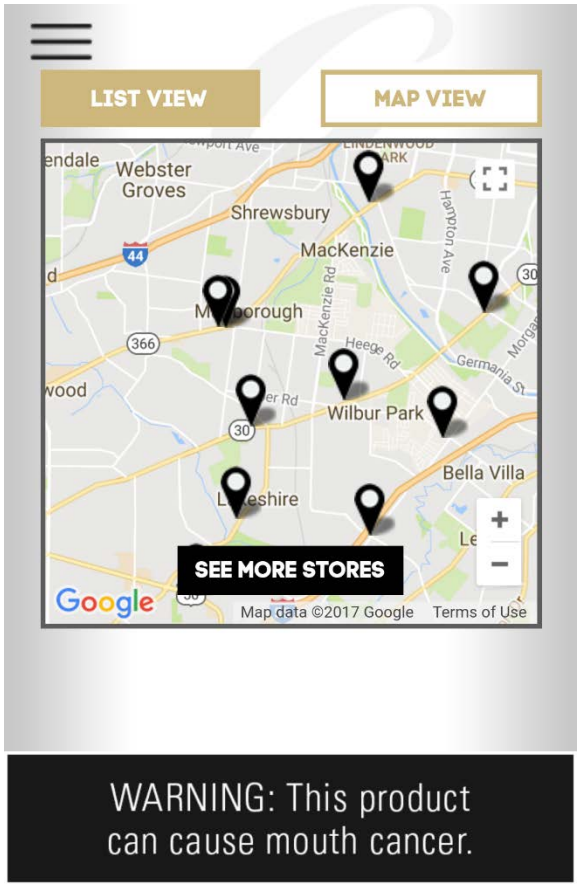


Chicago Pop-up Store Splash Page (June)



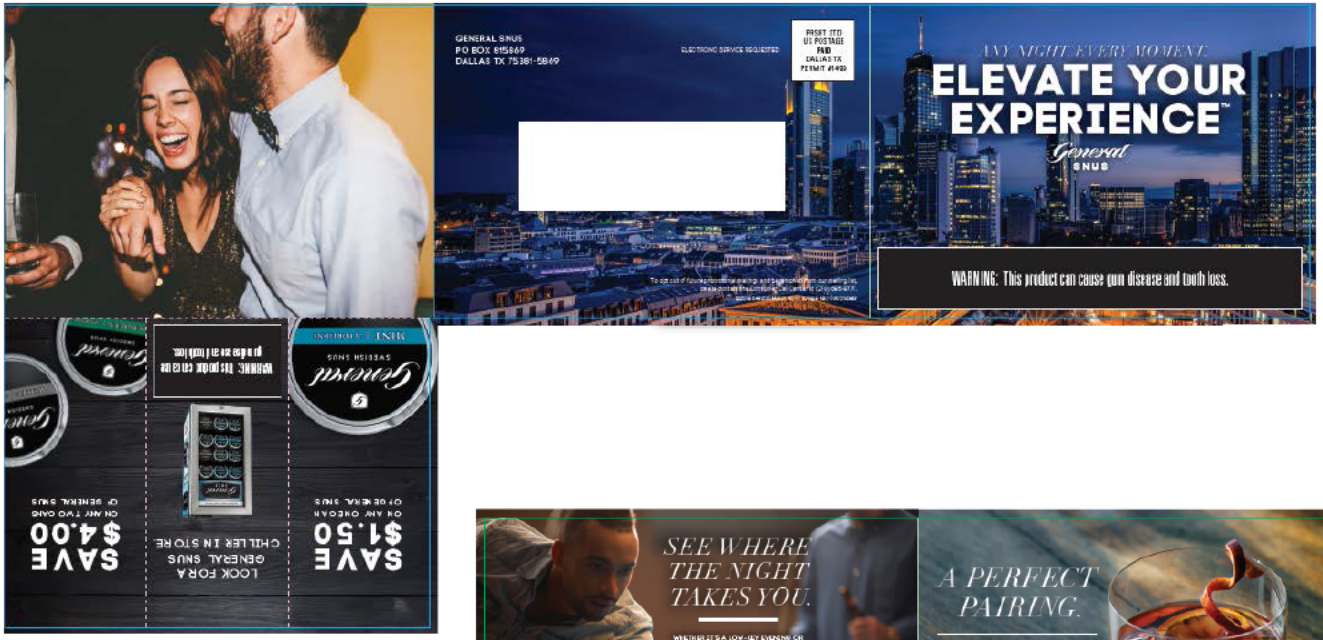
Mobile Store Locator Updates (June)





Direct Mail

Q3 Direct Mail



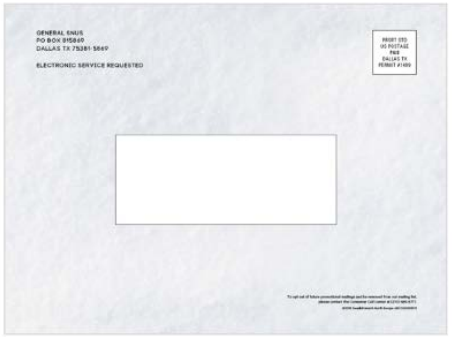


Q4 Direct Mail

FRONT



BACK



INSIDE



COUPON BACKS
AND STORE LOCATOR



Event Mailer (Jan)





Coupon Mailer (Jan)





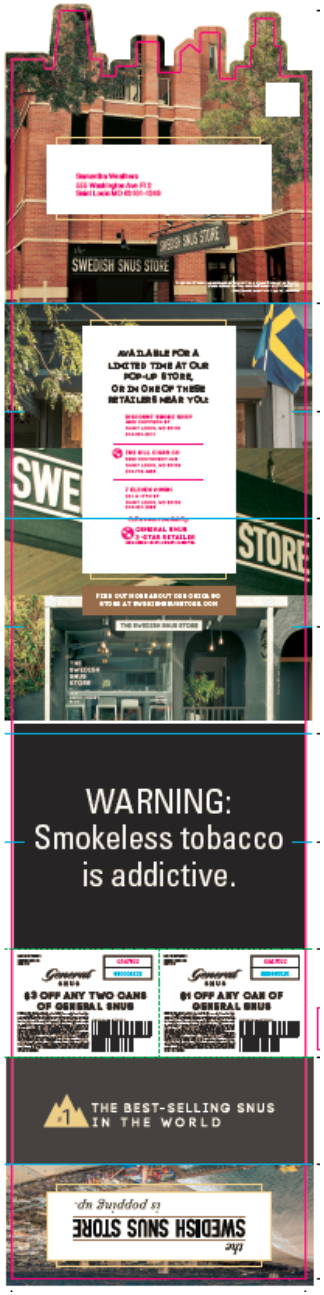
Birthday Mailer (Jan)





Q1 Direct Mail (March)







