Bull. Environ. Contam. Toxicol. (1997) 59:570-576

Bulletin of Environmental Contamination and Toxicology

Determination of Volatile *N*-Nitrosamines in Combustion Smoke Samples

H. Kataoka, M. Kurisu, S. Shindoh

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima,
Okayama 700, Japan

Received: 12 April 1997/Accepted: 11 June 1997

Volatile *N*-nitrosamines are well known as potent mutagens and carcinogens in experimental animals, and lead to a wide variety of tumours in many animals (Tricker and Preussmann 1991). These nitrosamines are widely distributed in various human environments such as foodstuffs (Hotchkiss 1987; Tricker and Preussmann 1991), drinking water (Tomkins and Griest 1996), drug formulations (Fine et al. 1977), agricultural chemical formulations (Frassanito et al. 1994), rubber products (Sen et al. 1987), tobacco and tobacco smoke (Bunneman et al. 1980; Tricker & Preussmann 1992) and ambient air (Fine et al. 1977; Fajen et al. 1979). *N*-Nitrosamines can be formed by the reaction of precursor secondary amines with nitrosating agents such as nitrite or nitrate found in the human diet (Tricker and Preussmann 1991), and *in vivo* in the stomach or small intestine of experimental animals (Lijinsky 1980; Van Stee et al. 1995). Moreover, it is considered that volatile *N*-nitrosamines in cigarette smoke are formed from decarboxylation of *N*-nitroso amino acids, pyrolytic nitrosation of secondary amines and concentrated decarboxylation/nitrosation of amino acids during combustion process (Tricker and Preussmann 1992). However, the presence and formation of these *N*-nitrosamines in other combustion smokes have not yet been reported.

Recently, we developed a selective and sensitive method for the determination of secondary amines by gas chromatography with flame photometric detection (FPD-GC), in which these compounds are analysed as their *N*-diethylthiophosphoryl (DETP) derivatives (Kataoka et al. 1995). Moreover, we developed a selective and sensitive method for the determination of volatile *N*-nitrosamines by using this technique after denitrosation with HBr, and applied it to the analysis of these *N*-nitrosamines in mainstream and sidestream smokes of cigarettes (Kataoka et al. 1996). In this paper, we investigated the presence and formation of volatile *N*-nitrosamines in combustion smokes of various materials such as foods, cigarettes, wood and rubber by using our developed FPD-GC methods.

Correspondence to: H. Kataoka

MATERIALS AND METHODS

N-Nitrosodimethylamine (NDMA) and *N*-nitrosomorphorine (NMOR) were purchased from Wako Pure Chemicals (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. *N*-Nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosopiperidine (NPIP) were purchased from Sigma (St. Louis, MO, USA). Each *N*-nitrosamine was dissolved in methylene chloride to make a stock solution at a concentration of 10 mg/mL and used after dilution with the same solvent to the required concentration. Phenylphosphonic acid diethyl ester (PPDE) as an internal standard (IS) for *N*-nitrosamine analysis was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and stocked as a 20 mg/mL solution in n-hexane. Dimethylamine (DMA) and diethylamine (DEA) as the hydrochlorides, di-*n*-butylamine (DBA), pyrrolidine (PYR), piperidine (PIP), morpholine (MOR), and *N*-methylcyclohexylamine (NMCHA) as an IS for secondary amine analysis were purchased from Nacalai Tesque. Each amine was dissolved in 0.05 *M* HCl containing 50% acetonitrile to make a stock solution at a concentration of 10 mg/mL and used after dilution with 0.05 *M* HCl to the required concentration. HBr (Nacalai Tesque) was used as a concentration of 47%. *o*-Phthaldialdehyde (OPA, Nacalai Tesque) was used as a 0.2 *M* solution in acetonitrile. Diethylchlorothiophosphate (DECTP, Tokyo Kasei Kogyo) was used as a 1% solution in acetonitrile after distillation. Peroxide-free diethyl ether was obtained from Dojindo Laboratories (Kumamoto, Japan), and the the diethyl ether containing 25% isopropanol was used as an extraction solvent. All other chemicals were of analytical-reagent grade.

