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# Concentrations of the Carcinogen 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone in Sidestream Cigarette Smoke Increase after Release into Indoor Air: Results from Unpublished Tobacco Industry Research

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## Abstract

Research has shown that the toxicity of sidestream cigarette smoke, the primary constituent of secondhand smoke, increases over time. To find potential mechanisms that would explain the increase in sidestream smoke toxicity over time, we analyzed unpublished research reports from Philip Morris Co. using the internal tobacco industry documents now available at the University of California San Francisco Legacy Tobacco Documents Library and other Web sites. Unpublished research from Philip Morris Tobacco Company shows that 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a highly carcinogenic tobacco-specific nitrosamine, can form in sidestream cigarette smoke after it has been released into ambient air. In experiments done between 1983 and 1997, Philip Morris scientists measured the concentra-

tion of NNK in sidestream smoke in a sealed stainless steel test chamber at initial particle concentrations of 24 mg/m<sup>3</sup> over the course of 6 to 18 h. They repeatedly showed that airborne NNK concentrations in sidestream cigarette smoke can increase by 50% to 200% per hour during the first 6 h after cigarettes are extinguished. Two experiments done in a real office showed that NNK concentrations increase for the first 2 h after cigarettes are extinguished. If NNK formation also occurs in the lower smoke concentrations observed in real smoking environments, these results suggest that nitrosation of nicotine and/or nicotine breakdown products in aging secondhand smoke is a significant contributor to nitrosamine exposure in humans. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1547–53)

## Introduction

Exposure to secondhand smoke causes ~53,000 of the 453,000 deaths caused each year by tobacco use in the United States (1, 2). The ratio of mortality between active smokers and nonsmokers who are exposed to secondhand smoke (8:1) is remarkable because, in terms of the total mass inhaled, the dose for smokers is substantially higher than for nonsmokers. Sidestream cigarette smoke, the smoke released into the air from burning cigarettes between puffs, comprises the majority of secondhand smoke (3). In previous studies of unpublished research from tobacco industry documents, we showed that fresh sidestream cigarette smoke is 3 to 4 times more toxic to laboratory animals than mainstream smoke (the smoke the smoker inhales) and that sidestream smoke becomes another 2 to 4 times more toxic as it ages (4, 5), making aged sidestream smoke about an order of magnitude more toxic (per unit mass) than fresh mainstream smoke.

To investigate how sidestream smoke might become more toxic as it ages, we searched the tobacco documents for research on the most toxic and carcinogenic compounds in sidestream smoke. Philip Morris conducted research on tobacco-specific nitrosamines (TSNA) that may explain this phenomenon. TSNA are a group of highly carcinogenic compounds that are formed exclusively from nicotine and other tobacco alkaloids. They are found in mainstream, sidestream,

and secondhand cigarette smoke. Common TSNA include 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL), *N*-nitrosoanabasine (NAB), and *N*'-nitrosoanatabine (NAT). NNK and its metabolic breakdown product NNAL are among the strongest nitrosamine carcinogens known (6). NNK causes lung adenomas and adenocarcinomas, whether it is administered p.o., i.p., or via the lung (6). NNK can also cause cancer of the nasal mucosa and liver (7). The NNK metabolite NNAL is consistently found in nonsmokers exposed to secondhand smoke (8). Kinetic studies of TSNA, done at Philip Morris between 1983 and 1997, show that NNK concentrations in sidestream smoke increase after the smoke is released into room air.

## Materials and Methods

**Tobacco Industry Document Searches.** We found reports documenting research on the kinetics of NNK formation in sidestream cigarette smoke by searching the ~45 million pages of tobacco industry documents made public as a result of litigation against the tobacco companies. Between February and November 2006, we searched the University of California San Francisco (UCSF) Legacy Tobacco Documents Library,<sup>1</sup> the UCSF British American Tobacco Documents Archive,<sup>2</sup> and Philip Morris documents<sup>3</sup> using standard strategies (9), starting with keywords "Tasso," "Poldi," "sidestream," "NNK," and "nitrosamine."