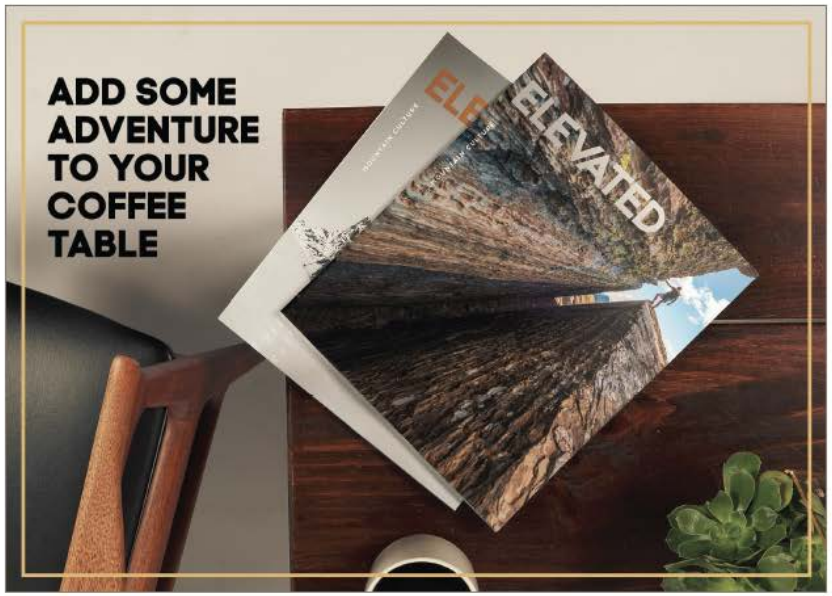
SWEDISH MATCH

Q2 Direct Mailer (June)





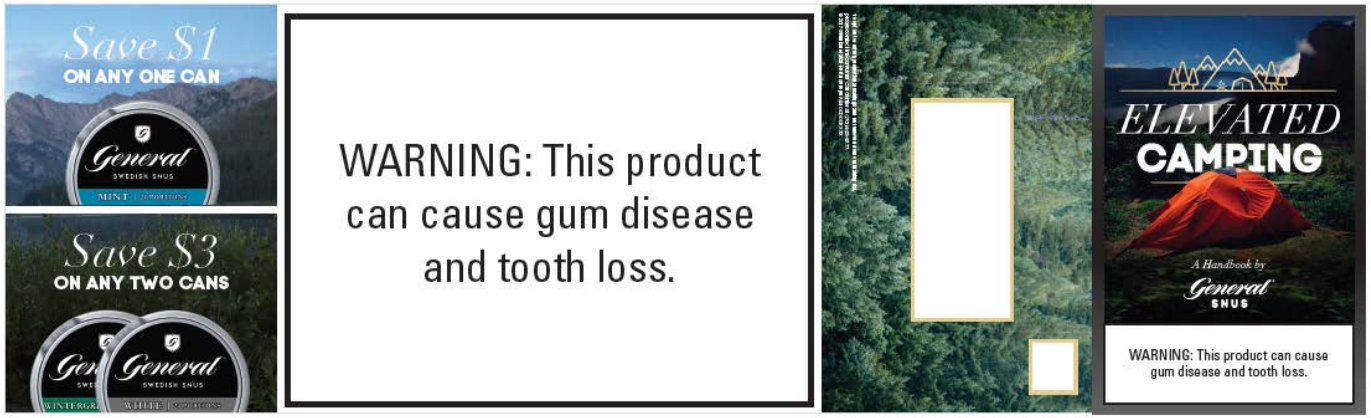
Elevated Postcard (June)





Q3 Direct Mail (August)





Social Media

Facebook Cover Photos







Facebook Cover Photos

Q1



Q1 (March)



Q2 2017



Facebook Cover Photos

Q3



Q4



October Facebook Posts

This October, we're hitting the road for the trip of a lifetime, and you're in the driver's seat. Where we go and what we do is all up to you. So start packing, because a new season of adventure begins in just three days…



WARNING: This product is not a safe alternative to cigarettes.

Your road trip with General Snus starts now.

The playlist is made, your friends are in the car, and you're almost ready to get out on the open road. All that's left for you to do is pack the camping cooler. As you place the cooler into the trunk, you look inside and realize you have a little bit more room. Do you toss in a few extra cans of your favorite snus, or throw in a few extra beers? Comment to cast your vote.



WARNING: This product is not a safe alternative to cigarettes.

Why choose when you can make room for both? The air is fresh, the sun is shining, and you feel great to be on the road with your friends. You haven't seen your buddy who made the playlist in a while, and you forgot about his questionable taste in music. He's blasting a mixtape from his old jam band that he sang in. Over the noise, he asks you about the cans you packed earlier. He's a smoker who hasn't tried snus before. Do you offer to pull over and grab him a fresh can out of the cooler in exchange for control of the music? Or, do you try to make good time on the road and talk about old memories instead to drown out the sound? Comment to cast your vote.



WARNING: This product is not a safe alternative to cigarettes.

Peace at last. You turn down the music as you pull over. It's the perfect time of day to hit the trails, and you're anxious to get back on the road. While you dig around in the trunk for a can, a girl approaches. She's having car trouble and asks for your help. Turns out she's a local who works at the canoe launch up the mountain, and she offers you free rentals if you'll give her a ride to the car repair shop a few miles back. It's a change in plans but a day on the water is tempting. Do you apologize and stay the course, or shake things up and tell her to hop in? Comment to cast your vote.



WARNING: This product is not a safe alternative to cigarettes.

She hops in the car and leads you off the main road in the direction of the car repair shop. As you weave your way down the dirt mountain lane, you notice a wild moose drinking out of a river nearby. Your friends back home will never believe this. Do you stop and try to snap a picture for proof? Or do you head to the shop in hopes of making it back to the canoe launch before closing time? Comment to cast your vote.



WARNING: This product is not a safe alternative to cigarettes.

There's no way you're missing out on this photo opp. By the time you stop the car and get out, several people have already gathered at the edge of the river. You and your friend both toss in a pouch before you reach for your cameras, and it catches the attention of a guy standing nearby. You hold out your open can and he gratefully reaches for a pouch. Once you're satisfied with your photo, you nod a goodbye and head back to the car. Your stop at the car repair shop is a short one, and you make it to the canoe launch well before closing time. Yet suddenly, just as you pull in, it starts to drizzle. You've been looking forward to canoeing all day, but this slight rain is making for the perfect fishing weather. Do you launch your canoes or cast your fishing lines?



WARNING: This product is not a safe alternative to cigarettes.

Fishing it is. After a successful afternoon, you head back in and catch sight of a familiar face setting up camp next to you. It's the guy you shared a can with back by the river. He waves you over, thanks you again, and invites you to join him and his friends for a bonfire. You're in. As you settle by the fire, someone asks if anyone plays guitar. You have one in the car, and your friend is always looking for ways to relive the jam band glory days. But someone else mentions an evening hike with spectacular views. Do you rock out by the fireside and enjoy the company of new friends, or do you head out and explore? Comment to cast your vote.



WARNING: This product is not a safe alternative to cigarettes.

What better way to end the day than with new friends. You grab your guitar, pass around a can of snus, and enjoy a night filled with good music, great company, and the kind of smoke you actually enjoy. And while the choices may end here, your road trip doesn't have to. What adventures are next for you and your can of General Snus?



WARNING: This product is not a safe alternative to cigarettes.