Food, cigarettes, mosquito repellent, wood chips, rubber and plastic were purchased at local retail markets and were treated for analyses on the same day. As shown in Figure 1, a cigarette was smoked with a laboratory-made smoking machine (Pieraccini et al. 1992), and mainstream- and sidestream-smokes were separately bubbled through 25 mL of 5% HCl containing 20 m*M* ascorbic acid in trapping bottles. Other samples (ca. 1-8 g) were heated on hot plates (250-270°C) inside the chamber of this machine and the resulting combustion smokes were bubbled through the same trapping solution.

An aliquot (0.5-2 mL) of each HCl solution containing combustion smoke was pipetted into a 10-mL Pyrex glass tube with a PTFE-lined screw-cap, and then extracted twice with half-volume of diethyl ether containing 25% isopropanol. After centrifugation at 2000 X g for 1 min, the organic layer (upper layer) was transferred to another tube, and the combined organic layer called the *N*-nitrosamine fraction. On the other hand, the remaining aqueous layer (under layer) was called the amine fraction. Both fractions were used as the sample for derivatization.

Derivatization of secondary amines and *N*-nitrosamines was performed by previously reported methods (Kataoka et al. 1995, 1996). For secondary amine analysis, to the amine fraction was added 0.1 mL of 10 µg/mL NMCHA (IS), and

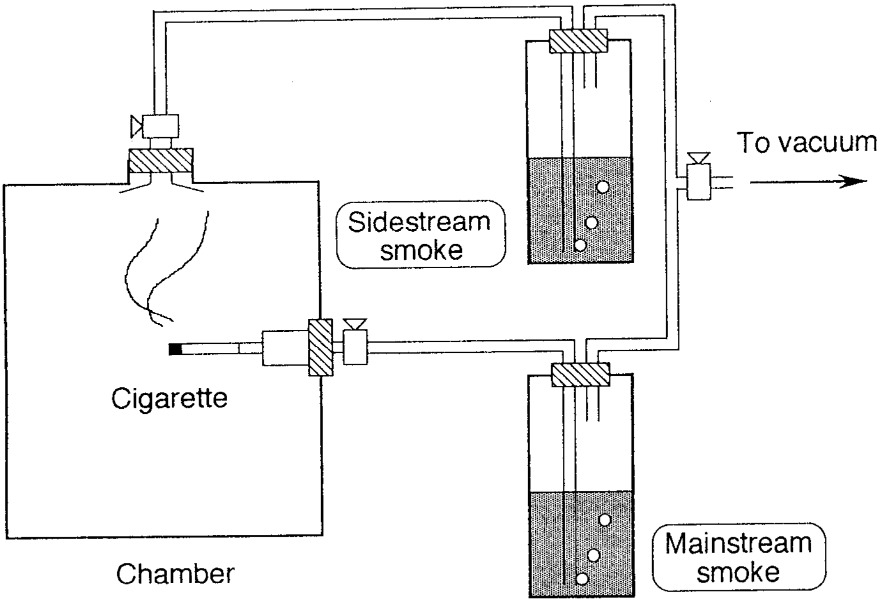

Figure 1. Laboratory-made smoking machine used to collect combustion smoke. For details see text.

the mixture was neutralized with 2 *M* NaOH. To the mixture was added 0.2 mL of 0.5 *M* phosphate buffer (pH 8) and 0.2 mL of 0.2 *M* OPA, and the mixture was allowed to stand for 2 min at room temperature. To the reaction mixture were added 0.2 mL of 10% Na_2CO_3 and 0.2 mL of 1% DECTP, and then the mixture was *N*-diethylthiophosphorylated by incubation at 60°C for 10 min after tightly capping. In order to remove the excess of reagent, the reaction mixture was incubated again at 60°C for 5 min after addition of 0.2 mL of 50 m*M* cysteic acid. The reaction mixture was extracted with 0.2-0.4 mL of *n*-hexane and 1 µL of this extract was injected into the FPD-GC system. On the other hand, *N*-nitrosamines were derivatized as secondary amines after denitrosation with HBr to produce the corresponding secondary amines. After evaporation to dryness of solvent in *N*-nitrosamine fraction at 80°C under a gentle stream of nitrogen (accomplished within a few minutes), 20 µL of HBr were added to the residue. Then the test tube was immediately capped and heated for 5 min at 100°C. After cooling, to the reaction mixture was added 0.5 mL of distilled water, and then the mixture was neutralized with 2 *M* NaOH. To this solution were added 0.2 mL of 1% DECTP and 0.2 mL of 10% Na_2CO_3, and then the mixture was *N*-diethylthiophosphorylated by the same procedure as secondary amine analysis. After cysteic acid treatment, the reaction mixture was extracted with 0.2-0.4 mL of *n*-hexane containing 0.4 µg of PPDE (IS) and 1 µL of this extract was injected into the FPD-GC system.