Except where noted, these studies were conducted at laboratories operated by Philip Morris subsidiary Fabriques

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<sup>1</sup> <http://www.legacy.library.ucsf.edu>

<sup>2</sup> <http://bat.library.ucsf.edu>

<sup>3</sup> <http://www.pmdocs.org>

de Tabac Reunie in Neuchâtel, Switzerland. The sidestream chamber in Neuchâtel was also used to test sidestream smoke of new products under development, especially low-visibility sidestream prototypes (10) that were being developed in an effort to develop a socially acceptable cigarette (11, 12). In 1988, Philip Morris built another chamber for analyses of aged sidestream cigarette smoke at the facilities in Richmond, VA, "to develop products which target the issue of reduced sidestream" (10). The chemical analyses done in the Richmond sidestream chamber included nitrosamine analysis, but we did not include results from this chamber in our analyses because we have not found a protocol specifying how long the smoke was aged in the Richmond chamber (Table 1).

We searched for evidence of similar kinetic studies of TSNA formation in secondhand smoke at other tobacco companies and did not find any. RJ Reynolds Tobacco Co. and British American Tobacco (BAT) measured TSNA in fresh sidestream smoke (13-16), and BAT measured it in secondhand smoke (17), but the only documents we found in the tobacco document collections as of November 2006 on the kinetics of TSNA formation in aging sidestream were from Philip Morris.

**Reproduction of Graphs from Tobacco Documents.** Data in many of the Philip Morris Co. reports is present solely in graphic format. We enlarged the graphs to read off the data points and converted the NNK units in the original to micrograms per cubic meter or nanograms per cubic meter to facilitate comparing results between studies.

**Philip Morris Nitrosamine Analysis Methods.** The chamber used was a modified stainless steel cold-storage room (Frigorex KZ 80) with an internal volume of 18.2 m<sup>3</sup>. Air exchange was not measured, but the door was tightly sealed and "every precaution was taken to make the room effectively air-tight" (18). The room was lit with a ceiling-mounted fluorescent fixture. Two 21-cm (19) ventilation ports entered the room: one for fresh air supply, one for exhaust. Three 1.5-in. stainless steel pipes extended through the wall, one for controlling the smoking machine and exhausting the mainstream smoke and two for sample tubes and cables. In the center of the room, there was a 60-cm-high stainless steel table supporting a modified 30-port Battelle smoking machine. The cigarettes were lit with a hydrogen flame which was ignited by a spark from a platinum wire. Extracted butts fell into a water-filled container, which was sealed with an automatically actuated lid, and ashes were constantly brushed into the same container through an opening in the lid (19). The room also

held two stainless steel stands for probes, cameras, and sampling lines. The air was originally mixed with two oscillating fans installed in opposite corners of the room. Later, the oscillating fans were replaced by a single large ceiling fan (19). The room surfaces were cleaned before each experiment with a 20% aqueous isopropyl alcohol solution (19).

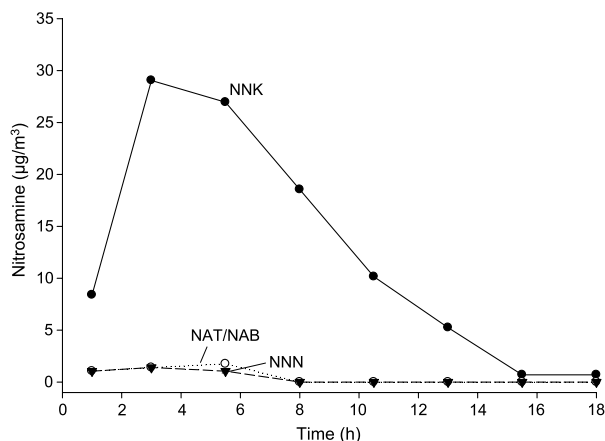
**Sidestream Smoke Generation.** Thirty (20) cigarettes were placed in the smoking machine, the room air was brought to 22°C and 60% relative humidity, and then the room was sealed for the remainder of the experiment. Thirty cigarettes were lit and smoked at the same time using the standard FTC method of one 35-mL puff of 2 s duration at 1-min intervals. Mainstream smoke was exhausted from the room. When the final cigarette was extinguished, the mixing fans were turned on and run for 1 min, and then the sampling pumps were started. The moment the pumps were started was defined as time 0.

