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Review

Methods for the determination of mutagenic heterocyclic amines and their applications in environmental analysis

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Abstract

The environmental analysis of heterocyclic amines is important to preserve human health, because all these compounds are potent mutagens and many are carcinogens in experimental animals. This review summarizes the analytical methods for the determination of mutagenic heterocyclic amines and their applications to various environmental samples. High-performance liquid chromatographic methods with ultraviolet, electrochemical and fluorescence detections are commonly used for the quantification of heterocyclic amines. Moreover, liquid chromatography–mass spectrometry, gas chromatography, gas chromatography–mass spectrometry, capillary zone electrophoresis and enzyme-linked immunosorbent assay are also developed. Heterocyclic amines in complex environmental matrices are present at low parts per billion or less, so that the sample preparation influences the reliable and accurate analysis of these compounds. Mutagenic heterocyclic amines are widely distributed in a number of ambient environmental components such as airborne particles, diesel-exhaust particles, cigarette smoke, cooking fumes, rain water, sewage water, incineration-ash and soil. These facts suggest that mutagenic heterocyclic amines are likely to be ubiquitous environmental pollutants and humans are continually exposed to these compounds in normal daily life. © 1997 Elsevier Science B.V.

Keywords: Reviews; Environmental analysis; Water analysis; Soil; Cigarette smoke; Diesel exhaust; Air analysis; Amines, heterocyclic

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1. Introduction

1.1. Heterocyclic amines from environmental sources and the need for developing an analytical method

As the problems of environmental pollution are very close to daily life of people everywhere in the world, the occurrence and toxicity of hazardous chemicals in the biosphere have received a great deal of attention in recent years. The environment contains a variety of naturally occurring and man-made mutagens and carcinogens to which humans are exposed. Among these compounds, a new series of heterocyclic amines has been found in cooked foods and pyrolysates of amino acids and proteins [1–9]. Up to the present, twenty-three heterocyclic amines have been isolated as potent mutagens in the Ames/*Salmonella* assay, and the structures of nineteen of them have been determined [8,9]. The abbreviations and structures of these compounds are shown in Tables 1 and 2. Many of these mutagenic heterocyclic amines have been isolated and identified not only from various proteinaceous foods including cooked meats and fish [4–9,11–13], but also from environmental components such as outdoor air [14–16], indoor air [15,17], diesel-exhaust particles [16,18], cigarette smoke [15,17,19–24], cooking fumes [25–27], rain water [14,28], incineration-ash [16] and soil [15]. Moreover, some mutagenic heterocyclic amines have been detected in biological samples such as urine, plasma, bile and faeces [10,29–32]. These facts suggest that heterocyclic amines may be emitted into the atmosphere through combustion of various materials such as foods, wood, grass, garbage and petroleum, and discharged into the water through domestic waste and human

waste, although their mechanisms have not been determined.

All the heterocyclic amines identified show high mutagenic activity in *Salmonella typhimurium* T98 (a detector of frameshift mutations) and *Salmonella typhimurium* T100 (a detector of base-pair change mutations) in the presence of a metabolic activation system (S9 mix) [1,2,4,5,8,12,33,34]. These heterocyclic amines are also mutagenic in mammalian cell systems and can produce chromosomal aberrations and sister chromatid exchanges in cultured cells [35–39]. Some heterocyclic amines have much higher mutagenic activity than typical mutagens/carcinogens such as aflatoxin B₁, AF-2 and benzo[*a*]pyrene. Among the known mutagenic heterocyclic amines, Trp-P-1, 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 when administered in the diet at concentrations of 100–800 ppm [1,4,5,8,34,40–42]. These compounds induced tumours in the liver, small and large intestine, Zymbal gland, clitoral gland, skin, oral cavity and mammary gland in rats, and the liver, forestmach, lung, hematopoietic system, lymphoid tissue and blood vessels in mice. IQ was also found to be carcinogenic in the monkey, inducing hepatocellular carcinomas when given by nasal-gastric incubation 5 times per week at 10 or 20 mg/kg [34,43]. Moreover, recent investigations revealed that heterocyclic amines also possess cardiotoxic effect [41] and various pharmacotoxicological activities such as convulsant activities [44,45] and potent inhibitory effects on platelet function and dopamine metabolism [45–48].

