

## SOME TOBACCO-SPECIFIC *N*-NITROSAMINES

Four tobacco-specific *N*-nitrosamines (TSNA), namely, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N'*-nitrosornicotine (NNN), *N'*-nitrosoanabasine (NAB) and *N'*-nitrosoanatabine (NAT) were considered by a previous Working Group in October 1984 (IARC, 1985). Since that time, new data have become available and are presented in this monograph.

In addition to these compounds, new TSNA have been identified (Figure 1) and their concentrations in tobacco and tobacco smoke have been assessed. The occurrence of 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid (*iso*-NNAC), 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (*iso*-NNAL) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in tobacco is reported in the monograph on Smokeless Tobacco; however, as a result of the limited data available, these TSNA have not been considered in the present evaluation.

This monograph does not consider the exposure of workers involved in the production of these compounds, which are used solely for laboratory research purposes.

### 1. Exposure Data

#### 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

##### 1.1 Chemical and physical data

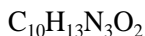
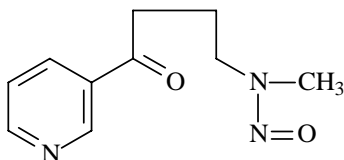
###### 1.1.1 *Synonyms and trade names*

*Chem. Abstr. Services Reg. No.:* 64091-91-4

*Chem. Abstr. Name:* 1-Butanone, 4-(methylnitrosoamino)-1-(3-pyridinyl)-

*IUPAC Systematic Name:* 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

*Synonym:* 4-(*N*-Methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone

1.1.2 *Structural and molecular formulae and relative molecular mass*

Relative molecular mass: 207.2

1.1.3 *Chemical and physical properties of the pure substance*

From Toronto Research Chemicals (2005, 2006), unless otherwise specified

- (a) *Description*: Light-yellow crystalline solid
- (b) *Melting-point*: 61–63 °C
- (c) *Spectroscopy data*: Infrared, nuclear magnetic resonance and mass spectra have been reported (IARC, 1985).
- (d) *Solubility*: Soluble in dichloromethane, dimethyl sulfoxide (DMSO), dimethylfuran, ethyl acetate and methanol
- (e) *Stability*: Sensitive to light

***N'*-Nitrosonornicotine (NNN)****1.1 Chemical and physical data**1.1.1 *Synonyms and trade names*

*Chem. Abstr. Services Reg. Nos.*: 80508-23-2; 16543-55-8<sup>1</sup>; 84237-38-7<sup>2</sup>

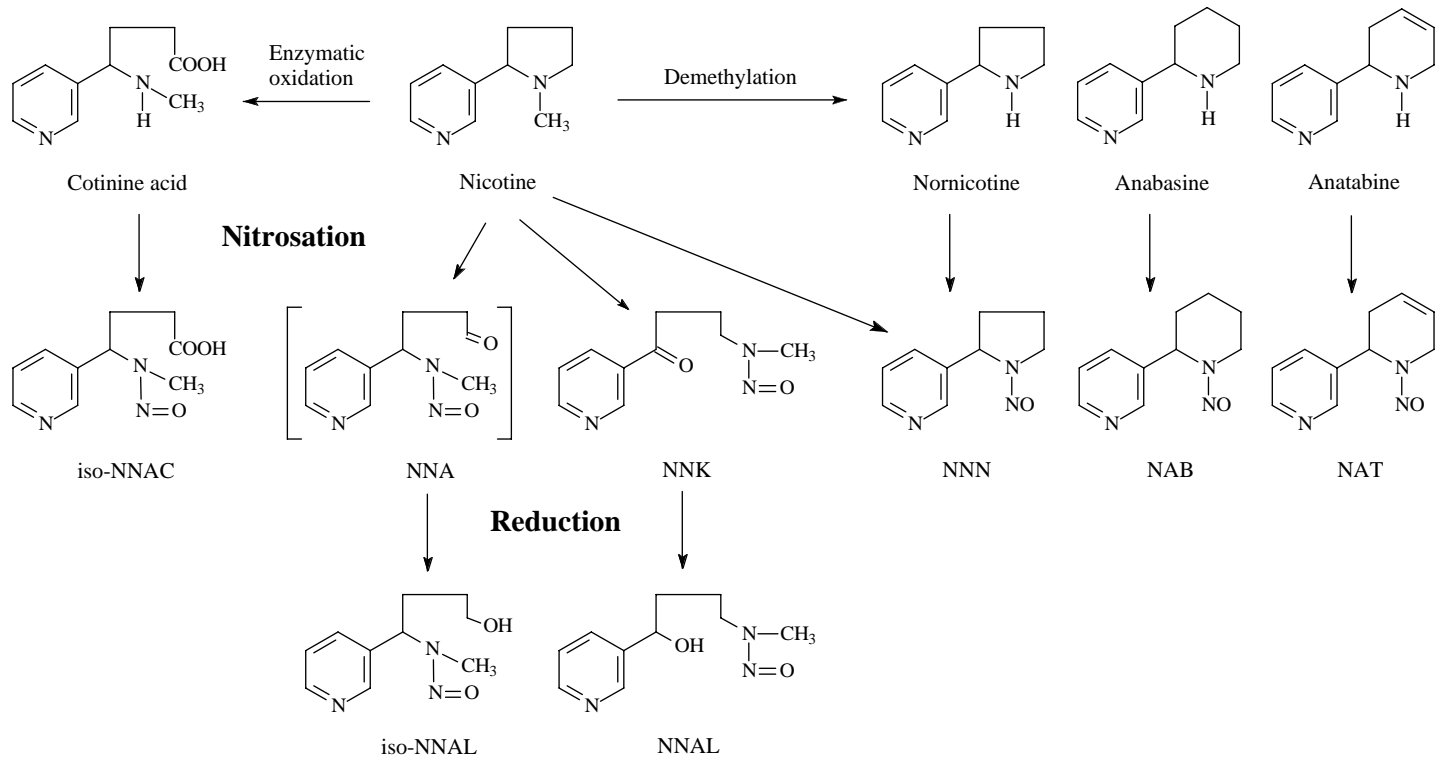
*Chem. Abstr. Names*: Pyridine, 3-(1-nitroso-2-pyrrolidinyl)-; pyridine, 3-(1-nitroso-2-pyrrolidinyl)-,(S)-<sup>1</sup>; pyridine, 3-(1-nitroso-2-pyrrolidinyl)-, (+,-)-<sup>2</sup>

*IUPAC Systematic Name*: 1'-Demethyl-1'-nitrosonicotine

*Synonyms*: 1'-Demethyl-1'-nitrosonicotine; 1'-desmethyl-1'-nitrosonicotine; 1'-nitroso-1'-demethylnicotine; nitrosonornicotine; *N*-nitrosonornicotine; 1'-nitrosonornicotine; 1-nitroso-2-(3-pyridyl)pyrrolidine; 3-(1-nitroso-2-pyrrolidinyl)pyridine

<sup>1</sup> The Chemical Abstracts Services Registry Number and Name refer to the (S) stereoisomer.

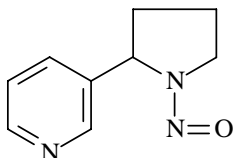
<sup>2</sup> The Chemical Abstracts Services Registry Number and Name refer to the racemic mixture that was synthesized and used in the biological studies reported in this monograph.

**Figure 1. Formation of tobacco-specific N-nitrosamines**

From Hoffmann *et al.* (1995)

*iso*-NNAC, 4-(methylnitrosamino)-4-(3-pyridinyl)butyric acid; *iso*-NNAL, 4-(methylnitrosamino)-4-(3-pyridinyl)-1-butanol; NAB, N'-nitrosoanabasine; NAT, N'-nitrosoanatabine; NNA, 4-(methylnitrosamino)-4-(3-pyridinyl)butanal; NNAL, 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridinyl)-1-butanone; NNN, N'-nitrosonornicotine

Note: NNA is a very reactive aldehyde and has therefore never been quantified in tobacco or tobacco smoke.

1.1.2 *Structural and molecular formulae and relative molecular mass*C<sub>9</sub>H<sub>11</sub>N<sub>3</sub>O

Relative molecular mass: 177.2

1.1.3 *Chemical and physical properties of the pure substance*

From Toronto Research Chemicals (2005, 2006), unless otherwise specified

- (a) *Description*: Light-yellow oil
- (b) *Boiling-point*: 154 °C at 0.2 mm (IARC, 1985)
- (c) *Melting-point*: 47 °C (IARC, 1985); 42–45 °C
- (d) *Spectroscopy data*: Mass, ultraviolet, infrared and nuclear magnetic resonance spectra have been reported (IARC, 1985).
- (e) *Solubility*: Soluble in acetone and chloroform
- (f) *Stability*: Hygroscopic

**N'-Nitrosoanabasine (NAB)****1.1 Chemical and physical data**1.1.1 *Synonyms and trade names*

*Chem. Abstr. Services Reg. Nos.*: 37620-20-5; 1133-64-8<sup>1</sup>; 84237-39-8<sup>2</sup>

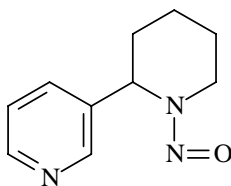
*Chem. Abstr. Names*: Pyridine, 3-(1-nitroso-2-piperidinyl)-; pyridine, 3-(1-nitroso-2-piperidinyl)-,(S)-<sup>1</sup>; pyridine, 3-(1-nitroso-2-piperidinyl), (+,-)-<sup>2</sup>

*UPAC Systematic Name*: 1-Nitrosoanabasine

*Synonym*: N-Nitrosoanabasine

<sup>1</sup> The Chemical Abstracts Services Registry Number and Name refer to the (S) stereoisomer.

<sup>2</sup> The Chemical Abstracts Services Registry Number and Name refer to the racemic mixture that was synthesized and used in the biological studies reported in this monograph.

1.1.2 *Structural and molecular formulae and relative molecular mass*C<sub>10</sub>H<sub>13</sub>N<sub>3</sub>O

Relative molecular mass: 191.2

1.1.3 *Chemical and physical properties of the pure substance*

From Toronto Research Chemicals (2005, 2006), unless otherwise specified

- (a) *Description*: Yellow oil
- (b) *Boiling-point*: 162 °C at 1 mm Hg (IARC, 1985); 165–167 °C at 0.25 mm Hg
- (c) *Optical rotation*: The specific rotation of NAB has been reported (IARC, 1985)
- (d) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectra have been reported (IARC, 1985).
- (e) *Solubility*: Soluble in chloroform and dichloromethane

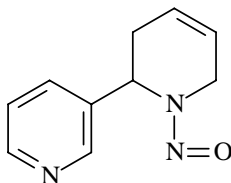
**N'-Nitrosoanatabine (NAT)****1.1 Chemical and physical data**1.1.1 *Synonyms and trade names*

*Chem. Abstr. Services Reg. No.*: 71267-22-6

*Chem. Abstr. Name*: 2,3'-Bipyridine, 1,2,3,6-tetrahydro-1-nitroso-

*IUPAC Systematic Name*: 1,2,3,6-Tetrahydro-1-nitroso-2,3-bipyridine

*Synonym*: NATB

1.1.2 *Structural and molecular formulae and relative molecular mass*C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>O

Relative molecular mass: 189.2

### 1.1.3 *Chemical and physical properties of the pure substance*

From Toronto Research Chemicals (2005, 2006), unless otherwise specified

- (a) *Description*: Pale-yellow oil
- (b) *Boiling-point*: 176 °C at 0.5 mm Hg (IARC, 1985)
- (c) *Spectroscopy data*: Infrared, mass and nuclear magnetic resonance spectra have been reported (IARC, 1985).
- (d) *Solubility*: Soluble in chloroform, ethanol and methanol
- (e) *Stability*: Sensitive to light

## 1.2 **Technical products and impurities, analysis, production and use**

### 1.2.1 *Technical products and impurities*

NNK, NNN, NAB and NAT are available commercially at a purity of 98%, in units of up to 1 g (Toronto Research Chemicals, 2006). NNK and NNN isolated from tobacco are mixtures of 72.7% E-isomer and 27.3% Z-isomer (Hecht *et al.*, 1977; Hoffmann *et al.*, 1980).