**Chemical Sampling and Quantification.** NNK sampling and quantification at Philip Morris followed approximately the same protocol in all of the analyses we discuss (19, 21-23). TSNA samples were trapped on an Extrelut-3 cartridge (Merck) impregnated with citrate/phosphate/ascorbic acid buffer (11.3 g citric acid monohydrate + 16 g disodium hydrogen phosphate dihydrate + 13.5 g ascorbic acid in 1 L of deionized water, pH unspecified) by pumping air through the cartridge at 2 L/min for 1 h. Sampling was started 1 min after the cigarettes were extinguished. The samples were eluted with 50 mL of dichloromethane/acetone (9:1). About 2 mL of internal standard solution (535 ng/mL *N*-nitrosodihexylamine in dichloromethane) was added, and the sample was concentrated to 2 mL. After concentration, aliquots of this extract were analyzed by gas chromatography on a 15 × 0.5-mm-wide i.d. bore DB-5 capillary column using a Thermal Energy Analyzer (TEA model 610 or 543, Thermo Electron Corp.) as a specific nitrosamine detector. Results were calibrated against standards obtained from Georg Neurath's laboratories (Hamburg) or from Isconlab (Heidelberg). The detection limit using this procedure was 15 to 50 pg per injection, corresponding to 15 to 50 ng per sample or 0.1 to 0.5 ng/L of air. Reproducibility was 5% to 10% over five to six replicates (21, 22).

NO concentrations were measured continuously with an NO/NO<sub>x</sub> analyzer CLD 502 (Tecan) and printed out on an HP 805B data logger 0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 h after smoking.

**Table 1. NNK formation over 6 h**

Year	Cigarette brand or filler type	NNK in first-hour sample (ng/m <sup>3</sup> )	NNK in 6-h sample (ng/m <sup>3</sup> )	5.5 h/0.5 h NNK	Average % increase in NNK/h
1986 (22)	Parisienne Super	4.0	24.5	6.1	122
	Marlboro Filter Box	2.5	14.1	5.6	111
	Gauloises Jaunes Filter	2.7	17.5	6.4	127
	Marocaine Super	2.3	15.6	6.7	134
	Select Filter	4.2	26.8	6.3	127
	Brunette Double Filter	4.4	10.1	2.3	46
	Flint Ultra	0.84	8.6	10.2	205
	Base Web	0.63	1.7	2.7	53
	C50	2.7	18.1	6.6	132
	Bright	3.2	17.3	5.5	109
	Burley	7.4	27.8	3.8	75
	Blended Oriental	0.63	3.5	5.7	113
	Expanded Bright Stems	0.63	3.2	5	100
	Blend	4.0	18.6	4.6	93
	Nicotine extracted	0.63	2.5	3.3	80
	1997 (19)	C20	1.4	4.4	3.3
Marlboro 100s		2.3	7.1	3.1	62
Virginia Slims		2.3	6.7	2.8	57
C50		1.9	4.9	2.6	52



**Figure 1.** NNK concentration in air polluted with sidestream cigarette smoke increases with time, whereas the concentrations of NNN and combined NAT/NAB do not. Ninety C20 cigarettes were smoked simultaneously. Time 0 is 1 min after all the cigarettes were extinguished. Each point represents a sample collected by pumping air through a solvent-impregnated cartridge at 2 L/min for 1 h (i.e., the point at hour 1 is from the same collected from  $t = 0$  to  $t = 1$  h). Reproduction of the graph from Philip Morris internal report (21) with the units converted to micrograms per cubic meter of NNK.

Nicotine air samples were drawn through an Extrelut 3 cartridge (Merck) coated with 4 mL of 0.05 N  $H_2SO_4$  by means of a Digitel LT 75 sampling pump for 5 min at 2 L/min. Aerosol particulate matter was determined by a TSI model 5000 tapered element oscillating microbalance.

## Results

**NNK Formation in Sidestream Smoke.** The first kinetic analyses of nitrosamine concentration in sidestream smoke at Philip Morris were part of a larger project, code named Poldi, on the effects of dilution and aging on the chemistry of sidestream cigarette smoke, done between 1982 and 1985 (18, 24). Using C20 and C50, two custom-made filtered research cigarettes designed to represent the most popular brands in Germany circa 1982 (25), they measured the concentration of aerosol particulate matter (APM), carbon monoxide, nicotine, NO,  $NO_2$ , ammonia, hydrocyanic acid, formaldehyde, acetaldehyde, phenol, cresol, benzopyrene, cadmium, NDMA, NNK, NNN, and NAB/NAT. As expected from basic chemistry principles, the concentrations of APM and nicotine decreased over time due to adsorption of organic compounds to the chamber walls. The concentration of NO decreased, whereas the concentration of  $NO_2$  increased. ( $NO_2$  forms from reaction of NO with oxygen or ozone.) Concentrations of CO, ammonia, hydrocyanic acid, formaldehyde, and acetaldehyde remained relatively constant.