However, significant risks on human health and environment through long-term exposure and bioaccumulation of heterocyclic amines are scientifically

Table 1
Isolated pyrolytic mutagens (Group I heterocyclic amines)

Chemical name	Abbreviation	Structure	Precursor	Reference
Pyridoindole				
3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole	Trp-P-1 *		Tryptophan	[49]
3-Amino-1-methyl-5H-pyrido[4,3-b]indole	Trp-P-2 *		Tryptophan	[49]
2-Amino-9H-pyrido[2,3-b]indole	AαC *		Soybean globulin	[50]
2-Amino-3-methyl-9H-pyrido[2,3-b]indole	MeAαC *		Soybean globulin	[50]
Pyridoimidazole				
2-Amino-6-methyl-dipyrido[1,2-α:3',2'-d]imidazole	Glu-P-1 *		Glutamic acid	[51]
2-Aminodipyrido[1,2-α:3',2'-d]imidazole	Glu-P-2 *		Glutamic acid casein	[51,52]
Phenylpyridine				
2-Amino-5-phenylpyridine	Phe-P-1		Phenylalanine	[49]
Tetraazafluoranthene				
4-Amino-6-methyl-1H-2,5,10,10b-tetraazafluoranthene	Orn-P-1		Ornithine	[53]
Benzimidazole				
4-Amino-1,6-dimethyl-2-methylamino-1H,6H-pyrrolo[3,4-f]benzimidazole-5,7-dione	Cre-P-1		Creatine	[54]

*=Carcinogen.

unclear. Therefore, the presence, identity and quantity of these compounds in the environment should be established to evaluate possible health hazards from heterocyclic amines. In many cases, the levels of environmental heterocyclic amines are very low, usually of the order of a few parts per billion or less. In the past, analytical methodology for the isolation and quantification of heterocyclic amines has not been well developed, although recently a significant improvement in these techniques has been reported. Therefore, it is a matter of urgency to develop a

useful method for monitoring the exposure levels of environmental heterocyclic amines.

1.2. Chemical structures and formation of heterocyclic amines

Mutagenic heterocyclic amines are formed during the heating process. This formation is dependent on time, temperature, pH, type and concentration of precursors and the presence of certain divalent ions [2,3,5,6,12,13]. These heterocyclic amines are classi-

Table 2
Isolated thermic mutagens (Group II heterocyclic amines)

Chemical name	Abbreviation	Structure	Precursor	Reference
<i>Imidazoquinoline</i>				
2-Amino-3-methylimidazo[4,5-f]quinoline	IQ *		Creatine, Gly, Phe, Ser, Pro, fructose, glucose	[55-58]
2-Amino-3,4-dimethylimidazo[4,5-f]quinoline	MeIQ *		Creatine, Ala, fructose	[59]
<i>Imidazoquinoxaline</i>				
2-Amino-3-methylimidazo[4,5-f]quinoxaline	IQx		Creatine, Gly, Ser, glucose	[60]
2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline	MeIQx *		Creatine, Gly, Ala, Thr, Lys, fructose, glucose, ribose	[56,60-66]
2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline	4,8-DiMeIQx		Creatine, Ala, Thr, Lys, fructose, glucose, ribose	[59-62,67]
2-Amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline	7,8-DiMeIQx		Creatine, Gly, glucose	[60,68]
2-Amino-4-hydroxy-methyl-3,8-dimethylimidazo[4,5-f]quinoxaline	4-CH2OH-8-MeIQx		Creatine, Thr	[69,70]
2-Amino-1,7,9-trimethylimidazo[4,5-g]quinoxaline	7,9-DiMeIQx		Creatine, Thr, glucose	[70]
<i>Imidazopyridine</i>				
2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine	PhIP *		Creatine, Phe, glucose	[58,71,72]
2-amino-1-methyl-6-(4'-hydroxyphenyl)imidazo[4,5-b]pyridine	4'OH-PhIP		Creatine, Tyr, glucose	[70,73,74]

*=Carcinogen.

fied into at least two groups due to the formation process. Heterocyclic amines formed at high temperatures above 300°C are known as 'pyrolytic mutagens' (group I) and those formed at temperatures less than 300°C are known as 'thermic mutagens' (group II). However, the formation of heterocyclic amines is almost negligible at 100°C. The structures of two groups are shown in Tables 1

and 2, respectively. All have a characteristic multi-ring aromatic structure with one or more nitrogen atoms in their ring system and exocyclic amino group.