### 1.2.2 *Analysis*

Standard methods for the analysis of NNK, NNN, NAB and NAT have been described previously (Egan *et al.*, 1983). Since that time, numerous studies on the levels of TSNA in tobacco and tobacco smoke have included descriptions of methods for the extraction and quantification of TSNA (Andersen *et al.*, 1989; Djordjevic *et al.*, 1989a; Fischer & Spiegelhalter, 1989; Fischer *et al.*, 1989a; Spiegelhalter *et al.*, 1989; Sharma *et al.*, 1991; Stepanov *et al.*, 2002; Ashley *et al.*, 2003; Jansson *et al.*, 2003; Wu *et al.*, 2003, 2004; Stepanov *et al.*, 2005; Wu *et al.*, 2005; Stepanov *et al.*, 2006a).

Risner *et al.* (2001) reported a collaborative investigation of methods for the determination of TSNA in tobacco. Seventeen laboratories around the world participated in this study and used seven different methods or variations thereof. The methods varied in sample preparation, conditions of analysis, compound detection and quantification of results. Morgan *et al.* (2004) reported the results from a collaborative study carried out by 15 different laboratories that used two different methods to determine TSNA. Both methods proved to be efficient for the determination of TSNA in a variety of tobacco types.

Methods for the assessment of NNAL, *iso*-NNAL and *iso*-NNAC in tobacco have also been described (Brunnemann *et al.*, 1987a; Djordjevic *et al.*, 1989a; Djordjevic *et al.*, 1993a).

Methods for determination of TSNA in mainstream tobacco smoke are also available on various websites, such as those from Health Canada ([http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/indust/method/index\\_e.html](http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/indust/method/index_e.html)) or from the United Kingdom Government Benchmark Study (<http://www.the-tma.org.uk/benchmark/>).

### 1.2.3 Production

NNK was first prepared by the reaction of sodium hydroxide and sodium nitrite with 4-(*N*-methyl)-1-(3-pyridyl)-1-butanone dihydrochloride (IARC, 1985). NNK is currently produced commercially (Toronto Research Chemicals, 2005, 2006).

NNN was first prepared by treating nornicotine with sodium nitrite in dilute hydrochloric acid (IARC, 1985). NNN is currently produced commercially (Toronto Research Chemicals, 2005, 2006).

NAB was first prepared by treating anabasine with sodium nitrite in dilute hydrochloric acid solution (IARC, 1985). NAB is currently produced commercially (Toronto Research Chemicals, 2005, 2006).

NAT was first prepared by the reaction of sodium nitrite with a solution of anatabine in hydrochloric acid (IARC, 1985). NAT is currently produced commercially (Toronto Research Chemicals, 2005, 2006).

*iso*-NNAC was synthesized from cotinine *via* 4-(methylamino)-4-(3-pyridyl)butyric acid and *N*-nitrosated. Currently, *iso*-NNAC, *iso*-NNAL and NNAL are also produced commercially as well (Toronto Research Chemicals, 2005, 2006).

### 1.2.4 Use

No evidence was found that NNK, NNN, NAB or NAT have ever been used other than in laboratory research that included animal studies (Hoffmann *et al.*, 1993a; Hecht, 1998).

## 1.3 Occurrence

The wide disparity in levels of TNSA in the mainstream smoke of cigarettes (Counts *et al.*, 2005; Gregg *et al.*, 2005; King *et al.*, 2007) and in smokeless tobacco products (Hoffmann *et al.*, 1995; Stepanov *et al.*, 2006a) is largely due to differences in the tobacco types used in any given product, agricultural practices, curing methods and manufacturing processes (IARC, 2004). While trace amounts of TSNA were measured in green tobacco leaves (Bhide *et al.*, 1987; Djordjevic *et al.*, 1989b), these compounds are formed from their alkaloid precursors and from nitrite or nitrate predominantly during tobacco curing, fermentation and ageing (Burton *et al.*, 1989a,b; Djordjevic *et al.*, 1993a). The type of tobacco (e.g., Burley, Bright, Virginia or Oriental), its nitrate and nitrite content, the mode of curing (e.g. air-, flue- or sun-curing) and the various steps of processing used are therefore the determining factors for the yields of TSNA in tobacco (Fischer *et al.*, 1989a; Bush *et al.*, 2001; Peele *et al.*, 2001). NNN, NAB and NAT are formed primarily from their corresponding secondary amines (namely nornicotine, anatabine and anabasine) in the early stages of tobacco curing and processing whereas the majority of NNK and some NNN are formed from the tertiary amine nicotine at the later stages of tobacco curing and fermentation (Spiegelhalder & Fischer, 1991).

Levels of NNN and other TSNA are consistently higher in Burley than in Bright tobacco, regardless of the curing method (Chamberlain & Chortyk, 1992; Morgan *et al.*, 2004). However, flue-curing of Bright tobacco produces nearly three times more TSNA than air-curing of the same tobacco. TSNA content varies between the parts of the plant. For example, NNK and NNN contents are higher in the midrib than in the lamina in air-cured tobacco, whereas the converse is observed in flue-cured tobacco. Flue-curing of Burley tobacco reduces the alkaloid content but greatly increases TSNA content in the lamina. Midribs from air-cured Burley leaves contain three times the TSNA concentrations of the lamina (Chamberlain & Chortyk, 1992). Analyses of 41 leaf segments from a dark air-cured tobacco variety (KY 171) revealed that concentrations of the individual nitrosamines were lowest at the tip and the periphery of the leaf (Burton *et al.*, 1992). The midvein contained the lowest concentrations of NNK, NNN and NAT at two-thirds of the length of the leaf; the highest concentrations were found at the base of the leaf. The correlation between nitrite nitrogen and TSNA was higher than that between alkaloid content and TSNA in the leaf.

DeRoton *et al.* (2005) summarized the major factors involved in the formation of TSNA in dark air-cured and Burley tobacco during curing and post-curing treatment. The main genetic trait involved in the formation of TSNA is the propensity of a variety of tobacco to convert nicotine to nor nicotine. In addition, the ability of a variety to lose water rapidly limits the formation of nitrite and hence also the formation of TSNA. Since TSNA are derived from tobacco alkaloids, agricultural practices that increase alkaloid concentrations in the tobacco leaves favour their formation. Air flow at the site of curing also influences the levels of TSNA: leaves that are cured in well-ventilated curing structures, such as plastic sheds, generally contain lower amounts of TSNA than those cured in barns. Levels of TSNA may increase after curing if tobacco leaves are stored under humid conditions (StAAF *et al.*, 2005) or in bales. The biological mechanism for TSNA formation in air-cured tobacco relates to the breakdown of plant cell membranes due to moisture loss, which makes the cell contents available to microorganisms that produce nitrite. That is, microbes generate nitrite as a by-product, and this becomes available to react with alkaloids to form TSNA. When curing is begun with relatively high humidity at the yellowing phase followed by a pronounced decline as cell breakdown begins, the TSNA content in tobacco will be lower. A uniform air-flow increases the rate and amount of moisture loss from the tobacco, and also reduces possible gas-phase reactions between alkaloids and gaseous nitric oxides.

Microorganisms appear to play a lesser role in TSNA formation in flue-cured tobacco. Morin *et al.* (2004) found that microbial populations were inversely correlated with concentrations of TSNA and with temperature. However, TSNA concentrations increased as temperature increased during curing. This finding supports the hypothesis that TSNA could result from the reaction of combustion gases (e.g. nitrogen oxides) with tobacco alkaloids during flue-curing. Removal of heating with propane as part of the curing process has been shown to reduce the levels of NNK and NNN substantially (Peele *et al.*, 2001; IARC, 2004).

In summary, a lesser degree of fertilization, particularly with products that contain nitrates, and careful manipulation of curing parameters and tobacco blending can lower the level of nitrosamines in tobacco products.

### 1.3.1 *Fresh tobacco*

The mean concentrations of NNK, NNN and NAT in the green leaves harvested at all stalk positions from the flue-cured tobacco plant NC-95 were 280, 260 and 790 ng/g dry tobacco, respectively (Djordjevic *et al.*, 1989b), and were six times higher in cured tobacco (namely 1810, 1560 and 6670 ng/g dry tobacco, respectively).

Bhide *et al.* (1987) reported the presence of NNK and NNN in green leaves of *N. tabacum* and *N. rustica* species grown in India in two different seasons. In one season, mature green leaves of *N. rustica* contained up to 2340, 46 100, 5200 and 23 700 ng/g tobacco (dry wt) NNK, NNN, NAB and NAT, respectively. One year later, tobacco harvested at the same location contained 352 ng/g tobacco NNK and 5730 ng/g tobacco NNN. These levels rose to 25 800 ng/g tobacco NNK and 15 000 ng/g tobacco NNN in sun-dried tobacco. In comparison, the levels of TSNA in sun-dried *N. tabacum* species grown in the same area during the same seasons were 37 ng/g tobacco NNK and 49 ng/g tobacco NNN.

### 1.3.2 *Cured tobacco*

A wide range of TSNA concentrations are found in cured tobacco, regardless of the type (Table 1). In each category of tobacco type, the range reflects the diversity of the tobacco variety, production year, climate, country of origin, agricultural practices including fertilization, post-harvesting and curing technologies, post-curing handling and storage conditions, as well as the analytical methods used and the reporting of the analytical results (e.g. ng/g dry tobacco wt versus ng/g wet tobacco wt).

The levels of total TSNA are highest in air-cured Burley tobacco and lowest in sun-cured Oriental (Turkish) tobacco. The highest levels of NNN were reported in Burley laminae and midribs (up to 8620 and 9080 ng/g dry tobacco, respectively). The highest reported concentrations of NNN were 1700 ng/g dry wt in flue-cured Bright tobacco and 420 ng/g dry wt in sun-cured Oriental tobacco. MacKown *et al.* (1988) also reported levels of NNN up to 3400 ng/g dry wt in reconstituted tobacco sheets that are used in cigarette blends.

The highest levels of NNK were reported in midribs of Burley tobacco (6660 ng/g dry tobacco) and laminae of Bright (Virginia) tobacco (2690 ng/g dry tobacco). It should be noted that levels of NNK in Burley midribs exceed those in the laminae (6600 versus 1370 ng/g dry wt). It should also be noted that NNK is a predominant TSNA in Bright tobacco (2690 ng NNK compared with 1370 ng NNN) while NNN is predominant in Burley tobacco (1370 ng NNK compared with 8620 ng NNN). Similarly to NNN, NAB and NAT are formed by nitrosation of a respective secondary amine alkaloid precursor

**Table 1. The concentration ranges of tobacco-specific *N*-nitrosamines in cured tobacco produced worldwide**

Tobacco type	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Oriental (Turkish)	Dry	ND–83 <sup>b</sup>	20–420	50	20–170		Djordjevic <i>et al.</i> (1991); Morgan <i>et al.</i> (2004)
	Wet	ND–70 <sup>c</sup>	20–460			20–200	Fischer <i>et al.</i> (1989a)
Bright (Virginia)	Dry	160–2690	240–1700	20–150	280–6670		Djordjevic <i>et al.</i> (1989b); Risner <i>et al.</i> (2001); Morgan <i>et al.</i> (2004)
	Wet	30–1100	10–600			30–950	Fischer <i>et al.</i> (1989a)
Burley (laminae)	Dry	ND–1370	1070–8620	200–223	4270–19 700		MacKown <i>et al.</i> (1988); Burton <i>et al.</i> (1989a); Risner <i>et al.</i> (2001); Morgan <i>et al.</i> (2004)
	Wet	100–1400	1300–8850			500–3600	Fischer <i>et al.</i> (1989a)
Burley (stems/midrib)	Dry	ND–6660	1500–9080	155	1800–20 940		MacKown <i>et al.</i> (1988); Burton <i>et al.</i> (1989a); Morgan <i>et al.</i> (2004)
Reconstituted tobacco sheets	Dry	490	3400		2500		MacKown <i>et al.</i> (1988)

NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; ND, not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosonornicotine

<sup>a</sup> Dry, ng/g dry wt; wet, ng/g wet wt

<sup>b</sup> Detection limit for NNK, < 10 ng/g tobacco

<sup>c</sup> Detection limit for NNK, < 50 ng/g tobacco

(Figure 1), and their accumulation in tobacco follows that of NNN: the highest concentrations were reported in Burley tobacco and the lowest in Oriental tobacco.