The effects of aging on one particular TSNA, however, were entirely unexpected (Fig. 1). In a report summarizing the

results of the TSNA experiments, Philip Morris scientist Charles Blake wrote "Over a period of 4 h a rapid increase in NNK concentration was observed and then a gradual decrease to almost 0 over the following 11 h. The decrease in NNK concentration was linked to the decrease in concentration of aerosol particulate matter, rather than decomposition (21)."

These findings generated interest at Philip Morris and a new project, code-named Tasso, was initiated in 1985 to "investigate the mechanism of NNK formation, to determine the reason for this unexpected behavior of NNK in aging sidestream, and finally, to attempt to suppress NNK formation of cigarette filler or paper modifications" (22).

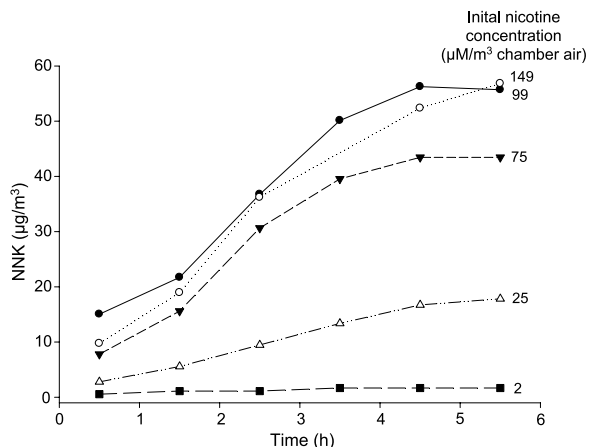
Project Tasso, also conducted at Philip Morris' Neuchâtel laboratory, tested eight popular brands of cigarettes from the Swiss market and many different experimental cigarettes. Our analysis of the data from reports in the tobacco documents shows that the concentration of NNK from sidestream smoke in the experimental chamber increased between 2- and 10-fold (Table 1). This factor of increase probably underestimates the true increase in NNK because the samples took an entire hour to collect, so the first measured NNK concentration is the average of the entire first hour. Increases in other TSNA were not detected.

**Mechanism of NNK Formation.** The chemical complexity of sidestream smoke, and the long sampling periods required to detect NNK at the time these experiments were done, limited the kinetic analyses available to the researchers at Philip Morris. Despite these limitations, some sound basic determinations were made. In the first study (Project Poldi), Philip Morris scientists observed that nicotine and nitric oxide (NO) concentrations decreased as NNK concentration increased, and that NNK formation ceased when the NO concentration in the room neared zero (21). NNK is believed to form primarily by nitrosation of nicotine or pseudoxy-nicotine during curing and combustion of tobacco (refs. 7, 26 p. 422). Pseudoxy-nicotine is a nicotine derivative that is formed during tobacco curing (26). NO and  $NO_2$  can react to form nitrous acid, a nitrosating agent (27). These observations were confirmed and extended in Project Tasso by testing the effects of varying the concentration of nicotine and NO in the cigarettes or the chamber.

Combining pure, vapor-phase nicotine and NO in the chamber without adding any sidestream smoke yielded very little NNK (Table 2), suggesting that nicotine and NO are not sufficient to form NNK, and that some other component of sidestream smoke is required. Cigarettes made with very low nicotine tobacco yielded almost no NNK (Fig. 2). Adding increasing amounts of pure nicotine to the cigarette filler increased the concentration of nicotine in the room at time 0 and the amount of NNK formed. Adding pure vapor-phase nicotine to the room air, before smoking the cigarettes, also increased the concentration of nicotine in the room at time 0 and amount of NNK formed (Fig. 3). The fact that the nicotine did not need to be supplied by the cigarette implies that nicotine did not need to be formed into an intermediate by combustion, but reacted directly with nitrosating agents in aging sidestream smoke.