GC analysis was carried out with a Shimadzu 14A gas chromatograph equipped with a flame photometric detector (P-filter). A fused silica capillary column (15 m X 0.53 mm I.D., 1.0 µm film thickness) of cross-linked DB-1701 (J & W, Folsom, CA, USA) was used. The operating conditions were as follows: column temperature, programmed from 100°C to 260°C at 10°C/min; injection and detector temperature, 280°C; nitrogen flow-rate, 10 mL/min. The peak heights of secondary amines or *N*-nitrosamines and each IS were measured and the peak height ratios against the IS were calculated.

RESULTS AND DISCUSSION

The volatile *N*-nitrosamines in environmental cigarette smoke, to which both smokers and non-smokers are exposed, have received a great deal of attention as a source of indoor air pollutions. The presence of NDMA, NMEA, NPYR and NPIP in cigarette smoke samples had already been reported (Bunneman et al. 1980; Tricker and Preussmann 1992). We also detected NDMA, NPYR and NPIP in the smoke samples of four commercial cigarettes in a previous study (Kataoka et al. 1996). In order to confirm the formation of these N-nitrosamines during smoking, *N*-nitrosamines and their precursor secondary amines were determined in the cigarette smoke and cigarette leaf extracts (extracted with 5% HCl containing 20 m*M* ascorbic acid). As shown in Figure 2, the contents of NDMA, NPYP and NPIP in cigarette smoke were found to be 2-25 times higher than those in cigarette leaves. On the other hand, the contents of precursor secondary amines for above *N*-nitrosamines in cigarette smoke were found to be lower than those in cigarette leaves, except for DMA which can be produced by pyrolysis of amino acids and proteins. MOR was also detected at low levels in both samples, but the corresponding *N*-nitrosamine, NMOR, was not detectable. These results indicate that the *N*-nitrosamines in cigarette smoke are formed from pyrolytic nitrosation of secondary amines during smoking. In fact, the percentage of NPYR formation by pyrolytic nitrosation of PYR in tobacco is estimated at about 37%, and it is considered that the nitrosation agent is NOx emitted during combustion of cigarettes. Furthermore, these results suggest that these *N*-nitrosamines may be formed through combustion of various materials containing secondary amines.

It is well known that secondary amines are present in various foods (Pfundstein et al. 1991; Kataoka et al. 1995) and environmental samples (Kataoka 1996). Therefore, we determined *N*-nitrosamines in combustion smokes of these samples containing secondary amines to elucidate a similar possibility of *N*-nitrosamine formation during combustion. Table 1 shows the *N*-nitrosamine contents in combustion smokes of various materials such as foods, wood and rubber. The data are represented as amount of nitrosamine in the smoke per amount of the burnt sample. Among six *N*-nitrosamines tested in this study, NDMA, NPYR, NPIP and NMOR were detected in these smoke samples, but NDEA and NDBA were not detectable. The combustion smoke of black pepper, containing high concentration