Some authors did not report concentrations of NAB and NAT separately but as a sum (Fischer *et al.*, 1989a). In some reports, TSNA values are expressed per dry tobacco wt while values are not adjusted for the moisture content in others. The variability of the data thus reflects not only diversity of tobacco specimens but also of analytical methods and instrumentation. There is an urgent need for standardization and validation of analytical methods for measurements of TSNA in tobacco, including reporting of results, to permit a meaningful comparison of data.

### 1.3.3 Cigarette tobacco

Table 2 presents a comparison of concentrations of NNK, NNN, NAB and NAT in tobacco from commercial cigarettes sold worldwide. NNK ranged from not detected to 10 745 ng/cigarette in cigarettes sold in Italy (Fischer *et al.*, 1990a). NNK was not detected in some cigarettes sold in the Central Europe, Middle East and Africa region, the European Union and the former USSR (Fischer *et al.*, 1990a; Djordjevic *et al.*, 1991; Counts *et al.*, 2005). High concentrations of NNK were also reported for cigarettes sold in India (4800 ng/g dry tobacco), the USA (1760 ng/g wet tobacco) and France (1530 ng/g dry tobacco).

NNN ranged from 20 ng/g dry tobacco for cigarettes sold in the former USSR (Djordjevic *et al.*, 1991) to 58 000 ng/g dry tobacco for cigarettes from India (Nair *et al.*, 1989). High concentrations of NNN were also reported in cigarettes sold in France (18 600 ng/g dry tobacco), the USA (up to 7900 ng/g dry tobacco), Germany (up to 5340 ng/cigarette), Poland (up to 4870 ng/g dry tobacco), Japan (up to 3892 ng/g dry tobacco), Norway (3736 ng/g dry tobacco) and Malaysia (up to 3350 ng/g dry tobacco).

NAB ranged from not detectable levels to 322 ng/g dry tobacco. The highest amount was measured in Philip Morris cigarettes marketed in the Central Europe, Middle East and Africa region (Counts *et al.*, 2005).

NAT ranged from 20 to 15 100 ng/g dry tobacco. The lowest concentrations were reported for cigarettes from the former USSR (Fisher *et al.*, 1990a; Djordjevic *et al.*, 1991) and the highest concentration for an Indian cigarette (Nair *et al.*, 1989). High levels of NAT were also reported in cigarettes from France, the Central Europe, Middle East and Africa region, the European Union, Japan, Taiwan (China) and the USA.

Ashley *et al.* (2003) measured TSNA in tobacco from cigarettes purchased in 21 countries. US brands of cigarettes marketed worldwide generally had higher levels than popular local cigarettes in many countries.

Higher TSNA concentrations were generally measured in the tobacco from non-filter cigarettes, especially those made of dark tobacco (Fischer *et al.*, 1989a; Tricker *et al.*, 1991). Among the 55 brands sold in Germany in 1987 (Table 3), the lowest amounts of NNK and NNN were measured in cigarettes made from Oriental tobacco (NNK, not detectable to 177 ng/cigarette; NNN, 45–432 ng/cigarette), followed by cigarettes made with Virginia tobacco (NNK, 170–580 ng/cigarette; NNN, 133–330 ng/cigarette) and

**Table 2. International comparison of the concentration ranges for preformed tobacco-specific *N*-nitrosamines in tobacco from commercial cigarettes**

Country	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Argentina	Dry	812	1866	357	1559		Counts <i>et al.</i> (2005)
Australia	Dry	490–1193	420–2888	NQ <sup>b</sup> –207	715–2366		Counts <i>et al.</i> (2005)
Austria	ng/cig	92–310	306–1122				Fischer <i>et al.</i> (1990a)
Belgium	ng/cig	219–594	504–1939				Fischer <i>et al.</i> (1990a)
Canada	ng/cig	447–884	259–982			564–1017	Fischer <i>et al.</i> (1990b)
CEMA	Dry	NQ <sup>b</sup> –1127	1094–3739	NQ <sup>b</sup> –322	1014–2989		Counts <i>et al.</i> (2005)
European Union	Dry	NQ <sup>b</sup> –860	332–2736	NQ <sup>b</sup> –262	423–2253		Counts <i>et al.</i> (2005)
France	Dry ng/cig	260–1530 57–990	4770–18 600 120–6019	100	1200–9970		Djordjevic <i>et al.</i> (1989a); Ohshima <i>et al.</i> (1985); Fischer <i>et al.</i> (1990a)
Germany	ng/cig Dry	ND–1120 <sup>c</sup> 445–469	45–5340 1355–1361	NQ <sup>b</sup>	1142–1207	ND–2490 <sup>c</sup>	Fischer <i>et al.</i> (1989a, 1990a); Tricker <i>et al.</i> (1991); Counts <i>et al.</i> (2005)
India	Dry ng/cig	40–4800 19–174	1300–58 000 68–730		800–15 100	98–519 <sup>c</sup>	Nair <i>et al.</i> (1989); Kumar <i>et al.</i> (1991)
Italy	ng/cig	153–10 745	632–12 454				Fischer <i>et al.</i> (1990a)
Japan	Dry	190–1171	360–3892	NQ <sup>b</sup> –320	300–3139		Djordjevic <i>et al.</i> (2000a); Counts <i>et al.</i> (2005)
Malaysia	Dry	434–923	2223–3350	NQ <sup>b</sup> –176	1302–2170		Counts <i>et al.</i> (2005)
Moldova	Wet	104–942	93–2090	ND–75	55–1290		Stepanov <i>et al.</i> (2002)
Netherlands	ng/cig	105–587	58–1647				Fischer <i>et al.</i> (1990a)
Norway	Dry	1124	3736	379	2945		Counts <i>et al.</i> (2005)

**Table 2 (contd)**

Country	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Poland	Dry ng/cig	70–660 140–450	670–4870 870–2760				Fischer <i>et al.</i> (1990a); Djordjevic <i>et al.</i> (2000b)
Sweden	ng/cig	192–569	544–1511				Fischer <i>et al.</i> (1990a)
Switzerland	ng/cig	450–554	1280–2208				Fischer <i>et al.</i> (1990a)
Taiwan	Dry	1194	3769	195	2883		Counts <i>et al.</i> (2005)
United Kingdom	ng/cig	92–433	140–1218				Fischer <i>et al.</i> (1990a)
USA	Dry Wet ng/cig	420–1270 1410–1760 433–733	880–7900 2590–4300 993–1947	ND–212 100–140	880–5810 1610–2660		Ohshima <i>et al.</i> (1985); Djordjevic <i>et al.</i> (1990); Fischer <i>et al.</i> (1990a); Djordjevic <i>et al.</i> (2000a); Stepanov <i>et al.</i> (2002); Counts <i>et al.</i> (2005)
Former USSR	Dry ng/cig	ND–40 <sup>d</sup> ND–150 <sup>c</sup>	20–420 60–850		20–170		Fischer <i>et al.</i> (1990a); Djordjevic <i>et al.</i> (1991)
Venezuela	Dry	591	2170	244	1339		Counts <i>et al.</i> (2005)

Adapted from IARC (2004)

CEMA, Central Europe, Middle East, Africa; NAB, *N*'-nitrosoanabasine; NAT, *N*'-nitrosoanatabine; ND, not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*'-nitrosornicotine; NQ, not quantifiable

<sup>a</sup> Dry, ng/g dry tobacco; ng/cig, ng/cigarette; wet, ng/g wet tobacco

<sup>b</sup> Limit for quantitation; NNK, 272 ng/g; NNN, 180 ng/g; NAB, 103 ng/g; NAT, 213 ng/g

<sup>c</sup> Detection limit for NNK, < 50 ng/cigarette; for NNN and for NAB + NAT < 25 ng/cigarette

<sup>d</sup> Detection limit for NNK, < 10 ng/g tobacco

**Table 3. International comparison of the concentration ranges of preformed tobacco-specific *N*-nitrosamines in tobacco from commercial cigarettes with a wide range of ISO/FTC nicotine and ‘tar’ mainstream smoke yields<sup>a</sup>**

Country (total no. of cigarette brands in the study)	Reported as <sup>b</sup>	Tobacco filler	F/NF	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Canada (25)	ng/cig	Ultra-low yield (V)	F	447–785	288–982			666–1017	Fischer <i>et al.</i> (1990b)
		Low yield (V)	F	510–884	292–527			586–978	
		Moderate yield (V)	F	569–705	337–407			666–779	
		High yield (V)	F	495–663	259–381			564–758	
Germany (20)	ng/cig	Blend	F	100–410	400–1390			220–1340	Tricker <i>et al.</i> (1991)
		Blend	NF	270–500	660–2670			460–1110	
		Dark	NF	800–960	4500–5340			1650–2330	Fischer <i>et al.</i> (1989a,b)
		Oriental	F + NF	ND–177	45–432			ND–575	
		Virginia	F + NF	170–580	133–330			253–630	
		American blend	F	160–696	500–2534			440–2490	
(55)		Dark	NF	370–1120	3660–5316			266–315	
Japan (6)	Dry	Low yield	F	190–330	810–1110	30–60	410–660		Djordjevic <i>et al.</i> (2000a)
		Medium yield	F	200–320	360–1040	30–70	300–620		
USA (13)	Dry	Ultra-low yield (AB)	F	500–580	1750–1980	ND	970–1080		Djordjevic <i>et al.</i> (1990, 2000a)
		Low yield (AB)	F	490–800	1900–3050	90–120	1030–1670		
		Moderate yield (AB)	F	420–890	1780–2890	70–110	1030–1680		
		High yield (AB)	NF	770–920	1290–2160	40–110	920–1170		
(11)	Wet	Quest 1,2,3 <sup>c</sup>	F	54–190	820–930	3–13	43–310		Stepanov <i>et al.</i> (2006a)
		Ultra-low yield (AB)	F	750–770	2800–2900	55–58	1100–1200		
		Low yield (AB)	F	550–680	2700–2800	51–61	1100–1300		
		Moderate yield (AB)	F	580–960	1100–2900	25–100	560–2300		

**Table 3 (contd)**

Country (total no. of cigarette brands in the study)	Reported as <sup>b</sup>	Tobacco filler	F/NF	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Philip Morris commercial brands (39)	Dry	Ultra-low yield (AB)	F	NQ <sup>d</sup> -1171	420-3892	NQ <sup>d</sup> -262	715-3139		Counts <i>et al.</i> (2005)
		Low yield (AB)	F	NQ <sup>d</sup> -1270	332-3438	NQ <sup>d</sup> -322	423-2676		
		Moderate yield (AB)	F	NQ <sup>d</sup> -1194	868-3769	NQ <sup>d</sup> -379	778-2945		

AB, American blend cigarettes; F, filter-tipped cigarettes; NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; ND, not detected; NF, non-filtered cigarettes; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosornicotine; NQ, not quantifiable; V, Virginia-type cigarettes

<sup>a</sup> Cigarettes were designated into classes based either on ISO/FTC nicotine smoke yields (Djordjevic *et al.*, 2000a) or ISO/FTC 'tar' smoke yields (IARC, 1986).