**Table 2. NNK formation with 400  $\mu\text{mol}/\text{m}^3$  NO and 55  $\mu\text{mol}/\text{m}^3$  vapor-phase nicotine added to chamber at  $t_0$  (22)**

Number of cigarettes smoked	NO ( $\mu\text{mol}/\text{m}^3$ )		Nicotine ( $\mu\text{mol}/\text{m}^3$ )		NNK (nmol/ $\text{m}^3$ )	
	0.5 h	4.5 h	0.0 h	3.5 h	0.5 h	4.5 h
0	351	186	54	24	4	5
5	310	178	55	28	24	79
10	294	190	52	26	53	120
20	305	149	51	25	55	145
30	392	215	51	22	25	155



**Figure 2.** Adding nicotine to cigarette filler increases the initial concentration (concentration at time 0) of nicotine in the chamber air and the concentration of NNK formed in the air over time. Higher initial nicotine concentrations yielded faster rates of NNK formation and higher total NNK concentrations. The lowest initial nicotine concentration is from a cigarette made of low-nicotine reconstituted tobacco. The 25- $\mu\text{mol/L}$  initial nicotine concentration is from a C50 test cigarette. The 75-, 99-, and 149- $\mu\text{mol/L}$  initial nicotine concentrations were achieved by adding 10, 30, and 40 mg nicotine, respectively, to the C50 filler. Reproduction of graph from Philip Morris internal report (22) with the units converted to micrograms per cubic meter of NNK.

This test did not rule out the possibility, however, that pseudoxyntocotine, present as a small percentage impurity in the purified nicotine added to the chamber and the cigarettes, was the true precursor of NNK. To test the potential role of pseudoxyntocotine, Philip Morris scientists spiked cigarettes with 20 mg of it and smoked them in the chamber. Adding pseudoxyntocotine to the cigarettes did not increase NNK concentrations (ref. 22; data not shown), suggesting that it is not an intermediate in NNK formation under these circumstances.

Given equal initial concentrations of nicotine, more NNK was formed from pure nicotine evaporated into the room than from nicotine released from cigarettes (Fig. 3). Because nicotine in sidestream cigarette smoke is partly in the gas phase and partly in the particulate phase, this finding suggested that nicotine reacts to form NNK preferably from the gas phase. At high concentrations of nicotine, whether from cigarettes or added nicotine vapor, the NNK concentration plateaued. There was always enough NO in the room to participate in the reaction (data not shown), suggesting that the sidestream smoke provided a third reactant or catalyst necessary to form NNK. After the reactant was consumed, or the catalyst was destroyed, NNK formation ceased.

To test whether the compound that nitrosated nicotine was NO or another compound formed during tobacco combustion, Philip Morris scientists added NO gas to the room before the cigarettes were smoked. Higher initial NO concentrations yielded more NNK (Table 3), which suggests that NO is involved in the reaction. In the first-hour sample, the NNK concentration had a linear relationship to the initial NO concentration (Fig. 4). In the sample collected from hours 5 to 6, the effect of NO on NNK concentration had the same slope as in the 1-h sample, except at high initial concentrations of NO, where it seemed to plateau. This result again suggests that there is a third substance in sidestream smoke necessary for NNK formation.

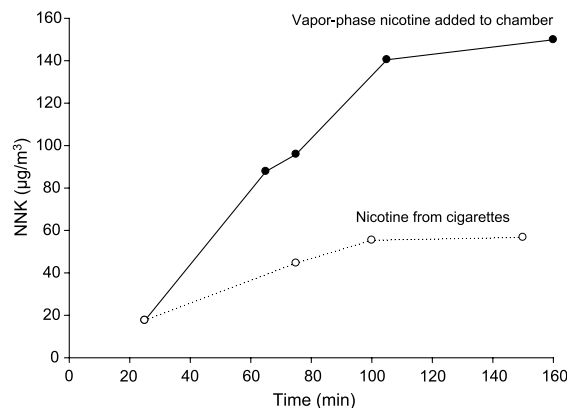
The ratio between nicotine concentration at time 0 (1 min after the 30 cigarettes had been extinguished) and the peak

concentration of NNK (1-h sample collected starting at 5 h) was  $\sim 0.003$ , indicating that at least 0.3% of the nicotine available at time 0 reacted to form NNK over the course of 6 h, assuming nicotine is the only precursor to NNK (22). Nicotine and NNK are both semivolatile organic compounds (vapor pressure between  $10^{-2}$  and  $10^{-8}$  kPa), which means that they may be found in both the particulate and vapor phase and tend to adsorb to surfaces (28), so NNK as well as nicotine was probably lost to adsorption. Thus, the amount of NNK measured may be less than the total amount produced. The ratio between NO concentration at the start of the reactions and the peak concentration of NNK was  $\sim 0.0005$ , so at least 0.05% of the NO available at time 0 reacted to form NNK.