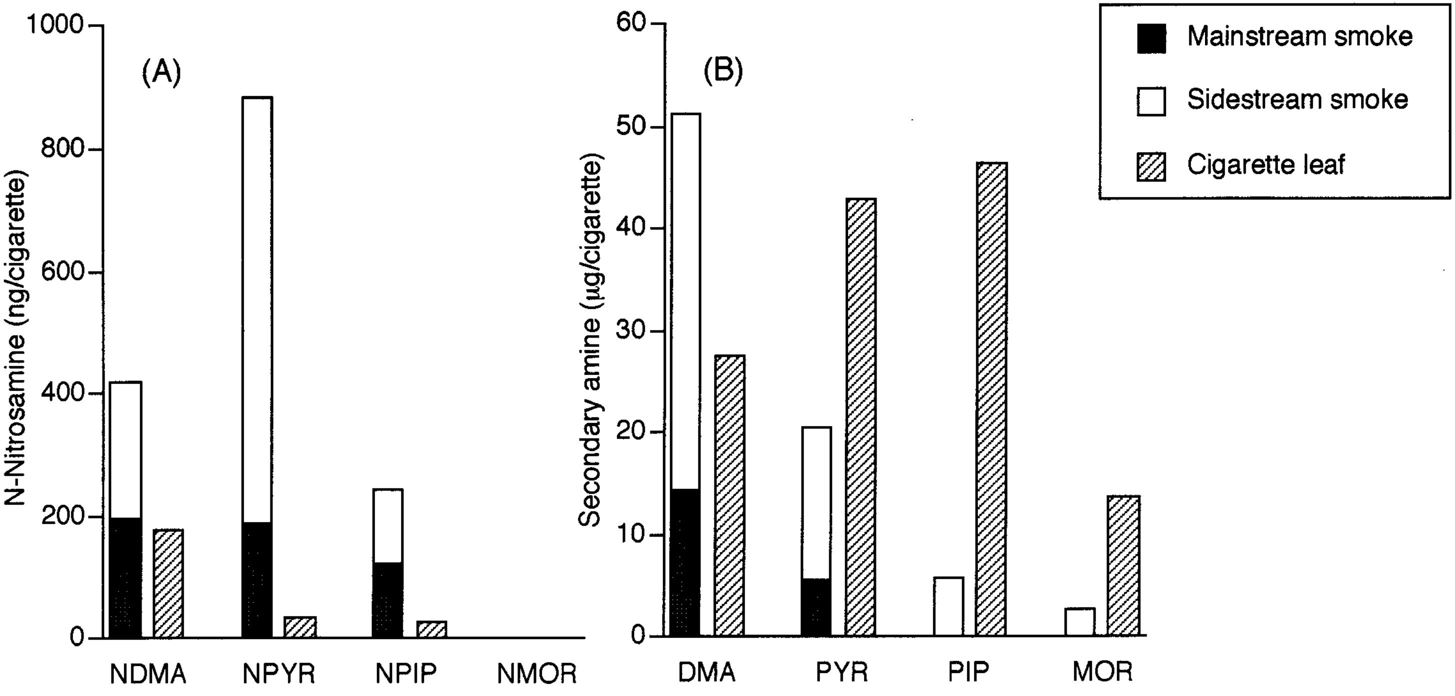

Figure 2. Distribution of (A) *N*-nitrosamines and (B) precursor secondary amines in cigarette smoke and cigarette leaf. *N*-Nitrosamines: NDMA=*N*-nitrosodimethylamine; NPYR=*N*-nitrosopyrrolidine; NPIP=*N*-nitrosopiperidine; NMOR= *N*-nitrosomorpholine. Secondary amines: DMA=dimethylamine; PYR=pyrrolidine; PIP=piperidine; MOR=morphorine.

Table 1. Contents of *N*-nitrosamines in combustion smokes of various samples

Sample	Content[a] (ng/g)			
	NDMA	NPYR	NPIP	NMOR
Black pepper	127 ± 4	750 ± 3	45030 ± 1140	ND[b]
Soy sauce	39 ± 2	64 ± 4	299 ± 6	ND
Semi-dried fish	46 ± 2	333 ± 4	ND	ND
Dried squid	2250 ± 59	2040 ± 20	516 ± 23	ND
Pork vienna	ND	37 ± 1	ND	ND
Chicken	ND	54 ± 3	155 ± 3	ND
Beef	ND	72 ± 3	147 ± 3	ND
Cigarette (mainstream)	278 ± 20	270 ± 17	231 ± 11	ND
Cigarette (sidestream)	318 ± 23	996 ± 79	231 ± 13	ND
Mosquito repellent	115 ± 18	17 ± 0	405 ± 24	ND
Hair	1073 ± 59	940 ± 28	540 ± 24	ND
Wood chips	56 ± 1	ND	21 ± 1	17 ± 1
Rubber	35 ± 3	ND	ND	7204 ± 545
Plastic	195 ± 6	ND	ND	ND