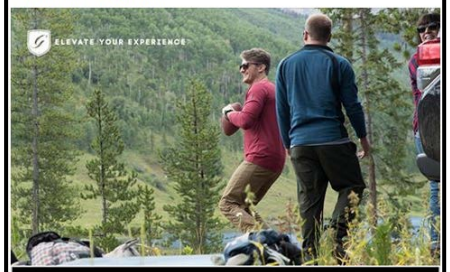
November Facebook Posts

Our friends over at FREESKIER Magazine are crowning North America's best après bars, and voting closes tomorrow. Visit apres.freeskier.com, choose a ski town, and vote for your favorites. The winners will be featured on an après party tour later this winter.

www.apres.freeskier.com

APRES.FREESKIER.COM

Play by your own playbook.



WARNING: This product is not a safe alternative to cigarettes.

Enjoy the great indoors.



WARNING: This product is not a safe alternative to cigarettes.

Four main ingredients. One exceptional experience. Discover what goes into a pouch of the world's best-selling snus:
www.GeneralSnus.com/Ingredients



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Four main ingredients. One exceptional experience. Discover what goes into a pouch of the world's best-selling snus:
www.GeneralSnus.com/Ingredients



WARNING: This product is not a safe alternative to cigarettes.

Always within reach.



WARNING: This product is not a safe alternative to cigarettes.

From one time-honored tradition to another. What are your favorite holiday traditions?



WARNING: This product is not a safe alternative to cigarettes.

Get out, with snus in.



WARNING: This product is not a safe alternative to cigarettes.

Dive into the exhilarating world of mountain culture with our new series "On the Mountain." Find it at www.GeneralSnus.com/OnTheMountain and check back regularly for new stories and spotlights.



WARNING: This product is not a safe alternative to cigarettes.

December 2016 Facebook Posts

In 1866, after more than four years of painstaking refinement, the world's best-selling snus was born. And now, in the last month of our 150th year, we're paying tribute to the qualities that truly make General Snus a tobacco experience like no other.



General's signature blend combines 22 of the world's most premium tobaccos, all of which are carefully selected by our experts. Learn more about our premium ingredients here: www.GeneralSnus.com/Ingredients



Because our authentic snus is crafted in Sweden, only ingredients that meet strict Swedish food-grade standards are used in our process. What kind of snus do you prefer? Like this post if you choose Swedish snus over American snus.



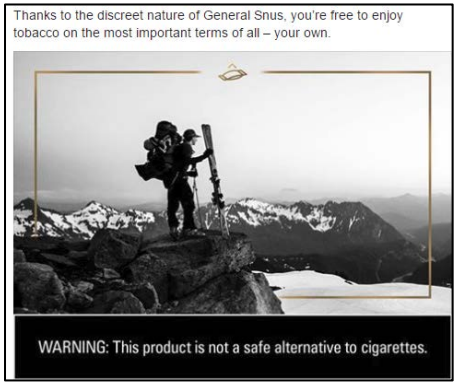
Every can of General is sold at 46 degrees Fahrenheit to ensure maximum freshness, moisture and flavor. Use the store locator to find our signature chiller near you: www.GeneralSnus.com/Find



Placing a pouch of General between your upper lip and gum means less saliva and no need to spit.
Like this post if you've tossed your spit cup, too.



Thanks to the discreet nature of General Snus, you're free to enjoy tobacco on the most important terms of all – your own.



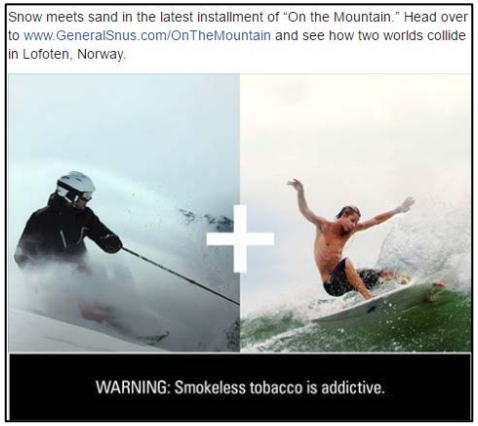
And here's to 150 more.



SWEDISH MATCH

January Facebook Posts













February Facebook Posts

Make the most of every moment, shadow or no shadow.



WARNING: Smokeless tobacco is addictive.

The only break you'll take today is the one at halftime. #GameOn #Smokeless



WARNING: Smokeless tobacco is addictive.

Sometimes a rescue team's hardest worker is also its smallest. Check out the latest installment of "On the Mountain" at www.GeneralSnus.com/OnTheMountain.



WARNING: Smokeless tobacco is addictive.

Smoke-free, spit-free, skip-free.



WARNING: Smokeless tobacco is addictive.

Satisfaction within reach.



WARNING: Smokeless tobacco is addictive.

The year is young and full of opportunity.
Where will 2017 take you?



WARNING: Smokeless tobacco is addictive.

You call the shots.
#Smokeless #Spitless



WARNING: Smokeless tobacco is addictive.

We'd like to thank you, our fans, for helping make General the world's best-selling snus.



WARNING: Smokeless tobacco is addictive.

SWEDISH MATCH

March Facebook Posts

There's no doubt. In winter, skiing owns the mountain. But when the snow starts to melt and spring hits, what will take its place? Trails, rivers, rocks or off-roads – this month, spring's best are going head-to-head, and it's up to you to decide which pursuit reigns supreme. Stay tuned, the matchup starts soon…



WARNING: Smokeless tobacco is addictive.

It's time to take a closer look at this season's contenders. First up, #Trails. Once they've thawed, mountain trails are your road of choice. Every walk is a hike waiting to happen, and the long way is the only way to get where you're going. Trekking, sky running and more – you're happiest with the sky above and the trails below.



WARNING: Smokeless tobacco is addictive.

In your world, every #River is a chance for you to leave land behind. Whether by canoe, kayak, raft or paddle board, there's no better view than the view from the water.



WARNING: Smokeless tobacco is addictive.

Bouldering, canyoning, trad or lead climbing. Where some see an obstacle, you see an opportunity. You live for the #Rocks that call mountains home, and you'd never get caught taking the easy way up… or down.



WARNING: Smokeless tobacco is addictive.

Happiest traveling the #OffRoads? If so, then you already know – it's better to carve your own path than to follow the trails. Whether mountain biking, four-wheeling or rock crawling, the only path worth choosing is the one you make for yourself.



WARNING: Smokeless tobacco is addictive.

The first Mountain Matchup is here: #Trails vs. #Rivers. Comment below to vote for the pursuit you think should own the mountain come spring.



WARNING: Smokeless tobacco is addictive.

Our second Mountain Matchup: #Rocks vs. #OffRoads. Who will go on to face #Trails at the peak of the bracket? Comment below to cast your vote.



WARNING: Smokeless tobacco is addictive.

It's the final Mountain Matchup: #Trails vs. #Rocks. The peak of the bracket is up for grabs, and it's up to you to decide who wins. Cast your final vote below to choose which pursuit will conquer the mountain.



WARNING: Smokeless tobacco is addictive.

The adventure enthusiasts have spoken. It's #Trails that will own the mountain come spring.
And for those who plan to hit them soon, make sure you've stocked up on his season's latest gear. Thanks for playing. http://www.backpacker.com/.../best-hiking-boots-and-shoes-of-...



WARNING: Smokeless tobacco is addictive.