The first group 'pyrolytic mutagens' can be structurally divided into five groups, pyridoindoles, pyridoimidazoles, phenylpyridine, tetraaza-fluoranthrene and benzimidazole. These heterocyclic amines

are formed by pyrolysing single amino acids such as tryptophan [49], glutamic acid [51], phenylalanine [49] and ornithine [53], single proteins such as soybean globulin [50] and casein [52], or creatine [54]. Many of these pyrolytic mutagens are demonstrated in cooked foods and cigarette smoke.

The second group 'thermic mutagens' can be structurally divided into three groups, imidazoquinolines, imidazoquinoxalines and imidazopyridines. The main precursors of these heterocyclic amines are creatine/creatinine, free amino acids and sugars [55–74]. The precursor undergoes further dehydration and cyclization to form the observed pyrrole and pyridine derivatives. The heterocyclic pyridines and pyrazines formed in the Maillard reaction between hexose and amino acids then undergo further transformation with participation of aldehydes and creatinine to produce imidazoquinolines and imidazoquinoxalines. Various thermic mutagens are formed from different amino acids used in the model systems and their mutagenic activities differed for these amino acid precursors [12]. Many of these thermic mutagens are demonstrated in various cooked foods.

1.3. Objective and scope of the review

The studies concerning food-borne heterocyclic amines, their chemistry and formation, their analytical techniques, their occurrence in food, their biological activities including mutagenicity, induction of DNA damage and carcinogenicity, have been summarized in many reviews [1–13,33,34,40–42,75–77]. However, actual status of mutagenic heterocyclic amines in a general environment except for food is not so well known, although mutagenic activity has been detected in various environments such as air [78–86] and water [87–95]. Therefore, this review is concerned with the utilization of analytical methods and occurrence of mutagenic heterocyclic amines in a general environment. The review consists of following two main parts. In the first part (Section 2), general aspects of the analytical method for the determination of heterocyclic amines are surveyed according to the instrument type. In the second part (Section 3), applications of the methods in environmental analysis are considered according to the matrix type.

2. Analytical methods for heterocyclic amines

In order to monitor mutagenic heterocyclic amines in the environment, it is necessary to develop a simplified method that can analyze these compounds simultaneously using a rapid, selective and sensitive analytical technique. In the past, the determination of heterocyclic amines has been carried out using various techniques such as high-performance liquid chromatography (HPLC) with ultraviolet (UV), electrochemical and fluorescence detection, liquid chromatography–mass spectrometry (LC–MS), gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), capillary zone electrophoresis (CZE) and enzyme-linked immunosorbent assay (ELISA). The analytical methods reported presently are listed in Table 3. Some of these methods for the analysis of food-borne heterocyclic amines are also reviewed by Knize et al. [76] and Gross and Fay [77].

2.1. High-performance liquid chromatography

HPLC is one of the most popular analytical techniques for heterocyclic amines and this is at the top of the list of available methods in Table 3. All the heterocyclic amines have characteristic UV spectra and high extinction coefficients, and they are also electrochemically oxidizable. Some of them fluoresce. Therefore, these compounds can be measured with UV, electrochemical and fluorescence detectors. On the other hand, off-line HPLC methods based on the measurement of mutagenic activity using the Ames test are also used. However, these HPLC–bacterial mutagenesis methods are not covered in this section since these are summarized by Knize et al. [76].

UV absorbance detection is the most popular method for monitoring the effluent from HPLC columns, and most of the heterocyclic amines can be simultaneously detected at 260–275 nm. The detection limits of these compounds are at the 0.2–7 ng level, but the detection selectivity is low for the determination in the sample matrix. Many reversed-phase silica columns are tested, but the TSK gel column shows the best peak symmetry and separation efficiency. Zhang et al. [97] reported that eleven mutagenic heterocyclic amines could be

Table 3

Analytical methods for heterocyclic amines. *Kataoka / J. Chromatogr. A 774 (1997) 121–142*