**Tests of Artifactual NNK Formation.** To test whether the NNK was forming in the collection traps instead of inside the chamber, Philip Morris chemists loaded test collection traps with pure nicotine or easily nitrosatable compounds such as morpholine (concentrations not stated) before pumping the chamber air through them for 1 h to collect an NNK sample (21). The extra nicotine added to the traps was not converted to NNK, and <1% of the added morpholine was nitrosated. This argues that that artifactual NNK formation in the collection traps is not the cause of the 2- to 10-fold increases in NNK concentration observed.

**Inhibition of NNK Formation.** Philip Morris tested the effect of adding isoprene gas, a radical scavenger, to the chamber. Isoprene inhibited the formation of NNK. They then tested the addition of squalene, an isoprene progenitor, to cigarettes. There was only a slight reduction in NNK formation. Adding antioxidant vitamins, such as ascorbic acid, ascorbyl palmitate, and tocopheryl acetate and retinyl palmitate, at 0.05 mmol per cigarette to high-nicotine cigarettes (made of burley tobacco) reduced NNK formation by 5% to 40% (Fig. 5).

**NNK Formation in a Furnished Office.** A stainless steel chamber is not a normal indoor smoking environment. Most people smoke in homes and workplaces with painted walls, window treatments, furnishings, and carpet. These normal environments have many more surfaces for sidestream smoke gases and particles to adsorb to and tend to have significantly



**Figure 3.** NNK concentration in 5.5-hour-old sidestream smoke versus initial nicotine concentration (concentration at time 0). More NNK forms in sidestream smoke when pure vapor-phase nicotine is added to the chamber before cigarette combustion than when nicotine is released only via cigarette combustion. The number of cigarettes smoked was equal. Reproduction of graph from Philip Morris internal report (22) with the units converted to micrograms per cubic meter of NNK.

**Table 3. NNK formation with varying initial concentrations of NO (22)**

Number of cigarettes smoked	NO $\mu\text{mol}/\text{m}^3$		Nicotine $\mu\text{mol}/\text{m}^3$		NNK $\text{nmol}/\text{m}^3$	
	0.5 h	3.5 h	0.0 h	3.5 h	0.5 h	3.5 h
30	124	50	47	27	17	84
30	475	207	48	23	49	157
30	814	306	51	27	82	184
30	1,186	401	51	28	117	188
30	1,087	397	54	31	170	216

higher rates of particulate material loss. In 1988, Philip Morris scientists tested NNK formation in an ordinary  $35\text{-m}^3$  office at the Neuchâtel laboratory (ref. 23; Fig. 6). The office air vents were taped closed, and the cigarettes were allowed to smolder freely in an ashtray rather than being smoked on a smoking machine. The air was mixed with a fan for 1 min before the start of sampling and then turned off. This experiment was run twice. The sampling pumps were started at different times in the two experiments, which explains why there is a sharp increase in concentration in experiment 1, but not experiment 2 (Fig. 6). There was no explanation given for the difference in total concentration between the two experiments. The results of these experiments show that NNK concentrations can increase after sidestream smoke is released into a normal office, despite rapid loss of particulate matter (23).

#### Sensitivity and Reproducibility of Nitrosamine Analyses.

The method used in these studies can detect as little as 0.50 ng NNK/L of room air (21). The reproducibility of the method at a given laboratory, in a given year, was about 10% over five repetitions (21, 22). The variability between laboratories, or at the same laboratory over a decade, was as high as 70% (10, 19, 29, 30). However, the finding that NNK concentrations increase as sidestream smoke ages was consistent between laboratories and across all analyses.

**Publication of NNK Results by Philip Morris.** Philip Morris did not publish their results on the formation of NNK in aging sidestream smoke. Philip Morris presented data from Project Poldi (on the general effects of aging on the chemistry of sidestream smoke) at the International Experimental Toxicology Symposium on Passive Smoking in Essen, Germany, in October 1986 but did not include data on TSNAs (31, 32). A manuscript listing TSNA concentrations for the earliest time point, but not the increase in NNK concentration over time, was submitted to *Beitrag für Tabakforschung* (a tobacco science journal) in 1988 (25). The manuscript was reviewed and returned with requests for minor revisions (33), but no published version appears in the journal archives,<sup>4</sup> the tobacco documents, or the open literature. No data from Project Tasso seem to have been presented publicly.