SWEDISH MATCH

April Facebook Posts

Last we checked, Sunday was still the weekend. Make the most of it.



WARNING: This product can cause mouth cancer.

The BEST-selling snus in the world, and still climbing. #SpeakSwedish



WARNING: This product can cause mouth cancer.

Winter is reaching its final leg, and so is our first edition of "On the Mountain." Make both last a little while longer with the latest post: www.GeneralSnus.com/OnTheMountain



WARNING: This product can cause mouth cancer.

When fresh mint leaves meet hand-picked tobacco, the result is a blend of cool peppermint flavor that makes General Mint a favorite among snus enthusiasts.



WARNING: This product can cause mouth cancer.

Serve up satisfaction.



WARNING: This product can cause mouth cancer.

Experience Sweden wherever you are with authentically Swedish General Snus.



WARNING: This product can cause mouth cancer.

Tobacco, water, salt. Our simple core ingredients create a signature taste that's anything but.



WARNING: This product can cause mouth cancer.

All the makings of a great day.



WARNING: This product can cause mouth cancer.

SWEDISH MATCH

May Facebook Posts

Even on a day like today, we're decidedly Swedish. #BottomsUp



WARNING: This product can cause mouth cancer.

The two most exciting minutes in sports are even better in person. Like this post if you're at the race today.



WARNING: This product can cause mouth cancer.

Sweet, like soon-to-be summer.



WARNING: This product can cause mouth cancer.

From sound check to encore.



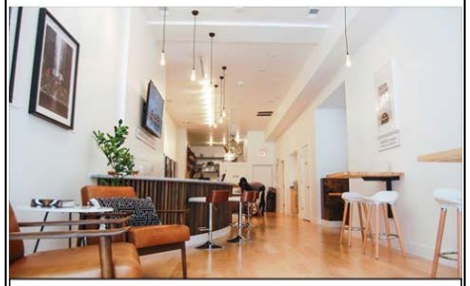
WARNING: This product can cause mouth cancer.

Whether it's a ride to work or the ride of your life, the journey is all yours. #NationalBikeMonth



WARNING: This product can cause mouth cancer.

In or near the Windy City this weekend? Stop by the Swedish Snus Store at 840 W. Armitage.



WARNING: This product can cause mouth cancer.

Every trip has its essentials. Make sure you have yours.



WARNING: This product can cause mouth cancer.

Experience is calling.



WARNING: This product can cause mouth cancer.

SWEDISH MATCH

June Facebook Posts

Take only your memories, and leave nothing behind. #DisposalLid



WARNING: This product can cause mouth cancer.

Experience the flavors of summer.



WARNING: This product can cause mouth cancer.

The first and second editions of ELEVATED magazine have arrived. Register at GeneralSnus.com to claim your complimentary copies. Already registered? Simply log in to request your special edition magazines.



WARNING: This product can cause mouth cancer.

To the experiences that lie ahead.



WARNING: This product can cause mouth cancer.

Enjoy every moment together.



WARNING: This product can cause mouth cancer.

No matter the season.



WARNING: This product can cause mouth cancer.

In Sweden, we've turned the longest day into the biggest celebration. Whatever you make of your Midsummer, we hope you make the most of it.



WARNING: This product can cause mouth cancer.

Headed down the coast, up to the mountains, or out into the woods this summer? Let the scenic routes (and General Snus) be your guide.

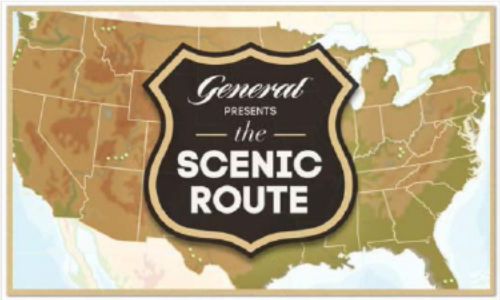


WARNING: This product can cause mouth cancer.

SWEDISH MATCH

July Facebook Posts

This summer, we're featuring nine of the country's most scenic routes. With each new post comes a new route and a new chance to comment about your [illegible] guide.



WARNING: This product can cause gum disease and tooth loss.

With endless acres of pine, expansive views of Lake Superior, and the Sawtooth Mountains dominating the horizon—there's seemingly no end to the natural beauty of Minnesota's North Shore Scenic Drive.



WARNING: This product can cause gum disease and tooth loss.

There are few places left where mans' intentions are still subject to nature's will. This canopy-covered route along Florida's eastern coast is one of those places. Get behind the wheel and travel to a place where time stands still.



WARNING: This product can cause gum disease and tooth loss.

With picturesque views of snow-covered peaks, cascading waterfalls and glacial lakes, Montana's Going-to-the-Sun Road deserves to be driven with purpose. The purpose of remembering every detail.



WARNING: This product can cause gum disease and tooth loss.

New Hampshire's colorful clearings, easily accessible waterfalls and breathtaking vistas call every Kancamagus Highway drive to become a hiker—if only for a day.



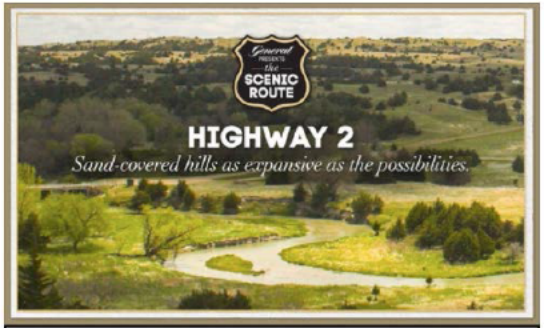
WARNING: This product can cause gum disease and tooth loss.

The only thing man-made along this Texas route is the road—a road as diverse in landscape as it is beautiful. So venture beyond the beaten path for a taste of true wilderness.



WARNING: This product can cause gum disease and tooth loss.

The Great Plains of Nebraska are turned upside down as endless flat fields of grain give way to hypnotic waves of sand. It's more than a nice roadside view. It's an unforgettable experience.



WARNING: This product can cause gum disease and tooth loss.

Sandstone arches rise from the thick, hilly forest that surrounds Kentucky's Route 77. The wildflowers and wildlife that line the road might tempt you to stop and explore. And that's exactly what you should do.



WARNING: This product can cause gum disease and tooth loss.

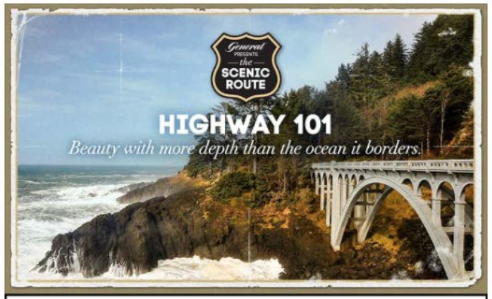
Route 12 winds and climbs through the narrow passes and plateau peaks of Utah's Red Rock Mountains. It's easy to get lost here. And that's just fine.



WARNING: This product can cause gum disease and tooth loss.

August Facebook Posts

Don't just pass through. Put the car in park and experience the beautiful sandy beaches and brimming tide pools that hug Oregon's iconic coast.



WARNING: This product can cause gum disease and tooth loss.

