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Determination of Mutagenic Heterocyclic Amines in Combustion Smoke Samples

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Heterocyclic amines formed during heating (cooking, broiling, frying or grilling) of various proteinaceous foods are well known as potent mutagens in the Ames/*Salmonella* assay (Felton & Knize 1991; Eisenbrand & Tang 1993; Stavric 1994). Up to the present, twenty-three heterocyclic amines have been isolated as mutagens and the structures of nineteen of them have been determined. Some heterocyclic amines have much higher mutagenic activity than typical mutagens/carcinogens such as aflatoxin B_1, AF-2 and benzo[a]pyrene. Among these amines, Trp-P-l, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx and PhIP have been verified to be carcinogenic in rats and mice, and IQ was also found to be carcinogenic in the monkey (Ohgaki et al. 1991; Eisenbrand & Tang 1993; Adamson et al. 1996; Sugimura et al. 1996). Many of these heterocyclic amines have been isolated and identified not only from cooked foods, but also from various environmental components such as airborne particles and diesel-exhaust particles (Manabe et al. 1991, 1993), cigarette smoke and indoor air (Manabe et al. 1992; Manabe & Wada 1990), cooking fumes (Thieband et al. 1995) and rain water (Wu et al. 1995). Moreover, it is considered that these environmental heterocyclic amines are likely to be emitted into the atmosphere through combustion of a large variety of combustible materials such as grass, wood, garbage and petroleum, and discharged into the water through rain, domestic waste and human waste. However, the data on the emission and discharge of these compounds into various environments are little.

Recently, we developed a selective and sensitive method for the determination of heterocyclic amines by gas chromatography with nitrogen-phosphorus detection (NPD-GC), in which these compounds are analysed as their *N*-dimethylaminomethylene derivatives (Kataoka & Kijima 1997). In this paper, we investigated the presence of mutagenic heterocyclic amines in combustion smokes of various materials such as cigarette, foods, wood and rubber by using our developed NPD-GC method.

MATERIALS AND METHODS

The heterocyclic amines used in this study are listed in Figure 1. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was purchased from Toronto Research

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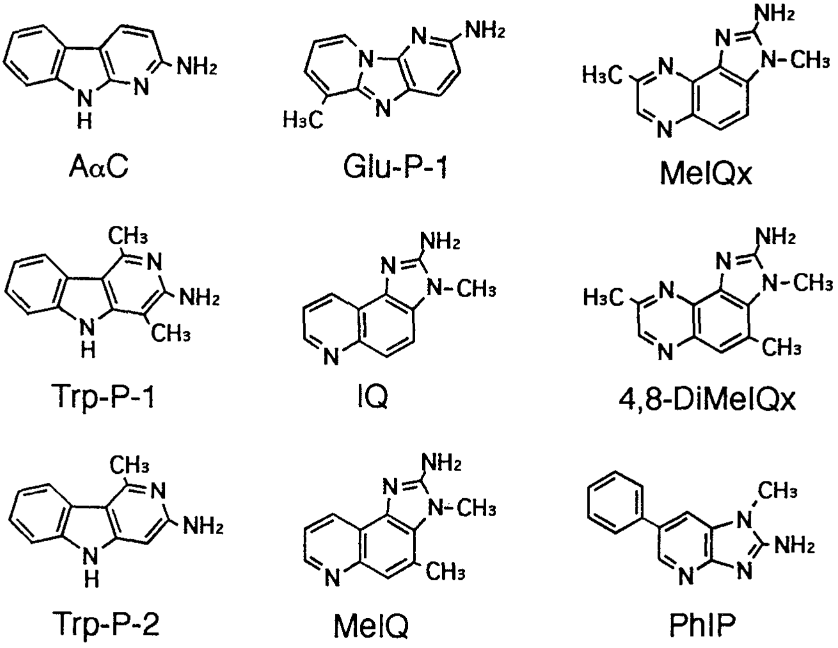

Figure 1. Chemical structures of heterocyclic amines used in this study.

Chemicals (Downsview, Canada). 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-9*H*-pyrido[2,3-b]indole (AαC) were purchased from Funakoshi Pharmaceutical Co. Ltd. (Tokyo, Japan). 3-Amino-1,4-dimethyl-5*H*-pyrido[3,4-b]indole (Trp-P-1) was purchased from Wako Pure Chemical Industries (Osaka, Japan). 3-Amino-1-methyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-2), 2-amino-6-methyldipyrido[1,2-α:3'2'-*d*]imidazole (Glu-P-1) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were kindly provided by Dr. H. Hayatsu, Professor of Okayama University. 2-Amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (TriMeIQx; Funakoshi) was used as an internal standard (IS). Each heterocyclic amine was dissolved in methanol to make a stock solution at a concentration of 0.1 mg/mL and used after dilution with methanol to the require concentration. N,N-Dimethylformamide dimethyl acetal (DMF-DMA) was purchased from Nacalai Tesque (Kyoto, Japan). Blue-rayon was obtained from Funakoshi. All other chemicals were of analytical-reagent grade.