Method	Detection ^a	Heterocyclic amine	Reference
HPLC	UV/ED	IQ, MeIQ, MeIQx, 4,8-DiMeIQx	Grivas and Nyhammar [96]
	UV	Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP	Zhang et al. [97]
	UV	Glu-P-1, Glu-P-2	Manabe et al. [98]
	UV	Trp-P-1, Trp-P-2, AαC, Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, 4,7,8-TriMeIQx, PhIP	Gross et al. [99,100]
	UV/FL	MeIQx (for UV); Trp-P-1, Trp-P-2, PhIP (for FL)	Ushiyama et al. [31]
	UV/FL	Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, IQ, MeIQ, IQx, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP (for UV); Trp-P-1, Trp-P-2, AαC, Glu-P-1, Glu-P-2, PhIP (for FL)	Gross et al. [101,103]
	UV/ED	IQ, MeIQ, 4,8-DiMeIQx, 7,8-DiMeIQx	Schwarzenbach and Gubler [104]
	UV/FL	IQ, MeIQ, MeIQx, 4,8-DiMeIQx (for UV); Glu-P-1, Glu-P-2, PhIP (For FL)	Jackson et al. [105]
	UV	IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP	Jagerstad et al. [106,107]
	UV/FL	Trp-P-2, AαC, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP (for UV); Trp-P-2, AαC, PhIP (for FL)	Thiebaud et al. [26]
	UV	IQ, MeIQx, 4,8-DiMeIQx, PhIP	Knize et al. [108]
	UV	IQ, MeIQx, 4,8-DiMeIQx, 4,7,8-TriMeIQx, PhIP	Karamanos and Tsegenidis [109]
	ED	IQ, MeIQ, 4,8-DiMeIQx	Takahashi et al. [110,111]
	ED	Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ, MeIQx	Billedeau et al. [112]
	ED	Trp-P-2, MeAαC, Glu-P-1, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP	Galceran et al. [113,114]
	ED	IQ, MeIQ, MeIQx	Van Dyck et al. [115]
FL	Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, PhIP	Manabe et al. [22,24,116]	
LC-MS	TSI (SIM)	IQ, MeIQ	Edmonds et al. [117,118]
	TSI (SIM)	Trp-P-1, Trp-P-2, IQ, MeIQx	Turesky et al. [119,120]
	TSI (SIM)	Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP	Gross et al. [103]
	ESI (Scan/SIM)	Trp-P-1, Trp-P-2, AαC, IQ, PhIP	Galceran et al. [121]
	ESI-MS-MS (SRM)	Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, IQ, MeIQ, IQx, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, 4,7,8-TriMeIQx, PhIP	Richling et al. [122]
GC	NPD	Trp-P-1, Trp-P-3, AαC, Glu-P-1, Glu-P-2, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP	Kataoka and Kijima [123]
GC-MS	NICI (SIM)	Trp-P-2, MeIQx, 4,8-DiMeIQx, PhIP	Murray et al. [124]
	EI (SIM)	MeIQx, 4,8-DiMeIQx	Vainiotalo et al. [25]
	NICI (SIM)	PhIP	Friesen et al. [32]
	NICI (SIM)	MeIQx, 4,8-DiMeIQx, PhIP	Tikkanen et al. [127]
CZE	UV	Trp-P-1, Trp-P-2, AαC, Glu-P-1, Glu-P-2, IQ, Iso-IQ, MeIQx, 4,8-DiMeIQx, 4,7,8-TriMeIQx, PhIP	Wu et al. [28,128,129]
Immuno-assay	ELISA	IQ, MeIQ, 4,8-DiMeIQx, PhIP	Vanderlaan et al. [130]– [132]
	ELISA	PhIP	Dragsted et al. [133]

^a UV: ultraviolet; ED: electrochemical; FL: fluorescence; TSI: thermospray ionization; ESI: electrospray ionization; MS-MS: tandem mass spectrometry; SIM: selected-ion monitoring; SRM: selected reaction monitoring; NPD: nitrogen-phosphorus detection; NICI: negative-ion chemical ionization; EI: electron ionization; ELISA: enzyme-linked immunosorbent assay.