<sup>b</sup> Dry, ng/g dry tobacco; ng/cig, ng/cigarette; wet, ng/g wet tobacco

<sup>c</sup> Nicotine-reduced cigarettes

<sup>d</sup> Limit for quantitation: NNK, 272 ng/g; NAB, 103 ng/g

American blend cigarettes (NNK, 160–696 ng/cigarette; NNN, 500–2534 ng/cigarette). The highest TSNA levels were reported in the dark tobacco cigarettes (NNK, 370–1120 ng/cigarette; NNN, 3660–5316 ng/cigarette) (Fischer *et al.*, 1989a,b; Tricker *et al.*, 1991).

Tobacco from ultra-low-, low-, medium- and high-yield cigarettes sold internationally, as ranked based on data obtained by the ISO/FTC (International Standardization Organization/Federal Trade Commission) machine-smoking method (IARC, 2004), contain similar amounts of preformed NNK and NNN regardless of the type of product (Table 3; Djordjevic *et al.*, 1990; Fischer *et al.*, 1990b; Djordjevic *et al.*, 2000a; Counts *et al.*, 2005; Stepanov *et al.*, 2006a). For example, levels of NNK and NNN in the tobacco of Canadian ultra-low-yield cigarettes ranged from 447 to 785 ng/cigarette and from 288 to 982 ng/cigarette, respectively, whereas high-yield cigarettes contained 495–663 ng/cigarette NNK and 259–381 ng/cigarette NNN (Fischer *et al.*, 1990b). Similar observation was reported for American blend cigarettes sampled in the USA (Djordjevic *et al.*, 1990, 2000a; Stepanov *et al.*, 2006a) or internationally (Counts *et al.*, 2005). Tobacco from Canadian brands with a wide range of nicotine and tar yields as measured by the ISO/FTC method contained lower amounts of preformed NNN (up to 982 ng/cigarette; Fischer *et al.*, 1990b) than US brands sold domestically or internationally (up to 3050 and 3892 ng/g dry tobacco, respectively; Djordjevic *et al.*, 1990, 2000a; Counts *et al.*, 2005). In contrast, NNK content in cigarette tobacco was of the same order of magnitude in the two countries (up to 884 ng/cigarette in Canadian cigarettes and up to 920 ng/cigarette in cigarettes sold in the USA). However, Philip Morris American blend cigarettes marketed worldwide contained the highest quantities of both NNK and NNN (up to 1270 and 3892 ng/g dry tobacco, respectively). Japanese and German cigarettes contained the lowest concentrations of preformed NNK (up to 330 ng/g dry tobacco).

Typically, levels of NNK in a given cigarette blend are lower than those of NNN except in cigarettes manufactured in Canada, the United Kingdom and Australia that use Bright Virginia flue-cured tobacco as a filler; in the latter brands, NNK is the dominant TSNA (Fischer *et al.*, 1989a,b, 1990b).

Cigarettes manufactured and marketed globally contain a wide range of TSNA. The dose of TSNA delivered in mainstream smoke is largely determined by their levels in the tobacco blend (Fischer *et al.*, 1990c; d'Andres *et al.*, 2003). In addition, exposure to TSNA depends on the relationship between the product and how it is used, that is, on smoking patterns and intensity (Djordjevic *et al.*, 2000c; Hecht *et al.*, 2005; Melikian *et al.*, 2007a,b).

### 1.3.4 Mainstream cigarette smoke

Monitoring of tobacco smoke has historically been limited to the measurement of carbon monoxide emissions from cigarettes using the ISO/FTC machine-smoking protocol, which does not reflect the characteristics of human smoking. During the past two decades, many studies have reported the occurrence of TSNA in mainstream smoke. Table 4

**Table 4. International comparison of the concentration ranges (ng/cigarette) of tobacco-specific N-nitrosamines in the mainstream smoke of commercial cigarettes (ISO/FTC machine-smoking method)**

Country	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Argentina	79.5	99.6	14.5	106.9		Counts <i>et al.</i> (2005)
Australia	12.4–106.1 25.7 <sup>a,b</sup>	5.0–151.8 22.4 <sup>a,b</sup>	2.0–22.3	8.0–134.7		Counts <i>et al.</i> (2005) King <i>et al.</i> (2007)
Austria	12–100	42–172				Fischer <i>et al.</i> (1990a)
Belgium	29–150	38–203				Fischer <i>et al.</i> (1990a)
Canada	6–97 50.9 <sup>a,c</sup>	4–37 25.6 <sup>a,c</sup>			9–82	Fischer <i>et al.</i> (1990b) King <i>et al.</i> (2007)
CEMA	18.0–75.0	42.5–147.5	6.0–18.5	38.6–129.2		Counts <i>et al.</i> (2005)
European Union	12.7–78.4	14.8–126.0	2.0–17.9	18.0–106.8		Counts <i>et al.</i> (2005)
Germany	ND <sup>d</sup> –470	5–855	6.6–9.0	39.9–52.6	6.6–520	Fischer <i>et al.</i> (1989b, 1990a); Tricker <i>et al.</i> (1991); Counts <i>et al.</i> (2005)
France	18.3–498	11–1000	15.4–18.7	127–182		Djordjevic <i>et al.</i> (1989a); Fischer <i>et al.</i> (1990a)
India	TR–73	6–401		3.8–99.4	18–146	Nair <i>et al.</i> (1989); Kumar <i>et al.</i> (1991)
Italy	8–1749	21–1353				Fischer <i>et al.</i> (1990a)
Japan	18.6–87.1	36–146	6.8–21	40.3–138		Djordjevic <i>et al.</i> (1996); Counts <i>et al.</i> (2005)
Malaysia	45.0–87.3	63.4–195.3	7.8–28.5	55.7–153.3		Counts <i>et al.</i> (2005)
Netherlands	5–102	9–163				Fischer <i>et al.</i> (1990a)
Norway	103.9	189.4	18.6	160.4		Counts <i>et al.</i> (2005)
Poland	36–990	68–2830				Fischer <i>et al.</i> (1990a); Gray <i>et al.</i> (1998); Djordjevic <i>et al.</i> (2000b)

**Table 4 (contd)**

Country	NNK	NNN	NAB	NAT	NAB + NAT	Reference
Sweden	27–84	44–141				Fischer <i>et al.</i> (1990a)
Switzerland	69–124	121–226				Fischer <i>et al.</i> (1990a)
Taiwan (China)	96.2	161.2	24.7	142.8		Counts <i>et al.</i> (2005)
Thailand	16–369	28–730			43.5–483	Brunnemann <i>et al.</i> (1996)
United Kingdom	5.2–500	6.5–257.6	1.1–44.2	7.8–148		Fischer <i>et al.</i> (1990a); Gregg <i>et al.</i> (2005)
USA	3–425	6–1007	ND–34.7	6–250	102–744	Adams <i>et al.</i> (1987a); Fischer <i>et al.</i> (1990a); Djordjevic <i>et al.</i> (1990); Brunnemann <i>et al.</i> (1994); Djordjevic <i>et al.</i> (1996)
Former USSR	4–55	23–389		71–196		Fischer <i>et al.</i> (1990a); Djordjevic <i>et al.</i> (1991)
Venezuela	64.2	100.1	13.3	78.6		Counts <i>et al.</i> (2005)

Adapted from IARC (2004)

CEMA, Central Europe, Middle East, Africa; NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; ND, not detected; NNK, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosoanornicotine; TR, trace amounts

<sup>a</sup> Reported as ng/mg tobacco

<sup>b</sup> Mean of 15 brands

<sup>c</sup> Mean of 21 brands

<sup>d</sup> Limit of detection for NNK, 40 ng/cigarette

presents an international comparison of TSNA concentration ranges in mainstream smoke of commercial cigarettes using the ISO/FTC machine-smoking method.

Similarly to cigarette tobacco filler, the levels of individual TSNA in mainstream smoke vary dramatically among products regardless of the country of origin. The emissions of NNK ranged from undetected to 1749 ng/cigarette (Italy; Fischer *et al.*, 1990a). The concentrations of NNN ranged from 4 ng/cigarette (Canada) to 2830 ng/cigarette (Poland; Gray *et al.*, 1998). The highest emissions of NNN were reported in the mainstream smoke of cigarettes sold in Germany (855 ng/cigarette), France (1000 ng/cigarette), the USA (1007 ng/cigarette), Italy (1353 ng/cigarette) and Poland (2830 ng/cigarette). The concentrations of NAB ranged from undetected to 44.2 ng/cigarette and those of NAT from 6 to 250 ng/cigarette.

The lowest emissions of TSNA were measured in the mainstream smoke from blended cigarettes sold in Australia, Austria, Canada, Japan, the Netherlands and Sweden, with upper values of 66–106 ng NNK. Surprisingly, levels of NNK and NNN in the mainstream smoke of two cigarette brands from India were very low despite the extremely high levels of preformed nitrosamines in tobacco (Nair *et al.*, 1989).

A comparative assessment of the composition of mainstream smoke from three popular brands of filter-tipped cigarette from the USA that were purchased on the open market in 29 countries worldwide showed from three- to ninefold differences in the yields of NNK and NNN within each brand. Yields of NNK and NNN were highly correlated ( $r = 0.88$ ; Gray *et al.*, 2000).

The parameters that affect smoke yields and compositions have recently been reviewed (Hoffman *et al.*, 2001; Borgerding & Klus, 2005). Measurements of smoke yields using the ISO/FTC machine-smoking method do not provide information that is representative of the exposure of a smoker. Machine-smoking protocols other than the ISO/FTC protocol have been examined, particularly those that have more intense puffing parameters which block some or all of the ventilation holes in cigarette filters. Examples include those developed by the Massachusetts Department of Public Health (MDPH) and by Health Canada (IARC, 2004). Table 5 depicts the differences in TSNA emissions in the mainstream smoke generated by machine smoking using the ISO/FTC, MDPH and Health Canada protocols. Thirty-nine Phillip Morris cigarettes marketed globally (Counts *et al.*, 2005) were grouped into three categories (moderate-yield, low-yield and very low-yield) based on the ISO/FTC emissions of tar, nicotine and carbon monoxide (IARC, 1986, 2004). The range of levels of NNK, NNN, NAB, NAT and total TSNA in the emissions of each product category was wide regardless of the machine-smoking protocol. The levels of emissions within each protocol increased from very low-yield cigarettes to moderate-yield cigarettes. Since the tobaccos in each product category contain similar amounts of preformed TSNA per gram of tobacco (Table 3), the differences in emission levels are due solely to the characteristics of the cigarette design such as the amount of tobacco, length and circumference of a cigarette, perforation of the cigarette and filter paper, and the type of material used for filtration (Hoffmann & Hoffmann, 2001; Hoffmann *et al.*, 2001; IARC, 2004; Borgerding & Klus, 2005; Counts *et al.*, 2005). It was calculated that, compared with the ISO/FTC conditions,

the MDPH method produces up to 2.2-fold higher yields of total TSNA for moderate-yield cigarettes, 2.7-fold higher yields for low-yield brands and threefold higher yields for ultra-low-yield brands. The ratios for the emissions using the Health Canada protocol follow the same trend but are somewhat higher (up to 2.4-, 3.4- and 5.2-fold, respectively) because of complete blockage of filter ventilation holes. Overall, MDPH and Health Canada protocols generally produce higher yields per cigarette and reduce the differences between brands in the yields.