[a] Mean ± SD (n=3). NDMA = *N*-nitrosodimethylamine; NPYR = *N*-nitrosopyrrolidine; NPIP = *N*-nitrosopiperidine; NMOR = *N*-nitrosomorphorine

[b] Not detectable.

of PIP (Kataoka et al. 1995), was found to contain very high concentration of NPIP. The combustion smoke of dried squid contained high concentration of NDMA and NPYR. The combustion smoke of hair also contained relatively high concentration of NDMA, NPYR and NPIP. NPYR was detected in most combustion smokes of foods. NDMA, NPYR and NPIP were widely distributed in the combustion smokes of various samples tested in this study, but NMOR was detected in the combustion smoke of rubber at high concentration. MOR has been used in the rubber and tire industry and NMOR has been detected in the factory air (Fajen et al. 1979). Furthermore, the presence of *N*-nitrosamines in the combustion smoke of mosquito repellent is noteworthy, because humans are continuously exposed to this smoke in the closed room. These results suggest that *N*-nitrosamines are ubiquitous air pollutants and humans are continually exposed to these compounds in normal daily life.

REFERENCES

Brunnemann KD, Fink W, Moser F (1980) Analysis of volatile *N*-nitrosamines in mainstream and sidestream smoke from cigarettes by GLC-TEA. Oncology 37: 217-222

Fajen JM, Carson GA, Roinbehler DP, Fan TY, Vita R, Goff UE, Wolf MH, Edwards GS, Fine DH, Reinhold V, Biemann K (1977) N-Nitrosamines in the rubber and tire industry. Science 205: 1262-1264

Fine DH, Rounbehler DP, Fan T, Ross R (1977) Human exposure to *N*-nitroso compounds in the environment. In: Hiatt HH, Watson JD, Winsten JA (ed) Origins of Human Cancer, Cold Spring Harbor Laboratory University of Tokyo Press, p 293-307

Frassanito R, Benfenati E, Ciotti G, Fanelli R (1994) A simple method for determination of *N*-nitrosamine traces in trifluralin samples by gas chromatography-mass spectrometry. Toxicol Environ Chem 45: 199-204

Hotchkiss JH (1987) A review of current literature on *N*-nitroso compounds. Adv Food Res 31: 53-115

Kataoka H, Shindoh S, Makita M (1995) Determination of secondary amines in various foods by gas chromatography with flame photometric detection. J Chromatogr 695: 142-148

Kataoka H, Shindoh S, Makita M (1996) Selective determination of volatile *N*-nitrosamines by derivatization with diethyl chlorothiophosphate and gas chromatography with flame photometric detection. J Chromatogr A 723: 93-99

Kataoka H (1996) Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis. J Chromatogr A 733: 19-34

Lijinsky W (1980) Significance of *in vivo* formation of *N*-nitroso compounds. Oncology 37: 223-226

Pfundstein B, Tricker AR, Theobald E, Spiegelhalder B, Preussmann R (1991) Mean daily intake of primary and secondary amines from foods and beverages in West Germany in 1989-1990. Food Chem Toxicol 29: 733-739

Pieraccini G, Luceri F, Moneti G (1992) New gas chromatographic/mass spectrometric method for the quantitative analysis of primary aromatic amines in main- and side-stream cigarette smoke. Rapid Comm Mass Spectrom 6: 406-409

Sen NP, Seamen SW, Kushwaha SC (1987) Improved method for determination of volatile nitrosamines in baby bottle rubber nipples and pacifiers. J Assoc Off Anal Chem 70: 434-438

Tomkins BA, Griest WH (1996) Determinations of *N*-nitrosodimethylamine at part-per-trillion concentrations in contaminated groundwaters and drinking waters. Anal Chem 68: 2533-2540

Tricker AR, Preussmann R (1991) Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. Mut Res 259: 277-289

Tricker AR, Preussmann R (1992) Volatile *N*-nitrosamines in mainstream cigarette smoke: occurrence and formation. Clin Invest 70: 283-289.

Van Stee EW, Sloane RA, Simmons JE, Moorman MP, Brunnemann KD (1995) Endogeneous formation of *N*-nitrosomorpholine in mice from $^{15}NO_2$ by inhalation and morpholine by gavage. Carcinogenesis 16:89-92