separated using a TSK gel ODS-120A column (30 cm×7.8 mm I.D., 10 μm particle size) with a gradient elution system within 100 min. Gross and Gruter [102] separated simultaneously twelve mutagenic heterocyclic amines and two non-mutagenic pyrolysis products, norharman and harman, using a TSK gel ODS 80TM column (25 cm×4.6 mm I.D., 5 μm particle size) with a ternary gradient elution system within 32 min. This method has been the most often used by other investigators. Furthermore, Gross et al. [103] achieved a more sensitive detection of these compounds using a narrow-bore column, a Vydac 201HS52 (25 cm×2.1 mm I.D.). As shown in Fig. 1, twelve mutagenic heterocyclic amines and norharman could be separated as symmetrical peaks within 26 min, and almost three-fold increased sensitivity could be achieved with this narrow-bore column as compared with the standard analytical column (4.6 mm diameter). However, working with narrow-bore HPLC columns requires the HPLC hardware optimized for low void volumes

and may not be within the reach of some instruments. Recently, Karamanos and Tsegenidis [109] indicated that the gradient elution tended to cause an increased baseline elevation during chromatography leading in the case of HPLC–UV techniques. They reported the separation of five thermic mutagen-type heterocyclic amines using a Supelcosil LC-8 column (25 cm×4.6 mm I.D., 5 μm particle size) with an isocratic elution. Furthermore, UV detection with a diode-array instrument, capable of recording the UV absorption spectra of a peak, gives not only the retention information but also the comparison of spectra for peak identification. This technique has been successfully used in the analysis of various samples [27,101,103,104,106,108].

Electrochemical detection of heterocyclic amines is based on the oxidization of these compounds at the operating potential. The choice of instruments and electrode material, and the composition of the mobile phase, are factors that influence both sensitivity and selectivity of the electrochemical analysis. Isocratic elution is usually used in the combination of HPLC and electrochemistry, because gradient elution cannot be used in a high sensitivity range with this detector. Therefore, it is difficult to analyze all heterocyclic amines in one run. Some thermic mutagen-type heterocyclic amines, IQ, MeIQ, MeIQx, 4,8-DiMeIQ and 7,8-DiMeIQx have been detected using this technique with a detection limit of 0.05–0.3 ng [96,104,110,111]. Moreover, Billedeau et al. [112] and Galceran et al. [113,114] reported the detection of not only thermic mutagens but also pyrolytic mutagens, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and MeAαC using this detector. However, these methods require two different conditions for the separation of these compounds. Galceran et al. used 50 mM ammonium acetate (pH 4.0)–acetonitrile (90:10, v/v) as mobile phase for the analysis of Glu-P-1, IQ, MeIQ, MeIQx, 4,8-DiMeIQx and 7,8-DiMeIQx, and 50 mM ammonium acetate (pH 6.0)–acetonitrile (70:30, v/v) as mobile phase for the analysis of Trp-P-2, PhIP, MeAαC, norharman and harman using a TSK gel ODS 80T column (25 cm×4.6 mm I.D., 5 μm particle size). Recently, Van Dyck et al. [115] achieved low detection sensitivity of 35–70 pg in IQ, MeIQ and MeIQx using a Spherisorb SXC 5U (ion-exchange type) column. The selectivity of electrochemical detection results

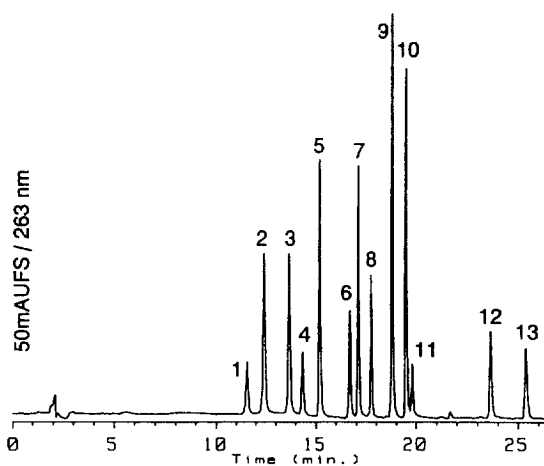


Fig. 1. Narrow-bore HPLC of heterocyclic amine standards. Instrument: HP1090. Chromatographic conditions: column: Vydac 201HS52, 250×2.1 mm I.D.; mobile phases A: 10 mM triethylammonium phosphate, pH 3.2, B: 10 mM triethylammonium phosphate, pH 4.5, C: acetonitrile. Flow-rate: 350 μl/min. Temperature: ambient. Mobile phase gradient program (Time: %A:%B:%C): 0 min: 99:0:1; 8 min: 0:89:11; 20 min: 0:52:48; 30 min: 0:10:90. Injected amount was ca. 20 ng per compound. Detection: UV 263 nm. Compound identification: 1=Glu-P-2; 2=IQ; 3=4-MeIQ; 4=Glu-P-1; 5=MeIQx; 6=Norharman; 7=4,8-DiMeIQx; 8=Harman; 9=Trp-P-2; 10=Trp-P-1; 11=PhIP; 12=AαC; 13=MeAαC. Reproduced from Ref. [77].