**Table 5. Comparison of the concentration ranges<sup>a</sup> of tobacco-specific *N*-nitrosamines (TSNA) in the mainstream smoke of 39 Philip Morris commercial brands marketed globally**

Measurement conditions <sup>b</sup>	Cigarette type <sup>c</sup>		
	Moderate-yield	Low-yield	Very low-yield
<b>ISO-condition</b>			
NNK (ng/cig)	27.7–107.8	12.7–75	18–53.9
NNN (ng/cig)	48.1–195.3	14.8–147.5	5–103.5
NAB (ng/cig)	6–28.5	2–18.5	2–16.3
NAT (ng/cig)	44.1–160.4	18–129.2	8–91.2
Total TSNA (ng/cig)	127.1–472.3	47.5–370.2	27.4–264.9
<b>MDPH-condition</b>			
NNK (ng/cig)	60.6–208.6	36.2–150.4	25.8–130.8
NNN (ng/cig)	100.4–374.2	26.5–262.4	16.3–219.7
NAB (ng/cig)	10.5–46.9	4–27.7	5.8–33
NAT (ng/cig)	91.8–295.3	37.5–204.7	31.9–196.3
Total TSNA (ng/cig)	273.5–892.1	104.2–643.4	88.6–571.1
<b>Health Canada-condition</b>			
NNK (ng/cig)	73–263	44.7–171.1	39.1–157.5
NNN (ng/cig)	114.5–410.6	30.6–359.1	20.6–277.8
NAB (ng/cig)	13.8–50.1	5.3–42.8	4.3–33.1
NAT (ng/cig)	105.3–345.1	43.5–283.9	49.3–240.3
Total TSNA (ng/cig)	312.4–1049.8	124.1–852.8	113.3–708.7
<b>Total TSNA ratios</b>			
MDPH/ISO	1.7–2.2	1.7–2.7	2.1–3.0
Health Canada/ISO	1.9–2.5	1.9–3.4	2.7–5.2

From Counts *et al.* (2005)

NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosoornicotine

<sup>a</sup>The measurements are the averages of seven replicates within each machine-smoking method.

<sup>b</sup>Health Canada machine-smoking method; ISO, International Standardization Organization machine-smoking method; MDPH, Massachusetts Department of Public Health machine-smoking method

<sup>c</sup>Cigarettes were designated into classes based on ISO/FTC 'tar' smoke yields (IARC, 1986).

Table 6 presents an overview of the range of TSNA concentrations in mainstream and sidestream smoke of 26 major US cigarette brands as determined using the MDPH machine-smoking protocol. The concentrations of each individual TSNA vary substantially between cigarette brands marketed in the USA; these values are comparable to those reported for Philip Morris American blended cigarettes marketed globally (Table 5; Counts *et al.*, 2005).

**Table 6. Range of concentration of tobacco-specific N-nitrosamines in the mainstream and sidestream smoke of 26 US commercial cigarette brands (MDPH machine-smoking method)**

	Concentration (ng/cigarette)		
	Mainstream	Sidestream	Sidestream/mainstream ratio
NNK	53.5–220.7	50.7–96.7	0.40
NNN	99.9–317.3	69.8–115.2	0.43
NAB	14.2–45.3	11.9–17.8	0.55
NAT	95.2–298.6	38.4–73.4	0.26

From Borgerding *et al.* (2000)

MDPH, Massachusetts Department of Public Health; NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrososornicotine

One study assessed the concentration of NNK in mainstream smoke using the smoking pattern of smokers. Among 133 smokers of low- or medium-yield cigarettes (Djordjevic *et al.*, 2000c), smokers of low-yield brands drew somewhat larger puffs (48.6 versus 44.1 mL) and more smoke both per cigarette (615 mL versus 523 mL) and daily (9.5 L versus 8.2 L) compared with smokers of medium-yield cigarettes; however, concentrations of NNK in mainstream smoke were marginally higher among smokers of medium-yield cigarettes compared with smokers of low-yield brands (250.9 versus 186.5 ng/cigarette).

The most recent report examined whether differences in gender and ethnicity exist in relation to exposure to selected mainstream cigarette smoke constituents as a result of variations in smoking behaviour or type of cigarettes smoked among 129 female and 128 male smokers (Melikian *et al.*, 2007a). Compared with men, women took smaller puffs (37.6 versus 45.8-mL puff;  $p = 0.0001$ ) of shorter duration (1.33 versus 1.48-s puff;  $p = 0.002$ ) but drew more puffs from their cigarettes (13.5 versus 12.0;  $p = 0.001$ ) and smoked less of their cigarette (left 36.3-mm butts or 40.2% of cigarette length versus 34.3-mm butts or 39.2% of cigarette length;  $p = 0.01$ ). The daily dose of smoke was significantly higher in men (9.3 versus 8.0 L;  $p = 0.02$ ). When data were stratified by ethnicity, no difference was found in puffing characteristics between Caucasian American and African-American smokers, except that women and men in the latter group smoked their cigarettes

to an equal length (butt lengths, 34.5 versus 33.9 mm;  $p = 0.93$ ). However, because African-Americans smoked fewer cigarettes, the daily smoke volume was significantly higher among Caucasian American smokers (8.61 versus 7.45 L for women; 10.6 versus 7.8 L for men). The emissions of selected compounds per cigarette, as determined by mimicking human smoking behaviours, were greater among the male smokers than among the female smokers and correlated significantly with delivered smoke volume per cigarette. Cigarettes smoked by women yielded 139.5 ng/cigarette NNK compared with 170.3 ng for men ( $p = 0.0007$ ). The gender differences with regard to cigarette smoke delivery were more profound in Caucasian Americans than in African-Americans. On average, the smoking behaviour of African-American men produced the highest emissions and that of Caucasian American women produced the lowest.

### 1.3.5 *Sidestream cigarette smoke*

Adams *et al.* (1987a) determined the levels of TSNA in the mainstream and sidestream smoke of four different types of US commercial cigarette brands (untipped and filter-tipped, with or without filter perforation) with a wide range of ISO/FTC yields. The highest levels of NNK and NNN were measured in the sidestream smoke of untipped cigarettes (1444 and 857 ng/cigarette, respectively). The amounts of NAB and NAT ranged from 125 to 783 ng/cigarette. It was noted that, with the ISO/FTC machine-smoking method, NNK was the predominant TSNA in sidestream smoke, while NNN was predominant in mainstream smoke. The data obtained in the Massachusetts Benchmark Study (Borgerding *et al.*, 2000) showed that, under more intense smoking patterns that included partial vent blocking, American blend cigarettes released 50.7–96.7 ng/cigarette NNK, 69.8–115.2 ng/cigarette NNN, 11.9–17.8 ng/cigarette NAB and 38.4–73.4 ng/cigarette NAT (Table 6). With more intense puffing and shorter smouldering time, lower amounts of NNK than NNN were released into the ambient air.

Emissions of TSNA from sidestream smoke are significantly lower when cigarettes are smoked using a more intense machine-smoking protocol compared with the standard ISO/FTC method. The explanation for this is that the physical and chemical processes that occur in a burning cigarette change with changing puffing intensity. Moreover, there is less time between puffs to allow for tobacco combustion in the smoldering phase and release of TSNA in ambient air.

For NNK only, the yields in sidestream smoke measured by the Health Canada method were up to 2.6-fold higher than those measured by the ISO method, but only for the 'extra light' and 'ultra light' brands (Government of British Columbia, 2002).

### 1.3.6 *Other smoked tobacco products*

#### (a) *Cigars*

Levels of NNK, NNN and NAT in cigars from the Netherlands were 2850–4250, 6750–53 000 and 4560–20 400 ng/g dry tobacco wt, respectively (Ohshima *et al.*, 1985).

One Indian cigar brand contained 8900 ng/g NNK, 25 000 ng/g NNN and 13 700 ng/g NAT fresh tobacco wt (Nair *et al.*, 1989).

Under standard ISO/FTC machine-smoking conditions, the levels of NNK in the mainstream smoke of premium cigars were 17 times higher than those of medium-yield cigarettes; NNN levels were 22.4 times higher (931 versus 41.5 ng/unit) (Rickert & Kaiserman, 1999).

Djordjevic *et al.* (1997) also reported high emissions of TSNA in the mainstream smoke of small, large and premium cigars generated by a standard ICCSS (International Committee for Cigar Smoke Study) machine-smoking method developed for cigars. Concentrations in the mainstream smoke ranged from 290 to 2490 ng/unit NNK, from 595 to 1225 ng/unit NNN and from 310 to 1145 ng/unit NAT. The NNK emissions from premium cigars exceeded the highest levels reported for cigarettes (see Table 4). Djordjevic *et al.* (1997) also explored the levels of TSNA in the mainstream smoke of small cigars using methods that mimic human smoking patterns and compared the data with those obtained by standard machine-smoking methods. Under human smoking conditions, the emissions of NNK, NNN and NAT were 1.7-fold, 2.1-fold and 1.8-fold higher, respectively, than those obtained under standard protocols.

#### (b) Bidis

*Bidis* originated in India and have been gaining popularity in the USA during the last decade, particularly among adolescents. The levels of preformed NNK, NNN and NAT in tobacco from *bidis* sold in India ranged from 400 to 1400 ng/g, from 6200 to 12 000 ng/g and from 9000 to 12 500 ng/g, respectively, reported as wet tobacco wt (Nair *et al.*, 1989). In the mainstream smoke of these *bidis*, the levels of NNK ranged from not detected to 40 ng/cigarette, those of NNN from 11.6 to 250 ng/cigarette and those of NAT from 9.9 to 175 ng/cigarette. These concentrations were comparable with those measured in the mainstream smoke of Indian cigarettes (Nair *et al.*, 1989; Pakhale & Maru, 1998).

Wu *et al.* (2004) analysed the TSNA content of tobacco filler and mainstream smoke from 14 brands of *bidis* purchased in Atlanta, GA (USA). In *bidi* tobacco filler, the levels of NNK ranged from 90 to 850 ng/g and those of NNN ranged from 150 to 1440 ng/g tobacco. These levels are slightly lower than those in typical American blended cigarettes (see Table 2) and substantially lower than those in *bidis* sold in India. In mainstream smoke from these *bidis*, the levels of NNK ranged from 8.56 to 62.3 ng/cigarette and those of NNN ranged from 2.13–25.9 ng/cigarette.

The wide variation in the TSNA levels most probably reflects the hand-rolled nature of *bidis*, which results in products that have a less homogeneous tobacco content and a wider variation in overall cigarette quality. Since *bidis* contain on average 215.3 mg tobacco versus 738 mg in commercial US cigarettes (Malson *et al.*, 2001), normalization of mainstream smoke emissions of TSNA per milligram of nicotine would give more realistic information on the ultimate levels of exposure to carcinogens (King *et al.*, 2007).

(c) *Chutta tobacco*

The levels of NNK, NNN and NAT in *chutta* tobacco were reported to be extremely high: from 12 600 to 210 300 ng/g, from 21 100 to 295 800 ng/g and from 89 200 to 686 800 ng/g, respectively, reported as fresh tobacco wt. The reverse smoker inhales both the mainstream and sidestream smoke. NNK, NNN and NAT levels in the mainstream smoke of *chutta* ranged from 150 to 2651 ng, from 289 to 1260 ng and from 431 to 1722 ng/*chutta*, respectively (Nair *et al.*, 1989). Stich *et al.* (1992) reported comparable levels of NNK, NNN and NAT in the mainstream smoke of *chutta*: 274–2520, 925–3910 and 141–1300 ng/cigarette, respectively.