There are routes that take you to destinations. And then there are routes that are destinations. No matter where this summer takes you, make sure to take the long way.



WARNING: This product can cause gum disease and tooth loss.

Truly the go-anywhere, do-anything tobacco.



WARNING: This product can cause gum disease and tooth loss.

Here's to bold ideas that get better with age. Check out the latest "On the Mountain" blog to learn more about the history behind America's National Parks and how to make the most of them today: www.GeneralSnus.com/OnTheMountain.



WARNING: This product can cause gum disease and tooth loss.

The weekend's almost here. Make your move.



WARNING: This product can cause gum disease and tooth loss.

Bergamot (n.) — a green citrus fruit the size of an orange; the unique hint of flavor behind General White and Original.



WARNING: This product can cause gum disease and tooth loss.

When nothing's holding you back, "going nowhere" takes on an entirely new meaning.



WARNING: This product can cause gum disease and tooth loss.

Whether you're backpacking through the mountains or at a backyard hangout with new friends, embrace every adventure.



WARNING: This product can cause gum disease and tooth loss.

Play your tobacco satisfaction close to the chest. #Smokeless #Spitless



WARNING: This product can cause gum disease and tooth loss.

What flavor will round out your summer experience?



WARNING: This product can cause gum disease and tooth loss.

SWEDISH MATCH

September Facebook Posts

Two popular flavors, with very distinct tastes. Which flavor do you have a taste for?



WARNING: This product can cause gum disease and tooth loss.

Whether it's in the fridge or on the counter, we all store our snus somewhere. Where do you keep yours after you buy it?



WARNING: This product can cause gum disease and tooth loss.

We're made for experiences indoors, outdoors, and most importantly, amongst friends. Where do you enjoy General?



WARNING: This product can cause gum disease and tooth loss.

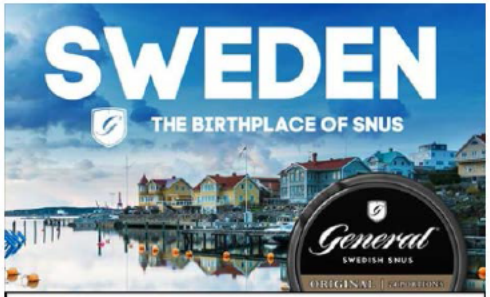
SWEDISH MATCH

The spice of variety or the comfort of tried and true. Which do you prefer?



WARNING: This product can cause gum disease and tooth loss.

Imitation truly is the sincerest form of flattery. But, it's not really snus unless it's Swedish snus.



WARNING: This product can cause gum disease and tooth loss.

This ban will restrict the sale of General Mint and Wintergreen at your local retailers. To speak up for your favorite flavors, contact City Council members or attend this week's meeting. Learn more: enoughisenoughstpaul.com



WARNING: This product can cause gum disease and tooth loss.

'Mint' doesn't mean small flavor. Try dry, smooth Mini Mint or bold, well-balanced Mint for a fresh release of peppermint taste.



WARNING: This product can cause gum disease and tooth loss.

No matter how you snus, or why you choose it. With General, the right experience is always right in front of you.



WARNING: This product can cause gum disease and tooth loss.

Do you enjoy your snus with coffee in the morning, or with a beer to end the day?



WARNING: This product can cause gum disease and tooth loss.

Experiential

Tent (April)













SWEDISH MATCH

Trailers Large (April)

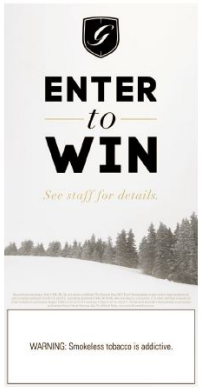




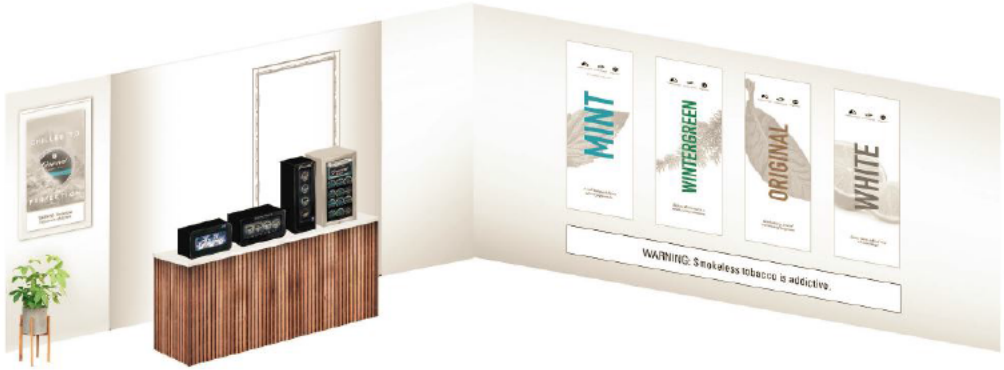








Trailers Large (Cont.)







Trailers Small (April)







Infographic (April)





SWEDISH MATCH

Media

Freeskier Après Tour (Sept-Dec 2016)















Freeskier Best Of (Sept-Dec 2016)





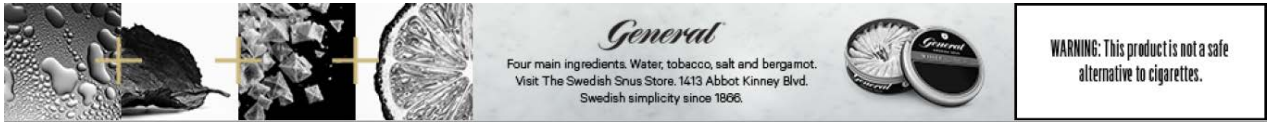


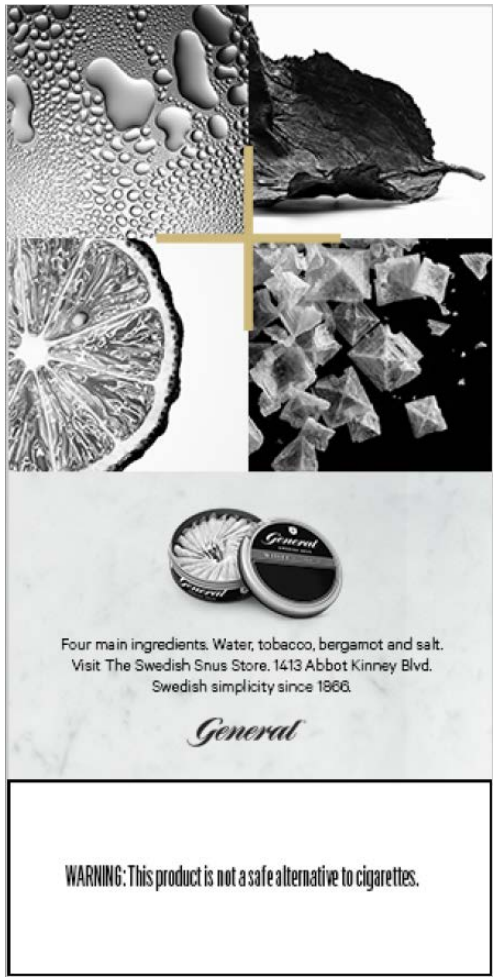


LA Confidential Banner Ads (Nov-Dec 2016)