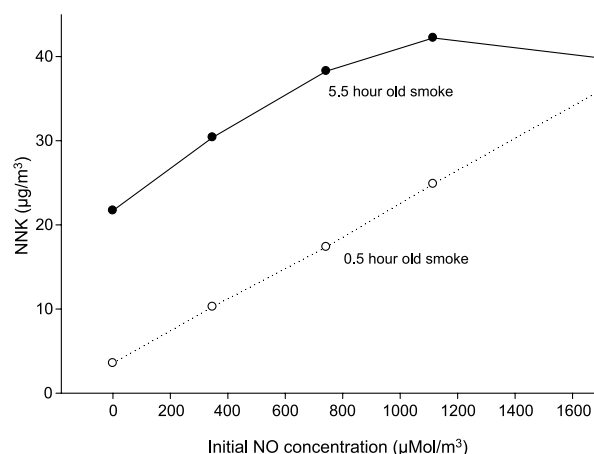
Philip Morris did, however, present and publish work that emphasized the rapid loss of SHS components from room air by adsorption and ventilation (34-38). Publications in 1998 and 2005 from Philip Morris on the toxicity of experimentally aged sidestream cigarette smoke do provide TSNA concentration data and show that NNK concentrations are elevated 10- to 20-fold relative to other TSNAs (39, 40). However, unless the readers of these publications were aware that the concentration of NNK was only slightly higher than that of other TSNAs in fresh sidestream smoke, the fact that NNK forms as sidestream smoke ages will not be evident.

## Discussion

This is the first published report of gas-phase formation of a nitrosamine in a pollutant mixture commonly present in indoor air. Most research has focused on nitrosation reactions in solution, but gas phase nitrosation has been reported in simulated outdoor atmospheres (41, 42) and in an industrial environment in East Germany (43). It is known that the concentration of NNK in sidestream cigarette smoke is higher than that in mainstream cigarette smoke (7, 44). Previous publications have also reported that the concentration of NNK in freshly generated sidestream cigarette smoke and in secondhand cigarette smoke in test chambers may be higher than the concentration of other TSNAs (39, 40). However, we believe that this is the first publication to disclose data showing that the concentration of NNK in sidestream cigarette smoke can increase after the smoke is released into a room.

Philip Morris' research shows that the concentration of NNK in sidestream cigarette smoke increases at rates between 50% and 200% per hour after smoke is released from the cigarette (Table 1). There is no corresponding increase in the concentration of other TSNAs (21, 22). The reaction that produces NNK depends on nicotine concentration, NO concentration, and the presence of sidestream cigarette smoke (22), but the reaction mechanism and intermediates are not yet known. NNK concentration peaked between 3 and 6 h in the stainless steel experimental chamber (21) and between 1 and 2 h in the office (23).

**Limitations.** The initial particle concentration in the experiments done in the stainless steel chamber in Neuchâtel was



**Figure 4.** Varying initial NO concentration, while holding initial nicotine concentration constant (at  $50 \mu\text{mol}/\text{m}^3$ ), reveals a linear relationship between initial NO and NNK concentration in the first 1-h sample (time 0 to 1 h average). In the sample taken from hour 5 to 6, the effect of initial NO concentration seems to plateau. Reproduction of graph from Philip Morris internal report (22) with the units converted to micrograms per cubic meter of NNK.

<sup>4</sup> <http://www.beitraege-bti.de>, accessed November 10, 2006.

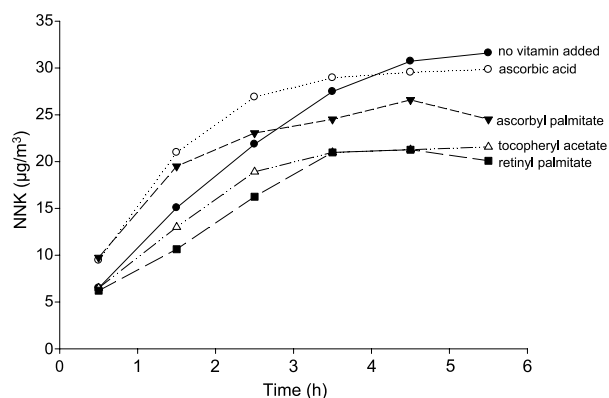
~24 mg/m<sup>3</sup> (21). This concentration, produced by burning 30 cigarettes in the 18.2-m<sup>3</sup> chamber, was chosen to optimize the detection of low concentrations of TSNA and became the standard protocol at Philip Morris. It is 10 to 20 times higher than the highest real-world concentrations reported in the open scientific literature (45, 46). The office experiments were done at initial particle concentrations of 21 mg/m<sup>3</sup> (23). It is possible that the chemical reactions forming NNK do not proceed at significant rates at the lower concentrations observed in real smoking environments.