from the fact that heterocyclic amines are oxidized at lower potentials than others, so that its selectivity is as valuable as its sensitivity. Most of the impurities detected as overlapping peaks with UV detection are not oxidized at the working potential and therefore do not obscure the detection of heterocyclic amines.

Fluorescence detection is usually used for the analysis of pyrolytic mutagen type heterocyclic amines and PhIP, because thermic mutagen type heterocyclic amines such as IQ, MeIQ and MeIQx have no fluorescence. The detection limits of Trp-P-1, Trp-P-2, A α C, MeA α C and PhIP are from 1–12 pg [22,24,31]. Gross and Gruter [102] reported the analysis of six mutagenic heterocyclic amines, norharman and harman using this technique. The compounds were separated using a TSK gel ODS 80TM column (25 cm \times 4.6 mm I.D., 5 μ m particle size) with a ternary gradient elution system and detected with different excitation wavelengths from 265–360 nm and different emission wavelengths from 390–450 nm for each compound. On the other hand, Schwarzenbach and Gubler [104] tried the possibilities of fluorogenic labeling of heterocyclic amines, but the derivatization of the amino group with a fluorescent reagent has not been successful.

2.2. Liquid chromatography–mass spectrometry

LC–MS ideally combines advantages of the gentle separation of HPLC with high sensitivity and selectivity of mass spectrometry. The mass spectrometer functions essentially as a molecular mass-specific detector, so that the interference levels for a complex sample matrix are reduced as compared with UV detection, and normally laborious and time-consuming isolation procedures can be reduced. Therefore LC–MS is one of the best on-line identification systems, because of its selectivity and relatively high sensitivity. During the last decade, improvements in LC–MS coupling have made it possible to apply this technique to the detection and quantification of heterocyclic amines. Few LC–MS data using such ionization interfaces as thermospray and electrospray are available on the analysis of heterocyclic amines.

The thermospray LC–MS ionization process produces abundant pseudo-molecular ions for this class of compounds and the base peaks in the mass spectra are detected at $[M+H]^+$. Heterocyclic

amines are stable towards the ionization process and do not undergo notable fragmentation [120]. Thus, single-ion monitoring of the $[M+H]^+$ ion of the respective heterocyclic amines can be used for analyses in complex matrices. Some investigators demonstrated the usefulness of a thermospray LC–MS ionization system to detect Trp-P-1, Trp-P-2, IQ, MeIQ and MeIQx in complex samples [103,117–120]. These heterocyclic amines were measured using stable isotopically-labeled internal standard [117,118,120]. Linear responses were obtained for calibration curves of the concentration range corresponding to 0.05–10 ng/ng deuterium-labeled internal standard. Millon et al. [119] achieved a low detection limit of 0.1 ng for Trp-P-1 and Trp-P-2. Gross et al. [103] used the thermospray LC–MS for the confirmation of eleven mutagenic heterocyclic amines, norharman and harman. Fig. 2 shows the separation of a mixture of these standards with UV and mass-selected ion monitoring. However, the application of LC–MS with thermospray ionization has been restricted to the detection of a single heterocyclic amine.

The electrospray LC–MS using soft ionization interface is opening powerful approaches for the analysis of low molecular weight trace constituents in complex matrices. The electrospray LC–MS ionization process can effectively transform the heterocyclic amines from solution to protonated ions in the gas phase. As a result, the heterocyclic amines give a simple mass spectrum in which the only peak is due to $[M+H]^+$. These compounds are stable towards the ionization process and do not undergo significant fragmentation except for IQ and 4,7,8-TriMeIQx, which show the $[MH-15]^+$ fragment. Galceran et al. [121] reported the simultaneous determination of five mutagenic amines, norharman and harman using the electrospray LC–MS with positive ionization. The detection limits for the seven compounds in the full-scan and selected-ion monitoring (SIM) modes were ranged from 0.12 to 2.2 ng and from 5.4 to 44 pg, respectively. Furthermore, the electrospray ionization can be used in combination with tandem mass spectrometry (MS–MS). Recently, Richling et al. [122] developed a sensitive and selective method for the simultaneous analysis of the sixteen most abundant heterocyclic amines by HPLC–electrospray MS–MS in combination with