(d) *Pipe tobacco*

Ohshima *et al.* (1985) analysed the TSNA content of pipe tobacco from France, the Netherlands and the United Kingdom. The concentrations of NNK ranged from not detected to 1130 ng/g, those of NNN from 3000 ng/g to 6880 ng/g and those of NAT from 1990 to 4850 ng/g dry tobacco wt, respectively. Chamberlain *et al.* (1988) reported levels of 300 ng/g tobacco NNK and 1800 ng/g tobacco NNN in pipe tobacco from the USA. TSNA emissions in the mainstream and sidestream smoke generated by pipe smoking have not been reported to date.

### 1.3.7 *Secondhand tobacco smoke*

The NNN concentrations measured in a poorly ventilated office where heavy smoking of cigarettes, cigars and pipes took place ranged from not detected to 6 ng/m<sup>3</sup> and those of NNK from not detected to 13.5 ng/m<sup>3</sup> (Klus *et al.*, 1992). The upper levels reported by Klus *et al.* (1992) and by Adlkofer *et al.* (1990) for 'heavily smoked rooms' (11 cigarettes smoked in 2 h in a 84-m<sup>2</sup> office) were lower than those measured inside bars, restaurants, trains, a car, an office and a smoker's home: NNN concentrations ranged from not detected to 22.8 ng/m<sup>3</sup> and NNK concentrations ranged between 1.4 and 29.3 ng/m<sup>3</sup> (Brunnemann *et al.*, 1992).

### 1.3.8 *Smokeless tobacco products*

The occurrence of TSNA in smokeless tobacco products is discussed extensively in the monograph on Smokeless tobacco. An international comparison of the concentration ranges in a variety of smokeless tobacco products is presented in Table 7. [TSNA concentrations are customarily expressed in micrograms per gram of dry (or wet) tobacco. To enable the comparison of the TSNA levels in cigarette and smokeless tobacco, the values in this table are expressed in nanograms per gram of dry (or wet) tobacco.] There is a very wide range of concentrations of TSNA in smokeless tobacco, which reflects the product category (e.g. chewing tobacco, moist snuff, dry snuff, new low-TSNA products), product characteristics in each category (e.g. short cut or long cut tobacco, flavourings), tobacco

**Table 7. International comparison of the concentration ranges of tobacco-specific N-nitrosamines in smokeless tobacco products (ng/g tobacco)**

Country	Type of product	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	References
Belgium	Chewing tobacco	Dry	130	7380	NR <sup>b</sup>	970 <sup>c</sup>	Ohshima <i>et al.</i> (1985)
Canada	Moist snuff	Dry	3200–5800	50 400–79 100	4000–4800	152 000–170 000	Brunnemann <i>et al.</i> (1985)
	Chewing tobacco	Dry	240	2090	100	1580	
Denmark	Chewing tobacco	Wet	19–1900	80–1600	30	180–2900	Österdahl <i>et al.</i> (2004)
Germany	Chewing tobacco	Dry	30–300	1420–2300	30–50	330–3700 <sup>b</sup>	Brunnemann <i>et al.</i> (1985); Tricker <i>et al.</i> (1988)
	Dry snuff	Dry	580–6430	2390–18 750	NR	1030–7830	Tricker & Preussmann (1991);
		Wet	100	680	NR	310	Österdahl <i>et al.</i> (2004)
India	Moist snuff	Wet	240	560	20	380	Stepanov <i>et al.</i> (2005)
	Chewing tobacco	Dry	130–600	470–850	30–70	300–500 <sup>b</sup>	Brunnemann <i>et al.</i> (1985); Tricker <i>et al.</i> (1988)
		Wet	2700–6500	15 300–24 400	NR	10 000–44 600	Nair <i>et al.</i> (1989)
	<i>Zarda</i>	Dry	220–24 100	400–79 000	NR <sup>b</sup>	780–99 100 <sup>c</sup>	Tricker & Preussmann (1988); Tricker <i>et al.</i> (1988)
		Wet	1070–3090	4810–19 900	190–1190	640–1980	Stepanov <i>et al.</i> (2005)
		NR		6550–7360			Gupta (2004)
	<i>Mishri</i>	Dry	294–1100	300–6995	NR	488–14 151	Tricker <i>et al.</i> (1988); Nair <i>et al.</i> (1987)
		Wet	870	4210	150	2550	Stepanov <i>et al.</i> (2005)
		NR		4020–4470			Gupta (2004)
	<i>Khiwam</i>	Dry	100–1030	2500–8950	NR <sup>b</sup>	1830–10 360 <sup>c</sup>	Tricker & Preussmann (1989)
	<i>Khaini</i>	Dry	110–5290	25 800–40 000	1240–2480	660–18 800	Stich <i>et al.</i> (1992)
		Wet	2340–28 400	39 400–76 900	3870–8830	4830–13 800	Stepanov <i>et al.</i> (2005)
	<i>Gutka</i>	Wet	40–430	90–1090	ND–50	10–80	Stepanov <i>et al.</i> (2005)
		NR	10 680–11 510	1870–5730	5750–6890	6270–6530	Gupta (2004)
	<i>Supari</i>	Wet	ND	ND	ND	ND	Stepanov <i>et al.</i> (2005)
		NR	4900–11 580	1920–2450	11 580–12 580	3600–4570	Gupta (2004)

**Table 7 (contd)**

Country	Type of product	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	References
India (contd)	Creamy snuff/ toothpaste	Wet	1310–12 500	2520–48 700	70–110	530–26 600	Stepanov <i>et al.</i> (2005); Nair <i>et al.</i> (1989) Gupta (2004)
			4380–4880				
	Snuff for inhalation	Wet	245 500	1 356 000	NR	1 857 000	Nair <i>et al.</i> (1989)
	Toothpowder <i>Tuibur</i>	Wet	ND	ND–40	ND	ND	Stepanov <i>et al.</i> (2005)
		NR		19 650–20 120			Gupta (2004)
Other tobacco	Wet	80–2610	1740–19 200	12–1570	350–11 900	Stepanov <i>et al.</i> (2005)	
Norway	Moist snuff	Wet	3300	21 000	1700	13 000	Österdahl <i>et al.</i> (2004)
South Africa	Low-TSNA moist snuff	Dry	270–290	1050–2070	90–110	890–1520	Brunnemann <i>et al.</i> (2004)
Sudan	<i>Toombak</i>	Dry	188 000–7 870 000	141 000–3 080 000	13 900–2 370 000	20 000–290 000	Idris <i>et al.</i> (1991); Prokopczyk <i>et al.</i> (1995)
Sweden	Moist snuff	Dry	190–2950	1120–154 000	40–1700	900–21 400 <sup>c</sup>	Brunnemann <i>et al.</i> (1985); Ohshima <i>et al.</i> (1985); Tricker <i>et al.</i> (1988); Hoffmann <i>et al.</i> (1991); Tricker & Preussmann (1991); Brunnemann & Hoffmann (1992); Djordjevic <i>et al.</i> (1993a); Connolly (2001) Österdahl & Slorach (1988); Österdahl <i>et al.</i> (2004)
		Wet	190–1300	490–4400	30–170	320–3500	
	Low-TSNA moist snuff	Wet	30–360	150–2300	10–130	60–980	Österdahl <i>et al.</i> (2004); Stepanov <i>et al.</i> (2006a)
	Chewing tobacco	Wet	10–460	700–1700	ND	1100–2100	Österdahl <i>et al.</i> (2004)
Thailand	Chewing tobacco	Dry	100	500	NR <sup>b</sup>	500 <sup>c</sup>	Tricker <i>et al.</i> (1988)

**Table 7 (contd)**

Country	Type of product	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	References
United Kingdom	Moist snuff	Dry	400–13 000	1100–52 000	86	2000–6500 <sup>c</sup>	Hoffmann <i>et al.</i> (1988); Brunnemann & Hoffmann (1992)
	Chewing tobacco	Dry	300	900	NR	1500 <sup>c</sup>	Tricker <i>et al.</i> (1988)
	Dry snuff	Dry	580–4300	2390–16 000	NR	1030–7830	Tricker & Preussmann (1991); Brunnemann & Hoffmann (1992)
USA	Moist snuff	Wet	260	1800	NR	820	Österdahl <i>et al.</i> (2004)
		Dry	ND–18 000	ND–147 000	20–10 670	240–339 000	Brunneman <i>et al.</i> (1985); Ohshima <i>et al.</i> , 1985; Adams <i>et al.</i> (1987b); Brunnemann <i>et al.</i> (1987a,b); Chamberlain <i>et al.</i> (1988); Tricker <i>et al.</i> (1988); Andersen <i>et al.</i> (1989); Djordjevic <i>et al.</i> (1989a); Hoffmann <i>et al.</i> (1991); Brunnemann & Hoffmann (1992); Prokopczyk <i>et al.</i> (1992); Djordjevic <i>et al.</i> (1993a); Hoffmann <i>et al.</i> (1995); Prokopczyk <i>et al.</i> (1995); Connolly (2001); Brunnemann <i>et al.</i> (2002, 2004)
	Low-TSNA moist snuff	Wet	60–13 000	710–63 000	14–2800	240–83 000	Österdahl <i>et al.</i> (2004); Stepanov <i>et al.</i> (2006a)
		Wet	32–33	620–640	17–18	310–320	Stepanov <i>et al.</i> (2006a)

**Table 7 (contd)**

Country	Type of product	Reported as <sup>a</sup>	NNK	NNN	NAB	NAT	References
USA (contd)	Chewing tobacco	Dry	ND–1100	670–6500	20–140	670–12 400	Brunnemann <i>et al.</i> (1985); Chamberlain <i>et al.</i> (1988); Andersen <i>et al.</i> (1989); Djordjevic <i>et al.</i> (1989a); Brunnemann & Hoffmann (1992)
	Dry snuff	Wet	80–110	250–1100	20	150–940	Österdahl <i>et al.</i> (2004)
		Dry	880–84 400	9370–116 100	520–1530	11 200–238 800 <sup>c</sup>	Adams <i>et al.</i> (1987); Brunnemann <i>et al.</i> (1987a); Andersen <i>et al.</i> (1989); Djordjevic <i>et al.</i> (1989a)
	Compressed tobacco lozenges	NR	37–43	19–56	7–8	12–17	Stepanov <i>et al.</i> (2006a)
Uzbekistan	<i>Naswar</i>	Dry	20–130	120–520	8–30	32–300	Brunnemann <i>et al.</i> (1985)

NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; ND, not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosoanornicotine; NR, not reported

<sup>a</sup> Reported as ng/g dry wt (dry) or wet wt (wet) tobacco

<sup>b</sup> The upper value reported as NAT also includes NAB.

<sup>c</sup> The upper value includes NAB + NAT.

blend, tobacco curing and manufacturing technologies, ageing, country of origin, year of production and analytical methods.

Because of the prohibition of the marketing of moist snuff in the European Union except in Sweden and in the EFTA (European Free Trade Association) country Norway (see the monograph on Smokeless tobacco, Section 1.5.3), data on the TSNA content of smokeless tobacco products marketed in the European countries beyond 1992 are limited. Chewing tobacco contained the lowest amounts of TSNA: NNK ranged from undetectable levels (USA) to 1900 ng/g wet wt (Denmark); NNN ranged from 80 ng/g wet wt (Denmark; Österdahl *et al.*, 2004) to 7380 ng/g dry wt (Belgium; Ohshima *et al.*, 1985); NAB ranged from undetectable levels to 140 ng/g dry wt (USA) and NAT ranged from 150 ng/g wet wt to 12 400 ng/g dry wt (USA).