Cigarettes, foods, mosquito repellent, wood chips and rubber were purchased at local retail markets and were treated for analyses on the same day. Cigarettes were smoked with a laboratory-made smoking machine developed by Pieraccini and co-workers (Pieraccini et al. 1992), and mainstream- and sidestream-smokes were separately bubbled through 50 mL of 0.1 *M* HCl containing 20 tiascorbic acid in trapping bottles. Other samples (ca. 1-8 g) were heated on a hot plate (250-270°C) inside the chamber of this machine and the resulting combustion smokes were bubbled through the same trapping solution.

Each combustion smoke solution was neutralized with 2 *M* NaOH, and then made up to 100 mL with distilled water. Heterocyclic amines in this smoke sample solution were extracted by Blue-rayon adsoption method (Hayatsu et al. 1992). Two parts of 50 mg Blue-rayon were added to the smoke sample solution and shaken with a shaker set at 300 r.p.m. (left and right) for 5 min each time. After filtration by suction, the combined Blue-rayon was washed twice with 200 mL of distilled water and dried with a paper towel. Then the heterocyclic amines adsorbed on blue rayon were eluted three times with 10 mL of MeOH-28% NH_3(50:1). The combined eluate was evaporated to dryness and the residue was dissolved in 4 mL of 0.1 *M* HCl. To this solution was added 10 ng of IS and the mixture was transfered to 10-mL Pyrex glass tube with a PTFE-lined screw-cap. After washing with 4 mL of n-hexane, the aqueous layer was adjusted to pH>10 with 28% NH_3 and then extracted twice with 4 mL of dichloromethane. After evaporation to dryness of the combined organic layer, the residue was dissolved in MeOH and used for derivatization of the sample. The clean-up process of combustion smoke sample is shown in Figure 2.

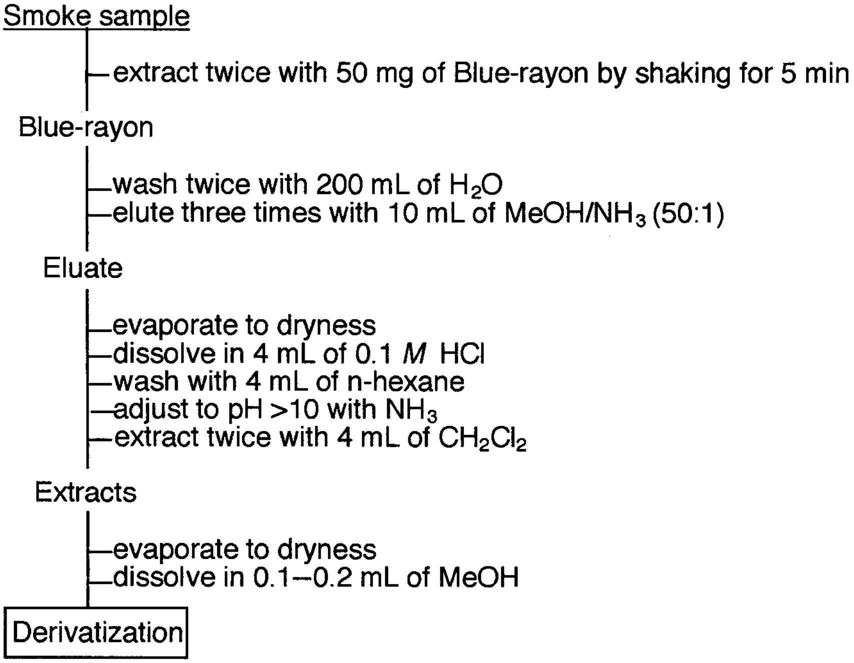

Figure 2. Clean-up of combustion smoke sample.

Derivatization of heterocyclic amines was performed by the previously reported methods (Kataoka & Kijima 1997). An aliquot of the sample containing 0.5-10 ng of heterocyclic amines was pipetted into a 10-mL Pyrex glass tube with a PTFE-lined screw-cap. To this solution was added 10 ng of IS (if necessary) and 10 µL of DMF-DMA, and the mixture (0.1-0.5 mL) was heated at 100°C for 15 min without capping the tube. After evaporation to dryness, the residue was dissolved in 20-40 µL of ethyl acetate and then 1 µL of this solution was injected into the gas chromatograph.