Although these studies were also done in chambers with little or no ventilation, average contemporary home ventilation rates range between 0.5 and 3 air changes per hour, with the majority of values falling below 1 air change per hour (47-49). At these low air exchange rates, it seems likely that NNK formation in the air might contribute significantly to NNK concentrations in homes and workplaces.

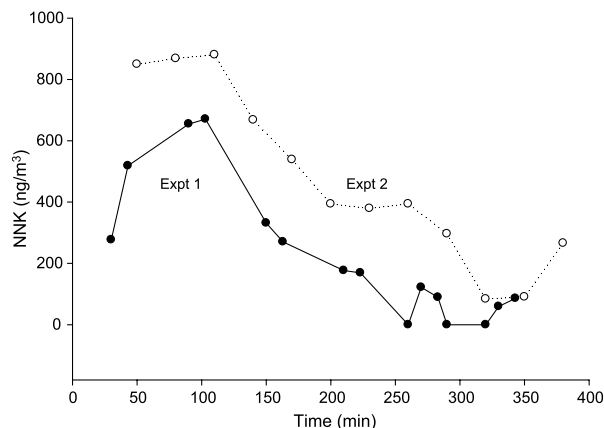
In a document titled "Plan for Project Tasso," dated 1992, a Philip Morris researcher states that "Although no direct, specific claims have yet been made about it in the literature, there is an increasing awareness among many ETS (environmental tobacco smoke) researchers of the possibility the nitrogen oxides could participate in a set of reactions with amines (both classes of compounds being important constituents of ETS), resulting in the formation of undesirable and products (nitrosamines) (50)."

Like the biological mechanisms driving nicotine addiction and the phenomena of compensation among smokers of light cigarettes, the formation of NNK in ambient sidestream smoke may have been common knowledge in the tobacco industry for years before the general scientific community was aware of it.

Our findings indicate that there is a need to study the kinetics of chemical reactions occurring in secondhand cigarette smoke in real-world environments. Methods in nitrosamine analysis have improved, and it is now more feasible to test TSNA concentrations in real smoking environments and at realistic experimental concentrations. NNK may not be the only carcinogenic or otherwise toxic compound formed as secondhand smoke ages. In previous papers (4, 5), we presented data from published and unpublished Philip Morris research which showed that sidestream smoke becomes 2 to 4 times more toxic (per unit mass) to the respiratory epithelium as it ages. These findings were based on short-term inhalation experiments with laboratory animal models and scored hyperplasia and dysplasia. Long-term inhalation experiments with laboratory animal models have not consistently shown the types of tumors that are associated with NNK



**Figure 5.** Addition of 0.05 µmol antioxidant vitamin to high-nicotine, burley cigarette filler reduced NNK formation between 5% and 40%. Reproduction of graph from Philip Morris internal report (22) with the units converted to micrograms per cubic meter of NNK.



**Figure 6.** NNK can also form in sidestream smoke in a furnished office, despite the large increase in adsorptive losses in the more complex physical environment. Experiment 1 shows a larger increase in NNK concentration because sampling began immediately after the cigarettes were extinguished. In experiment 2, sampling began 21 min after the cigarettes were extinguished. Reproduction of graph from Philip Morris internal report (23) with the units converted to micrograms per cubic meter of NNK.

exposure, so the contribution of elevated NNK concentrations in aging smoke to toxicity is not clear.

The precedent established in previous toxicologic experiments on sidestream cigarette smoke, by the tobacco industry (39, 40, 51-53) and by independent scientists (54-61), of aging smoke in a controlled manner before testing biological effects is sound. It would be useful for these researchers to validate their experimental proxies for SHS by comparing proxy NNK concentrations to those found in the real world.

Our analysis of Philip Morris' results suggests that NNK formation in aging secondhand smoke may contribute to nitrosamine exposure in humans. If NNK forms in the air in real smoking environments, and other nitrosamines do not, then exposure to NNK will be much higher than predicted from the concentration ratios in fresh smoke. Finally, these results are further evidence that smoke-free policies, governing all workplaces and public places, are the best method to protect the public from the known dangers of exposure to secondhand smoke.

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