The levels of NNK, NNN, NAB and NAT in moist snuff were, in general, substantially higher than those in chewing tobacco; upper concentrations reached 18 000 ng/g dry tobacco NNK (USA; Connolly, 2001), 154 000 ng/g dry tobacco NNN (Sweden; Ohshima *et al.*, 1985), 10 670 ng/g dry tobacco NAB (USA; Brunnemann & Hoffmann, 1992) and 339 000 ng/g dry tobacco NAT (USA; Ohshima *et al.*, 1985). Levels in Indian moist snuff were surprising low (Stepanov *et al.*, 2005).

High levels of TSNA in dry snuff were also measured in the USA: NNK, up to 84 400 ng/g dry tobacco; NNN, up to 116 100 ng/g dry tobacco; NAB, up to 1530 ng/g dry tobacco and NAT, up to 238 000 ng/g dry tobacco. In Indian snuff used for inhalation, TSNA levels were even higher: 245 000 ng/g fresh tobacco NNK, 1 356 000 ng/g fresh tobacco NNN and 1 875 000 ng/g fresh tobacco NAT (Nair *et al.*, 1989).

Although there has been a decline in the concentrations of TSNA in smokeless tobacco products in Sweden and the USA since the 1980s (Djordjevic *et al.*, 1993b; Brunnemann *et al.*, 2004; Österdahl *et al.*, 2004), the trend may not apply to other products and countries. For example extremely high levels (in milligrams) of TSNA were measured in *toombak* (Idris *et al.*, 1991; Prokopczyk *et al.*, 1995; Idris *et al.*, 1998): NNK, up to 7 870 000 ng/g dry tobacco; NNN, up to 3 085 000 ng/g dry tobacco; NAB, up to 237 000 ng/g dry tobacco and NAT, up to 290 000 ng/g dry tobacco. In contrast, very low levels of TSNA have been reported in *naswar*: NNK, 130 ng/g dry tobacco; NNN, up to 520 ng/g dry tobacco; NAB, 30 ng/g dry tobacco; and NAT, 300 ng/g dry tobacco.

In recent years, some manufacturers of smokeless tobacco products have used novel tobacco curing and processing technologies to produce moist snuff with lower TSNA. These products are marketed in Norway, South Africa, Sweden, the USA and other countries (Connolly, 2001; Brunnemann *et al.*, 2004; Österdahl *et al.*, 2004; Stepanov *et al.*, 2006a; McNeill *et al.*, 2007). Levels of NNK and NNN in moist snuff produced by the new manufacturing process (Gothia, 2004) have been reported to be up to 45 times lower than those in leading products manufactured under standard process (Connolly, 2001). In Sweden, all moist snuff brands on the market in 2002 contained low levels of TSNA. NNN concentrations in moist snuff have decreased consistently from 1983 to 2002 in this country (Österdahl *et al.*, 2004). However, low-TSNA Swedish *snus*, purchased both in Sweden and the USA between 2003 and 2005, contained up to 360 ng/g wet

tobacco NNK, up to 2300 ng/g wet tobacco NNN, up to 130 ng/g wet tobacco NAB and up to 980 ng/g wet tobacco NAT (Stepanov *et al.*, 2006a). Since the values were not adjusted for the moisture content, the concentrations may be even higher. Other new smokeless tobacco products (e.g. compressed tobacco lozenges such as Ariva<sup>®</sup>, Stonewall, Hard Snuff) contain even lower concentrations of NNK, NNN, NAB and NAT (Table 7).

In India, smokeless tobacco products account for over one-third of all tobacco used (see the monograph on Smokeless tobacco). Traditional forms of smokeless tobacco include betel quid with tobacco, tobacco with lime and tobacco tooth powder but a variety of new products are gaining popularity (Gupta & Ray, 2003). Table 7 presents an overview of TSNA concentrations in a variety of Indian products. The highest levels of NNK, NNN, NAB and NAT were reported in *zarda* and *khaini* (Tricker & Preussmann, 1988; Tricker *et al.*, 1988; Stepanov *et al.*, 2005). Other products also contain high levels of TSNA (Gupta, 2004; Stepanov *et al.*, 2005). In contrast, *gutka*, moist snuff and chewing tobacco contain significantly lower levels of TSNA (Stepanov *et al.*, 2005). Some tobacco products consumed in India, such as *supari* and toothpowder, did not contain quantifiable levels of TSNA (Stepanov *et al.*, 2005). Brown and black *mishri*, a half-burnt tobacco product commonly used as a dentifrice in some parts of India, were reported to contain significantly higher amounts of TSNA compared with unburnt tobacco: NNK, up to 1100 ng/g dry wt; NNN, up to 6995 ng/g dry wt; and NAT, up to 14 150 ng/g dry wt (Nair *et al.*, 1987; Tricker *et al.*, 1988). In addition to TSNA, *mishri* contains high concentrations of benzo[*a*]pyrene (from 27 to 119 µg/g; Nair *et al.*, 1987).

Hoffmann *et al.* (1995) compared the concentrations of TSNA between two major categories of moist snuff that contain high and low levels of unprotonated nicotine (Table 8). In the high-level unprotonated nicotine brands, concentrations were in the range of: NNK,

**Table 8. The levels of tobacco-specific *N*-nitrosamines (TSNA) in five leading brands of moist snuff purchased in the USA, 1994**

Product	TSNA (µg/g dry wt)				
	NNK	NNN	NAB	NAT	Total TSNA
Skoal Bandits Straight <sup>a</sup>	0.92 ± 0.26	5.09 ± 1.03	0.13 ± 0.03	2.05 ± 0.60	8.19 ± 1.72
Hawken Wintergreen <sup>a</sup>	0.23 ± 0.04	3.07 ± 0.3	0.13 ± 0.02	0.63 ± 0.15	4.08 ± 0.44
Skoal Original Fine Cut Wintergreen <sup>b</sup>	1.25 ± 0.13	8.18 ± 1.33	0.37 ± 0.09	5.10 ± 1.01	14.90 ± 2.50
Copenhagen Snuff <sup>b</sup>	1.89 ± 0.62	8.73 ± 1.44	0.50 ± 0.12	6.13 ± 1.02	17.24 ± 2.97
Kodiak Wintergreen <sup>b</sup>	0.55 ± 0.15	6.3 ± 1.06	0.32 ± 0.10	3.79 ± 1.25	10.96 ± 2.44

From Hoffmann *et al.* (1995)

NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrososornicotine

<sup>a</sup> Brands with low levels of unprotonated nicotine

<sup>b</sup> Brands with high levels of unprotonated nicotine

550–1890 ng/g dry tobacco; NNN, 6300–8730 ng/g dry tobacco; NAB, 320–500 ng/g dry tobacco; and NAT, 3790–6130 ng/g dry tobacco. In the low-level unprotonated nicotine brands, concentrations were in the range of: NNK, 230–920 ng/g dry tobacco; NNN, 3070–5090 ng/g dry tobacco; NAB, 130 ng/g dry tobacco; and NAT, 630–2050 ng/g dry tobacco. Thus, the three leading snuff brands in the USA (Copenhagen, Skoal Fine Cut and Kodiak) that made up 92% of the market in 1995 not only had a high pH and contained high levels of nicotine and unprotonated (free) nicotine, but also contained high concentrations of carcinogenic TSNA in comparison with the moist snuff brands that ranked fourth and fifth — Hawken and Skoal Bandits (3% of the US market).

A high-pH (7.99) and high-moisture (52.7%) moist snuff brand purchased in the USA in 2000 contained 2500 ng/g NNK, 15 400 ng/g NNN, 1200 ng/g NAB and 18 500 ng/g NAT, reported as dry tobacco wt; in contrast, a low-pH (5.84) and low-moisture (24%) moist snuff brand contained 500 ng/g NNK, 3100 ng/g NNN, 200 ng/g NAB and 800 ng/g NAT (Brunnemann *et al.*, 2002). TSNA levels in low-moisture brands are generally more consistent over time while they fluctuate dramatically in high-moisture brands.

In general, TSNA levels per unit dose are higher in smokeless tobacco compared with the levels in the mainstream cigarette smoke as determined by the ISO/FTC machine-smoking method (see Tables 4 and 7). Even in Swedish *snus*, levels of TSNA are comparable with those in cigarette smoke (e.g. the highest reported ISO/FTC NNN yield in the mainstream smoke was 1353 ng/cigarette versus 2300 ng/g *snus*; Stepanov *et al.*, 2006a).

#### 1.4 Biomonitoring in saliva, urine and other tissues

Analysis of TSNA and their metabolites in saliva, urine and blood has proven to be extremely useful in estimating human exposure to these carcinogens. While studies of levels in saliva and blood have been somewhat limited, there is extensive literature of urinary NNAL and NNAL-glucuronides (NNAL-Gluc), metabolites of NNK (see Section 4.1), in smokers, smokeless tobacco users and nonsmokers exposed to secondhand smoke. Studies relevant to the toxicokinetics (absorption, distribution, metabolism and excretion) of TSNA in humans and to the mechanisms of carcinogenesis by these compounds are discussed in Section 4. This section presents data that relate tobacco exposure to TSNA and their metabolites. Levels of NNK, NNN, NAB and NAT in the saliva of tobacco users are summarized in Table 9. Only studies published since the previous evaluation (IARC, 1985) have been included.

##### 1.4.1 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its metabolites

###### (a) NNK and its metabolites in saliva

NNK has been detected in the saliva of snuff dippers, users of *khaini*, of *gudhaku* chewers of betel quid with tobacco and users of *toombak* (Table 9; Nair *et al.*, 1985; Bhide *et al.*, 1986; Österdahl & Slorach, 1988; Idris *et al.*, 1992; Hoffmann *et al.*, 1994). Levels in snuff-dippers ranged from not detected to 201 ng/g saliva and those in *khaini* users were

**Table 9. Concentrations of NNK, NNN, NAB and NAT in the saliva of users of various forms of tobacco**

Reference	Country of study	Tobacco product	Unit	Mean concentration (range) or range			
				NNK	NNN	NAB	NAT
Nair <i>et al.</i> (1985)	India	Betel quid with tobacco	ng/mL saliva	0.34 (0–2.3)	7.5 (1.6–14.7)	–	4.8 (1.0–10.9)
		Chewing tobacco		ND	33.4 (16.5–59.7)		29.8 (13.5–51.7)
		Cigarette		ND	ND	–	ND
Bhide <i>et al.</i> (1986)	India	Betel quid with tobacco	ng/g saliva	–	3–85.7	ND–40	–
		<i>Mishri</i>		ND	23.7 (14.3–43.5)	ND	–
		<i>Khaini</i>		ND–28.5	91.9 (10.0–430)	–	ND–133.0
Brunnemann <i>et al.</i> (1987c)	Canadian Inuits	Snuff	ng/g saliva	56 (ND–201)	980 (115–2601)	–	1318 (123–4560)
Österdahl & Slorach (1988)	Sweden	Snuff	ng/g saliva	ND–16	3–140	–	4–85
Idris <i>et al.</i> (1992)	Sudan	<i>Toombak</i>	ng/mL saliva	ND–6689	582–20988	46–1944	ND–471
Stich <i>et al.</i> (1992)	India	<i>Khaini</i>	ng/mL saliva	ND–180	150–1580	13–90	86–690
		<i>Gudhaku</i>	ng/mL saliva	ND–10	15–88	1–15	9–55
		<i>Chutta</i> (reverse smoking)	ng/g saliva	–	45–5890	–	ND–1880

NAB, *N'*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; ND, not detected; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosoornicotine

up to 180 ng/g saliva. The highest levels were found in *toombak* users (up to 6690 ng/g saliva). Levels of NNK were generally 10–100 times greater in the saliva of *toombak* users than in users of other types of smokeless tobacco (Idris *et al.*, 1992; Hoffmann *et al.*, 1994).

NNAL, a metabolite of NNK, was also detected in the saliva of *toombak* users at levels as high as 3270 ng/g saliva (Idris *et al.*, 1992).