GC analysis was carried out with a Hewlett-Packard 5890 Series II gas chromatograph equipped with an electronic pressure control (EPC) system, a split/splitless capillary inlet system, and a nitrogen-phosphorus detector (NPD). Two connected fused-silica capillary columns (J & W, Folsom, CA, USA) containing DB-1 (10 m X 0.25 mm I.D., film thickness 0.25 µm) and DB-17ht (10 m X 0.25 mm I.D., film thickness 0.15 µm), respectively, with a two-way press fit fused-silica tube were used. The operating conditions were as follows: column temperature, programmed at 10°C/min from 230 to 280°C programmed at 25°C/min from 280 to 330°C and held at 330°C for 1 min; injection and detector temperature, 340°C. The inlet helium pressure (flow-rate) controlled with EPC, was programmed at 10 kPa/min from 180 (2.05 mL/min) to 230 kPa (2.5 mL/min), programmed at 25 kPa/min from 230 (2.5 mL/min) to 280 kPa (2.9 mL/min) and held at 280 kPa (2.9 mL/min) for 1 min. Make-up gas flow-rate: 30 mL/min; split ratio: 10:1. A chromatographic run (run made with no sample injected) data was subtracted from sample run data to remove baseline drift (usually caused by column bleed) using a single-column compensation function and then base-line corrected data was recorded on the chromatogram. The peak height ratios of heterocyclic amines and the IS were measured and the peak height ratios against the IS were calculated to construct calibration curves.

RESULTS AND DISCUSSION

Heterocyclic amines are present in the environment at low parts per billion or less. Therefore it is not too much to say that the clean-up procedure for the complex sample matrix greatly influences the reliability and accuracy of the analysis for these compounds. In order to achieve an efficient isolation and preconcentration of heterocyclic amines, we used the Blue-rayon adsorption method developed by Hayatsu and co-workers (Hayatsu 1992). Blue rayon, rayon bearing covalently linked copper phtharocyanine trisulphonate as ligand, can selectively adsorb heterocyclic amines and other mutagens/carcinogens having polycyclic planar molecular structures, so that its batch-wise use is an effective means to concentrate heterocyclic amines in aqueous solution. As shown in Table 1, overall recoveries of heterocyclic amines in cigarette smoke by this technique were 32-105%, and relative standard deviations were 0.1-6.4% (*n*=3). The recoveries of these amines were influenced from coexisting substances, except for AαC and Trp-P-2.

Table 1. Recoveries of heterocyclic amines added to cigarette smoke samples

Amine	Added (ng/cigarette)	Recovery^a (%)	
		Mainstream smoke	Sidestream smoke
AαC	10	105.1±0.3	95.2±0.1
Glu-P-1	10	76.9±2.0	47.9±0.5
Trp-P-1	10	68.1±1.3	47.6±0.6
Trp-P-2	10	104.4±0.8	86.4±5.5
IQ	10	74.3±0.8	32.4±0.8
MeIQ	10	84.7±3.6	44.2±0.3
MeIQx	10	81.1±0.1	43.2±0.1
DiMeIQx	10	74.7±0.1	48.4±0.3
PhIP	10	81.5±0.5	77.9±0.6

^{a}Mean ± SD (n=3).

Heterocyclic amines isolated by Blue-rayon adsorption method could be easily converted into their *N*-dimethylaminomethylene derivatives by a previously reported NPD-GC method (Kataoka & Kijima 1997). Figure 3 shows the typical chromatograms obtained from cigarette sidestream smoke and combustion smoke of semi-dried fish. NPD-GC method was selective and sensitive for heterocyclic amines, and combustion smoke samples could be analyzed without any interference from coexisting substances, although some unknown peaks were observed on the chromatogram. The quantitation limits of heterocyclic amines in combustion smoke samples were ca. 50 pg/g.

Table 2 shows the heterocyclic amine contents in combustion smokes of various materials such as cigarette, foods, wood and rubber. The data are presented as amount of heterocyclic amine in the smoke per amount of the burnt sample, after correction for recoveries of these compounds from each sample. Among nine heterocyclic amines tested in this study, IQ was detected in most combustion smokes of these samples, but MeIQx and DiMeIQx were not detectable. Cigarette sidestream smokes contained high concentration of AαC and PhIP. Although AαC, MeAαC, Trp-P-1, Trp-P-2, IQ and PhIP have been detected in cigarette smoke (Manabe & Wada 1990; Manabe et al. 1993), we newly detected MeIQ.The combustion smoke of semi-dried fish also contained relatively high concentration of AαC, Trp-P-2 and PhIP. AαC, Trp-P-1, IQ and MeIQ were widely distributed in the combustion smokes of various samples tested in this study, but Glu-P-1 was detected in the combustion smoke of black pepper at high concentration. Furthermore, the presence of AαC and IQ 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 heterocyclic amines are ubiquitous air pollutants and humans are continually exposed to these compounds in normal daily life.