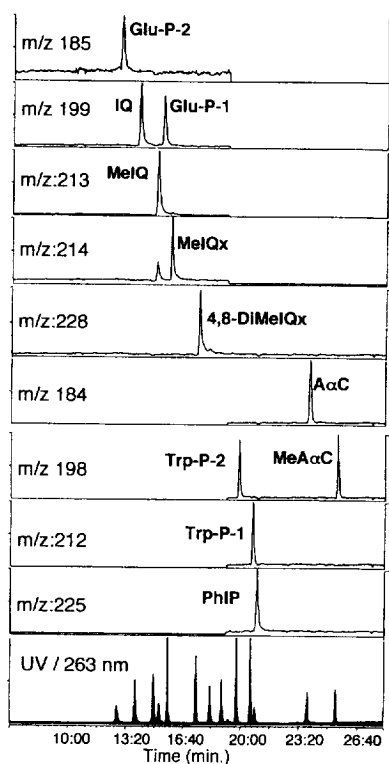


Fig. 2. HPLC separation of 11 heterocyclic amine standards with thermospray MS detection. Standards were separated using a TSK ODS80 HPLC column (250×4.6 mm I.D.) and a ternary mobile phase composed of two 10 mM ammonium acetate buffers (A=pH 3.2, B=pH 4.5) and acetonitrile (C). Flow-rate: 1 ml/min. Temperature: ambient. Mobile phase gradient program (Time: %A:%B:%C): 0 min: 95:0:5; 10 min: 83:0:17; 10.1 min: 0:83:17; 22 min: 0:45:55; 25 min: 0:10:90. The lowest panel shows the chromatogram observed with UV detection, whereas the upper panels show the chromatograms observed in the various mass selective detection channels recorded on a TSQ-7000 (Finnigan) instrument. Reproduced from Ref. [77].

selected reaction monitoring. The detection limits by this technique ranged from 0.5 to 250 pg.

2.3. Gas chromatography

GC has been widely used for amine analysis because of its inherent advantages of simplicity, high resolving power, high sensitivity and low cost [134]. Most heterocyclic amines are polar and less volatile, and tend to elute as broad and tailing peaks due to the strong adsorption to the column and injector during GC analysis. Therefore, they cannot be

detected in low concentration without derivatization. Derivatization of amines may be employed not only to reduce the polarity but also to improve the volatility, selectivity, sensitivity and separation of these amines [134]. Thus several derivatizing agents such as acetic, trifluoroacetic anhydride, heptafluorobutyric anhydride, pentafluorobenzyl bromide, 3,5-bistrifluoromethylbenzyl bromide and 3,5-bistrifluoromethylbenzoyl chloride have been tested for the analysis of some heterocyclic amines [25,29,32,110,124–127]. However, acylation with acid anhydrides yielded derivatives with very poor GC properties. The alkylation products with pentafluorobenzyl bromide, 3,5-bistrifluoromethylbenzyl bromide and 3,5-bistrifluoromethylbenzoyl chloride had good GC properties for some heterocyclic amines. However, these methods gave a mixture of mono- and di-alkylated forms and were used for GC–MS analysis but not for GC analysis. Recently, Kataoka and Kijima [123] developed a simple and rapid derivatization method for GC analysis of mutagenic heterocyclic amines. Ten heterocyclic amines were converted into their *N,N*-dimethylaminomethylene derivatives with *N,N*-dimethylformamide dimethyl acetal and measured by GC with nitrogen–phosphorus selective detection (NPD) using two-connected fused-silica capillary columns containing DB-1 (10-m×0.25-mm I.D., 0.25- μ m film thickness) and DB-17ht (10-m×0.25-mm I.D., 0.15- μ m film thickness). As shown in Fig. 3, these heterocyclic amines were separated within 7 min, although A α C and Glu-P-2 coeluted. The detection limits of these compounds ranged from 2 to 15 pg.