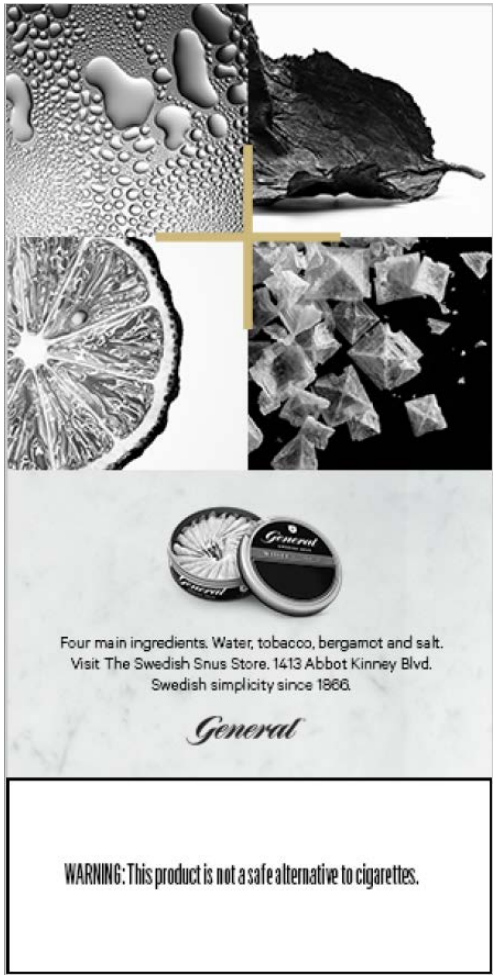


LA Weekly Banner Ads (Sept-Dec 2016)





LA Weekly Banner Ads (Sept-Dec 2016)



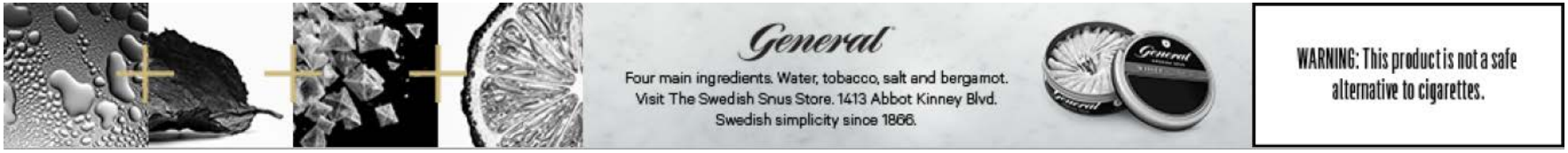




LA Weekly Banner Ads (Sept-Dec 2016)

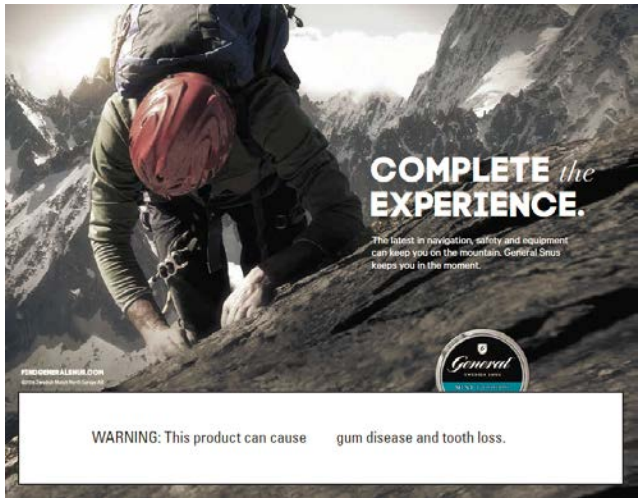


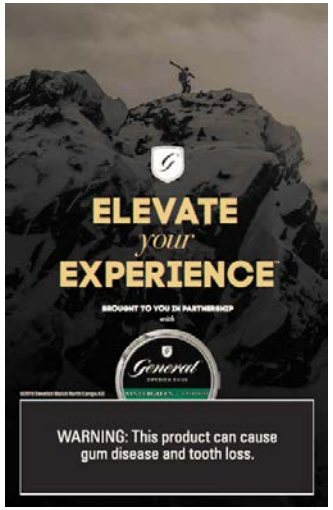




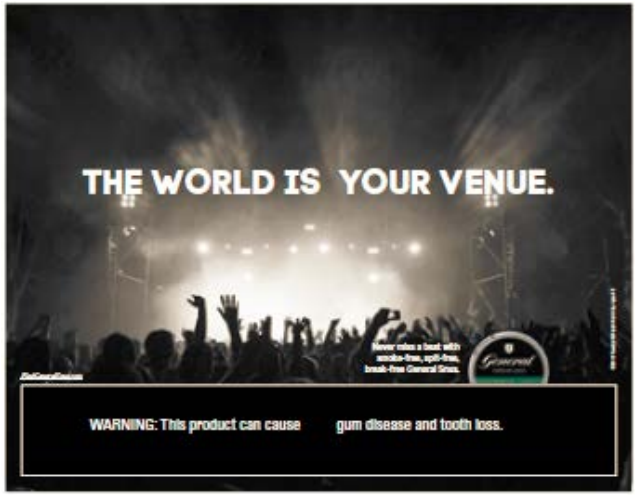
Freeskier Best Of (Sept & Oct 2016)





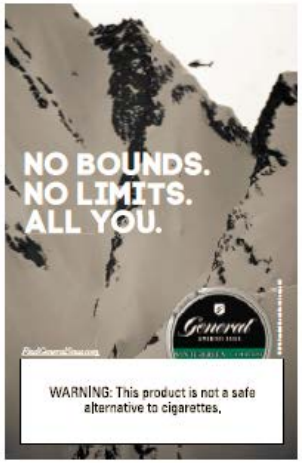




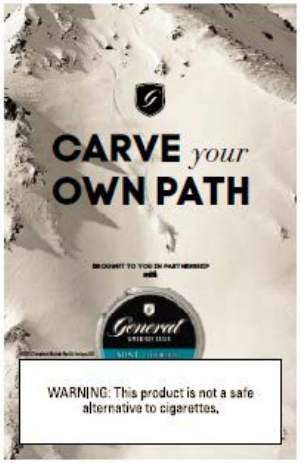




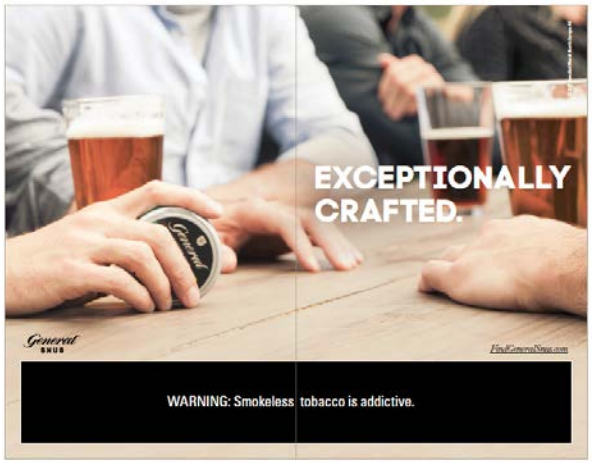
Freeskier Best Of (Nov & Dec 2016)