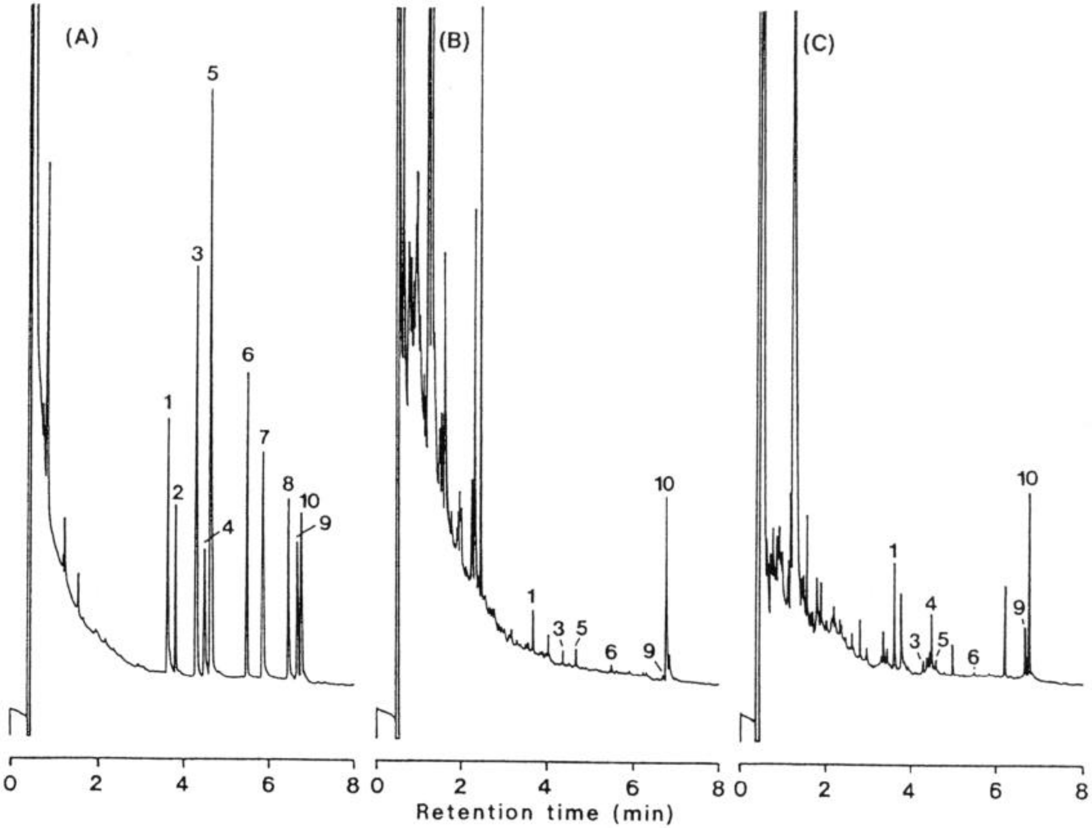

Figure 3. Gas chromatograms obtained from (A) standard heterocyclic amines, (B) cigarette sidestream smoke (C) combustion smoke of semi-dried fish. GC conditions are given in the text. Peaks: 1=AαC, 2=Glu-P-l, 3= Trp-P-l, 4=Trp-P-2,5=IQ, 6=MeIQ, 7=MeIQx, 8=DiMeIQx, 9=PhIP, 10=TriMeIQx (IS).

Table 2. Contents of heterocyclic amines in combustion smokes of various samples

Sample	Content[a] (ng/g)								
	AαC	Glu-P-1	Trp-P-1	Trp-P-2	IQ	MeIQ	MeIQx	DiMeIQx	PhIP
Cigarette A									
Mainstream	1.96±0.08	ND[b]	0.33±0.01	ND	0.33±0.01	0.28±0.003	ND	ND	ND
Sidestream	5.00±0.07	ND	0.27±0.002	ND	0.61±0.01	0.45±0.01	ND	ND	1.48±0.03
Cigarette B									
Mainstream	ND	ND	0.74±0.04	ND	0.49±0.02	0.75±0.01	ND	ND	ND
Sidestream	6.51±0.09	ND	0.37±0.002	ND	0.16±0.002	0.25±0.004	ND	ND	2.09±0.05
Black pepper	1.60±0.17	4.06±0.72	0.51±0.08	0.66±0.03	0.35±0.06	ND	ND	ND	2.24±0.44
Semi-dried fish	3.86±0.77	ND	0.79±0.08	3.70±0.18	0.22±0.04	0.19±0.01	ND	ND	3.92±0.03
Wood chips	ND	ND	ND	ND	2.26±0.34	ND	ND	ND	ND
Rubber	ND	ND	0.61±0.07	1.98±0.07	0.19±0.03	0.15±0.02	ND	ND	ND
Mosquito repellent	10.07±0.27	ND	ND	ND	1.94±0.02	ND	ND	ND	ND

[a] Mean ± SD (*n*=3).

[b] Not detectable.

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