(b) *NNK and its metabolites in cervical mucus and pancreatic juice*

NNK was detected in 16 samples of cervical mucus from 15 women who were smokers at concentrations of 11.9–115 ng/g mucus (two samples were collected from one smoker at different times) and in nine of 10 samples from nonsmokers at concentrations of 4.1–30.8 ng/g mucus; the concentrations of NNK in specimens from cigarette smokers were significantly higher than in those obtained from nonsmokers (Prokopczyk *et al.*, 1997).

NNK was detected in 15/18 samples of pancreatic juice from smokers at concentrations of 1.4–604 ng/mL and in six of nine samples from nonsmokers (range of concentrations, 1.13–97 ng/mL); the levels were significantly higher in smokers than in nonsmokers. NNAL was present in 11/17 samples from smokers and in three of nine samples from nonsmokers (Prokopczyk *et al.*, 2002).

(c) *NNAL and NNAL-Gluc in urine*

Unchanged NNK is not detected in urine (Hecht *et al.*, 1999a).

Several studies have quantified NNAL and NNAL-Gluc in human urine. Levels of total NNAL (NNAL plus NNAL-Gluc) are generally 2–4 pmol/mg creatinine in smokeless tobacco users, 1–4 pmol/mg creatinine in smokers and 0.02–0.07 pmol/mg creatinine in nonsmokers exposed to secondhand smoke. Several studies have quantified these metabolites in 24-h urine. Baseline levels of excreted NNAL and NNAL-Gluc are typically about 1 nmol NNAL/24 h and 2.2 nmol NNAL-Gluc/24 h (Hecht *et al.*, 1999a); levels of total NNAL generally reported were 6.6 nmol/24 h in smokeless tobacco users, 3–4 nmol/24 h in smokers and 0.03–0.13 nmol/24 h in nonsmokers exposed to secondhand tobacco smoke. Occasionally, however, as in the case of *toombak* users, far higher levels of total NNAL in urine have been observed.

(i) *Smokers*

The earlier literature on NNAL and NNAL-Gluc in the urine of smokers has been reviewed (Hecht, 2002; IARC, 2004). In a study of 274 smokers of cigarettes in the contemporary yield ranges of the German market and 100 nonsmokers, total NNAL levels were significantly higher in smokers than in nonsmokers (Scherer *et al.*, 2007).

Total NNAL has been shown to correlate with number of cigarettes smoked per day but the increase was not linear (Joseph *et al.*, 2005). No statistically significant differences in urinary levels of total NNAL were observed in urine samples from 175 smokers of regular, light or ultra-light cigarettes (Burns *et al.*, 2001; Harris *et al.*, 2004; Bernert *et al.*, 2005; Hecht *et al.*, 2005).

Two studies investigated the effects of reducing the number of cigarettes smoked per day on levels of NNAL and NNAL-Gluc. Decreases in levels of NNAL, NNAL-Gluc and total NNAL were observed, but these were generally modest, and were always proportionally less than the reduction in the number of cigarettes smoked per day (Hurt *et al.*, 2000; Hecht *et al.*, 2004a).

Levels of total NNAL were significantly reduced in smokers who switched from their customary brand to the Omni cigarette (a cigarette with reduced levels of NNK) and were significantly lower than those in smokers who stopped with the aid of a nicotine patch (Hatsukami *et al.*, 2004).

A comparison of total NNAL in the urine of Caucasians and African-Americans demonstrated that mean concentrations were greater in African-American men for each cigarette smoked; no difference was seen in women (Muscat *et al.*, 2005).

(ii) *Nonsmokers exposed to secondhand tobacco smoke*

Levels of total NNAL in the urine of nonsmokers exposed to secondhand tobacco smoke are typically about 1–5% of those in smokers (Hecht, 2002; IARC, 2004). In more recent studies, total NNAL was quantified before and after a 4-h visit to a gambling casino where smoking was allowed (Anderson *et al.*, 2003) and in nonsmokers who worked in restaurants and bars that allow smoking (Tulunay *et al.*, 2005). Both studies showed significant increases in urinary levels of total NNAL after exposure to secondhand smoke.

Levels of total NNAL in the urine of 144 infants (< 1 year old) averaged  $0.083 \pm 0.200$  pmol/mL urine; the mean number of cigarettes smoked per week by any family member in the home or car when the infant was present was significantly higher in the families of children with detectable levels of NNAL compared to those with undetectable levels. The levels of NNAL detected in the urine of these infants was higher than those in most other field studies of exposure to secondhand tobacco smoke (Hecht *et al.*, 2006).

In another study, total NNAL was detected in 69/80 urine samples from Moldovan children. The mean level ( $0.09 \pm 0.077$  pmol/mL) was comparable with those observed in previous studies of secondhand smoke exposure (Stepanov *et al.*, 2006b).

(iii) *Smokeless tobacco users*

Snuff dippers/tobacco chewers in the USA excreted 6.6 nmol/24 h total NNAL (NNAL plus NNAL-Gluc) in urine (Hecht *et al.*, 2002). In one study of snuff dippers and tobacco chewers, urinary excretion of total NNAL averaged 4.4 pmol/mg creatinine. Levels in 23 snuff-dippers (5.9 pmol/mg creatinine) were significantly higher than those in 13 tobacco chewers (2.1 pmol/mg creatinine) (Kresty *et al.*, 1996).

Seven *toombak* users excreted an average of 1270 pmol/mL urine total NNAL, which was approximately 300 times that excreted by snuff-dippers/chewers (Murphy *et al.*, 1994).

Among snuff dippers in the USA, total daily dip duration, total daily dipping time and number of dips per day were significantly correlated with levels of total NNAL (Lemmonds *et al.*, 2005). Levels of total NNAL correlated with the number of tins used per day in one study (Hecht *et al.*, 2002) but not in another (Lemmonds *et al.*, 2005). Total levels of NNAL

were significantly lower in users of smokeless tobacco after they switched to Swedish snuff or to nicotine patch; the overall mean level of total NNAL among subjects who used a nicotine patch was significantly lower than that among those who used snuff (Hatsukami *et al.*, 2004).

Levels of NNAL and NNAL-Gluc were quantified in the urine of 420 smokers and 182 smokeless tobacco users (Hecht *et al.*, 2007). Levels of total NNAL/mL urine and levels of total NNAL/mg creatinine, adjusted for age and sex, were significantly higher in smokeless tobacco users than in smokers ( $p < 0.001$ ).

(d) *Total NNAL in blood*

A liquid chromatography–electrospray ionization tandem mass spectrometry method was developed for the analysis of total NNAL in plasma. Levels averaged  $42 \pm 22$  fmol/mL in 16 smokers; total NNAL was not detected in the plasma of nonsmokers. Levels were 1–2% of those found in urine (Carmella *et al.*, 2005, 2006).

(e) *Total NNAL in toenails*

Total NNAL, nicotine and cotinine were analysed in human toenails by liquid chromatography–electrospray ionization tandem mass spectrometry in 35 smokers. Mean total NNAL was  $0.41 \pm 0.67$  pg/mg toenail (Stepanov *et al.*, 2006c).

#### 1.4.2 *N'-Nitrosonornicotine (NNN)*

(a) *Saliva*

Formation of additional quantities of NNN by the reaction of salivary nitrite with nicotine or nornicotine during the oral use of snuff or during tobacco chewing has been implied from in-vitro studies (Hoffmann & Adams, 1981).

NNN has been detected in the saliva of snuff dippers (Brunnemann *et al.*, 1987c; Österdahl & Slorach, 1988; Hoffmann *et al.*, 1994), chewers of betel quid with tobacco (Nair *et al.*, 1985; Bhide *et al.*, 1986), users of *khaini* (Bhide *et al.*, 1986; Stich *et al.*, 1992), users of *gudakhu* (Stich *et al.*, 1992), users of *mishri* (Bhide *et al.*, 1986), reverse smokers of *chutta* (Stich *et al.*, 1992) and users of *toombak* (Idris *et al.*, 1992) (Table 9). The exceptionally high levels of NNN in the saliva of *toombak* users reflects the unusually high concentrations of NNN (mg/g) in this product (Idris *et al.*, 1991).

(b) *Urine*

Levels of NNN in the urine of 14 smokers ranged from not detected to 0.43 pmol/mg creatinine (mean  $\pm$  standard deviation (SD),  $0.086 \pm 0.12$  pmol/mg) and those of NNN-*N*-glucuronide (NNN-*N*-Gluc) ranged from not detected to 0.36 pmol/mL creatinine (mean  $\pm$  SD,  $0.096 \pm 0.11$  pmol/mL). The corresponding values in 11 smokeless tobacco users were 0.03–0.58 pmol/mg creatinine (mean  $\pm$  SD,  $0.25 \pm 0.19$  pmol/mg) and 0.091–0.91 pmol/mg creatinine (mean  $\pm$  SD,  $0.39 \pm 0.27$  pmol/mg) (Stepanov & Hecht, 2005).

(c) *Pancreatic juice*

NNN was found in two of 17 samples of pancreatic juice from smokers (68 and 242 ng/mL) (Prokopczyk *et al.*, 2002).

1.4.3 *N*'-Nitrosoanabasine (NAB)

(a) *Saliva*

NAB was detected in the saliva of chewers of betel quid with tobacco (Bhide *et al.*, 1986), *toombak* (Idris *et al.*, 1992), *khaini* (Stich *et al.*, 1992) and *gudhaku* (Stich *et al.*, 1992) (Table 9).

(b) *Urine*

NAB and NAB-*N*-glucuronide (NAB-*N*-Gluc) have been found in the urine of smokers and smokeless tobacco users. Levels in 14 smokers ranged from not detected to 0.019 pmol/mg creatinine (mean  $\pm$  SD, 0.003  $\pm$  0.006 pmol/mg) NAB and from not detected to 0.14 pmol/mg creatinine (mean  $\pm$  SD, 0.038  $\pm$  0.039 pmol/mg) NAB-*N*-Gluc. Corresponding levels in 11 smokeless tobacco users ranged from not detected to 0.11 pmol/mg creatinine (mean  $\pm$  SD, 0.037  $\pm$  0.034 pmol/mg) and 0.028–0.44 pmol/mg creatinine (mean  $\pm$  SD, 0.19  $\pm$  0.16 pmol/mg), respectively (Stepanov & Hecht, 2005).

1.4.4 *N*'-Nitrosoanatabine (NAT)

(a) *Saliva*

NAT has been detected in the saliva of snuff dippers (Brunnemann *et al.*, 1987c; Österdahl & Slorach, 1988; Hoffmann *et al.*, 1994), *toombak* users (Idris *et al.*, 1992), *khaini* tobacco users (Bhide *et al.*, 1986; Stich *et al.*, 1992), *gudhaku* users (Stich *et al.*, 1992) and users of betel quid with tobacco (Nair *et al.*, 1985) (Table 9).

(b) *Urine*

NAT and NAT-*N*-glucuronide (NAT-*N*-Gluc) were detected in the urine of smokers and smokeless tobacco users. Levels in 14 smokers ranged from not detected to 0.31 pmol/mg creatinine (mean  $\pm$  SD, 0.067  $\pm$  0.104 pmol/mg) NAT and from not detected to 0.43 pmol/mg creatinine (mean  $\pm$  SD, 0.12  $\pm$  0.11 pmol/mg) NAT-*N*-Gluc. Corresponding levels in 11 smokeless tobacco users were 0.020–0.15 pmol/mg creatinine (mean  $\pm$  SD, 0.069  $\pm$  0.046 pmol/mg) and 0.08–2.78 pmol/mg creatinine (mean  $\pm$  SD, 1.36  $\pm$  1.06 pmol/mg), respectively (Stepanov & Hecht, 2005).