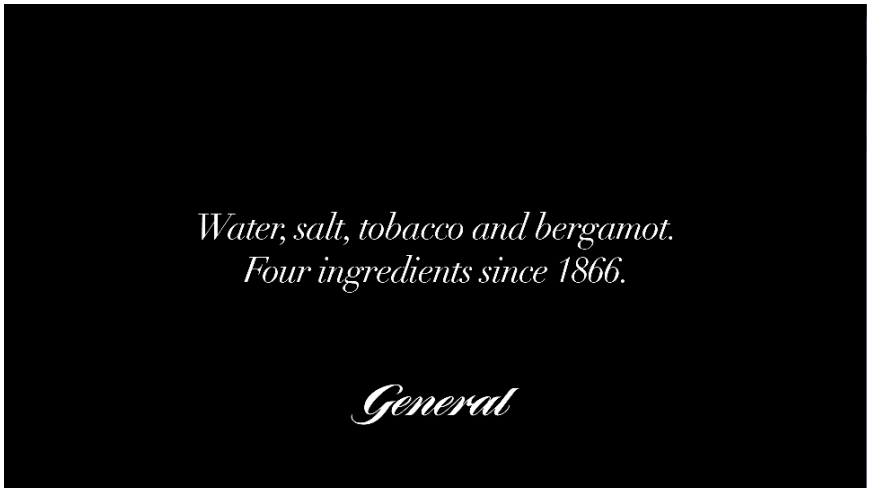






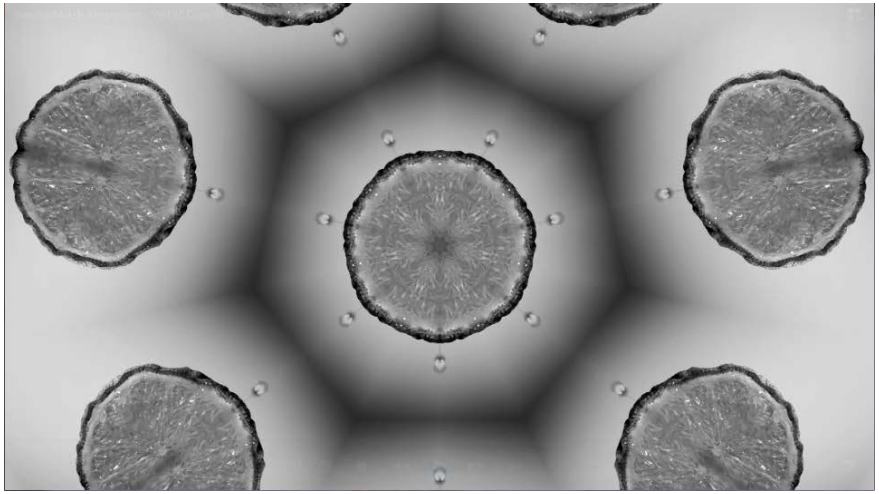


LA Pop-Up Store
4 Ingredients Video









Freeskier Exposure Contest Ads

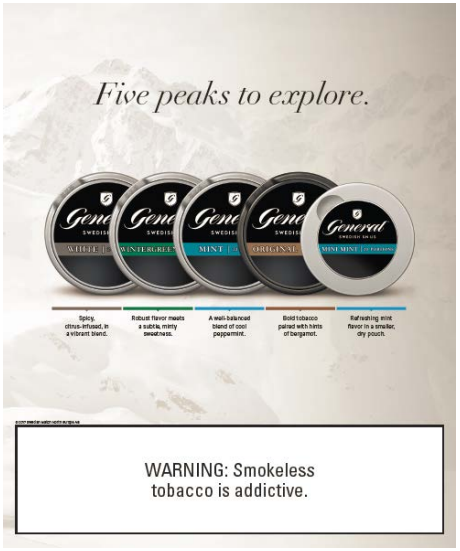






Exposure Ads (Feb)





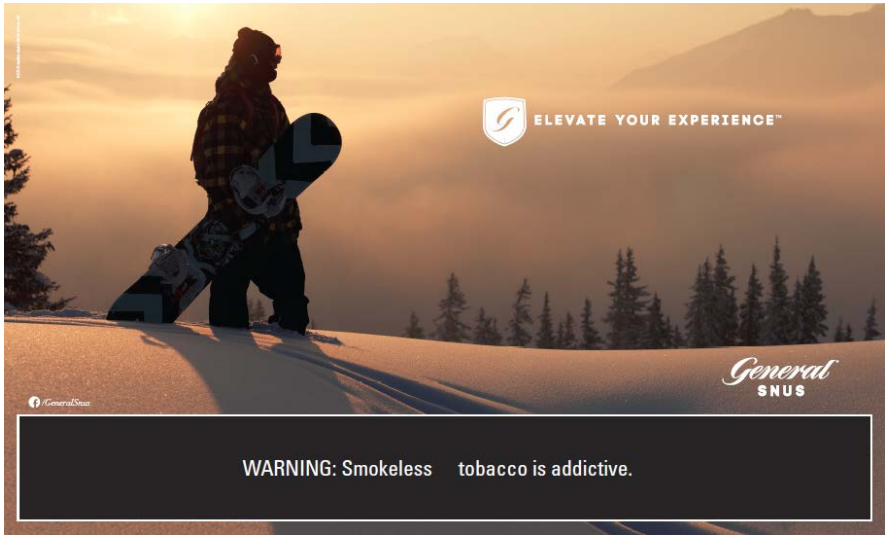




SWEDISH MATCH

Exposure Ads (Cont.)





Nordic Reach/Nordstjernan Print Ads (April)









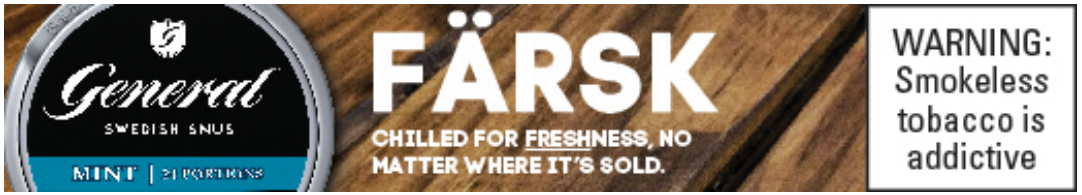


Nordic Reach Digital Banner Ads (April)











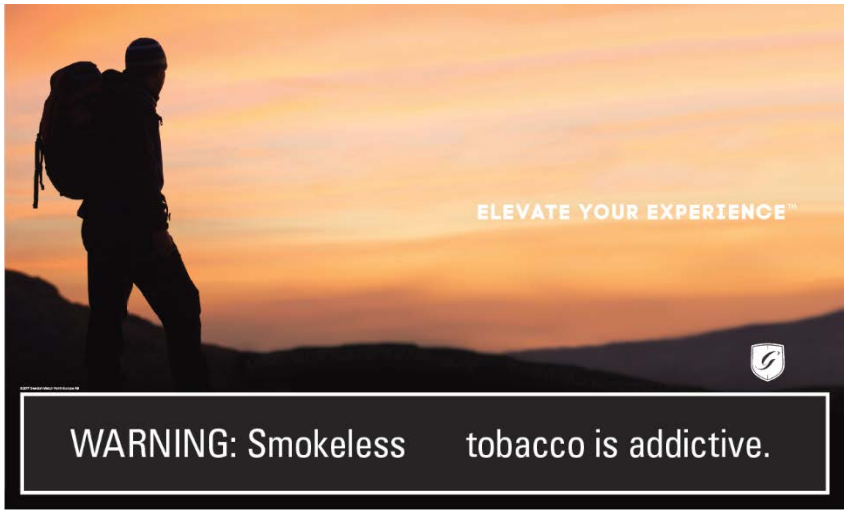








Elevated Magazine Ads (May)







SWEDISH MATCH

Elevated Magazine Ads (Cont.)



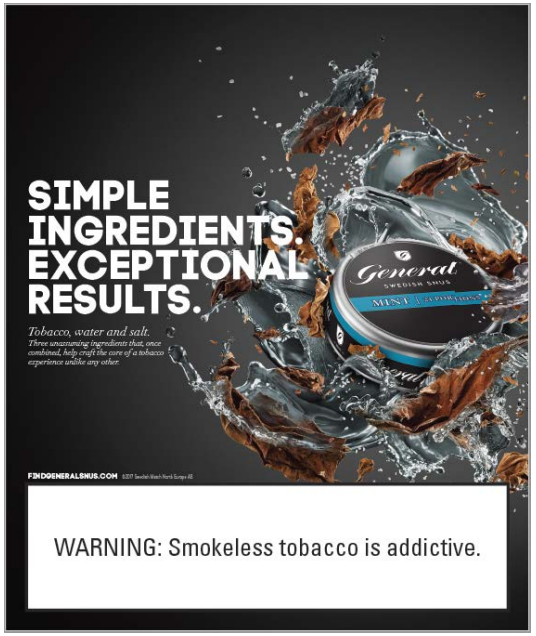


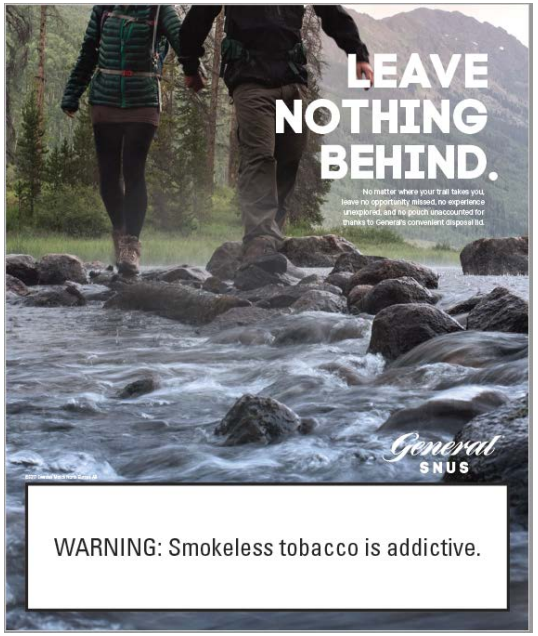


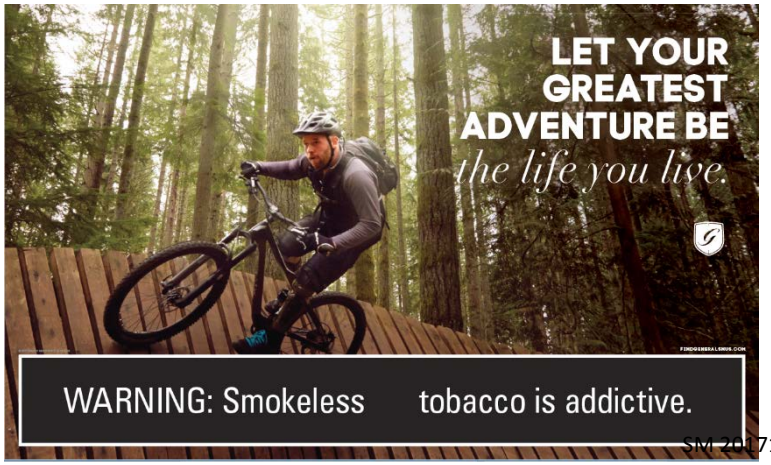


SWEDISH MATCH

Elevated Magazine Ads (Cont.)



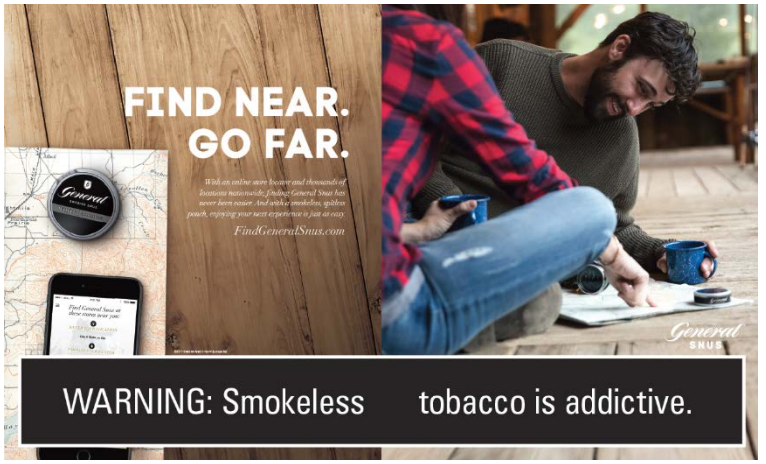




SWEDISH MATCH

Elevated Magazine Ads (Cont.)





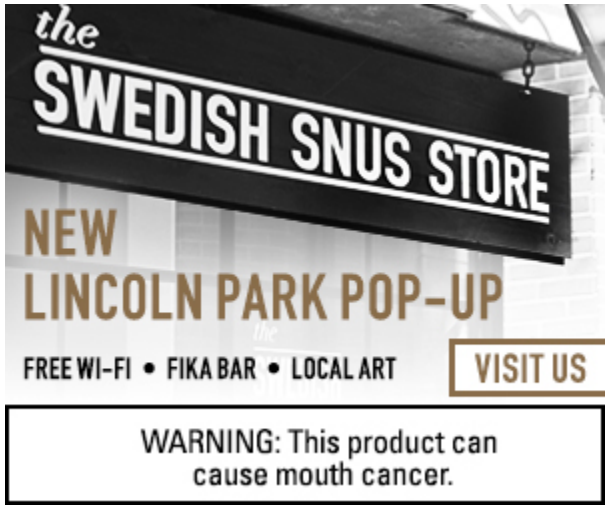


Chicago Store Ads and Support (May)



Print Ad







Banner Ads

Chicago Store Ads and Support (Cont.)





Take One Card



Infographic Ad







Banner Ads

SWEDISH MATCH

Freeskier Summer Shootout Print Ads

SPREAD 1



SINGLE PAGE 1



SINGLE PAGE 2



SINGLE PAGE 3



SINGLE PAGE 4



SPREAD 2



Freeskier Summer Shootout Digital Ads







Freeskier 20th Anniversary Ads