2.4. Gas chromatography–mass spectrometry

GC–MS ideally combines the advantages of the high resolution of capillary GC with the high sensitivity and selectivity of MS. A magnetic sector instrument is used for positive ion electron ionization MS, which yields excellent fragmentation patterns, with further conformation achieved using chemical ionization of the sample with a quadrupole instrument. Since chemical ionization MS is a much softer ionization method, it has the advantage of producing far less fragmentation of the compound and so allows a greater chance of the molecular ion being

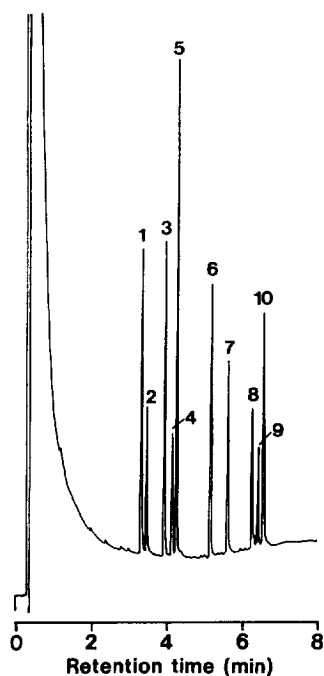


Fig. 3. Typical gas chromatogram obtained from standard heterocyclic amines. Instrument: Hewlett–Packard 5890 Series II gas chromatograph equipped with an electronic pressure control (EPC) system and a split/splitless capillary inlet system. GC conditions: column, two-connected fused-silica capillary column containing DB-1 (10 m \times 0.25 mm I.D., 0.25 μ m film thickness) and DB-17ht (10 m \times 0.25 mm I.D., 0.15 μ m film thickness); 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 (controlled with EPC): programmed at 10 kPa/min from 180 to 230 kPa, programmed at 25 kPa/min from 230 to 280 kPa and held at 280 kPa for 1 min. Make-up gas flow-rate: 30 ml/min. Split ratio: 10/1. Detection: nitrogen-phosphorus selective detection. Injected amount was ca. 250 pg per compound. Peaks: 1=A α C+Glu-P-2; 2=Glu-P-1; 3=Trp-P-1; 4=Trp-P-2; 5=IQ; 6=MeIQ; 7=MeIQx; 8=4,8-DiMeIQx; 9=PhIP; 10=4,7,8-TriMeIQx (internal standard). Reproduced from Ref. [123].

present, which can aid interpretation. Negative ion chemical ionization (NICI) is known to be highly sensitive and selective to electron-capture compounds. The GC–MS can be usually operated in two modes, total ion scanning and SIM. For SIM, only the base peaks are chosen to obtain the highest possible sensitivity. Thus GC–MS is one of the best on-line identification systems because of selectivity

and sensitivity, but it requires conversion into volatile derivatives before analysis.

Few GC–MS–SIM data using positive ion electron ionization and negative ion chemical ionization on the analysis of heterocyclic amines after derivatization are available. Murray et al. [124] developed a sensitive and selective method for the determination of Trp-P-2 as its 3,5-bistrifluoromethylbenzoyl derivative by NICI–MS–SIM. Furthermore, they reported the determination of MeIQx, 4,8-DiMeIQx and PhIP as their 3,5-bistrifluoromethylbenzyl derivatives by the same technique [125–127]. Although these heterocyclic amines give a mixture of mono- and di-bistrifluoromethylbenzyl derivatives, the spectra of the di-bistrifluoromethylbenzyl derivatives possess high mass fragment ions suitable for SIM work. Fig. 4 shows SIM traces of the derivatized standards of some heterocyclic amines and their isotopic-labeled internal standards. When ions m/z 438, m/z 452 and m/z 449 were specially monitored, the detection limits of MeIQx, 4,8-DiMeIQx and PhIP were 1 pg. A heterocyclic amine and its isotopic-labeled analogue as an internal standard show a similar retention time, so that these compounds can be conveniently monitored in a single chromatographic run and accurately quantified. Vainiotalo et al. [25] analyzed MeIQx and 4,8-DiMeIQx as the same derivatives by electron ionization MS–SIM. The detection limits for these compounds are 2 pg. On the other hand, Friesen et al. [32] developed a NICI–MS–SIM method for the determination of PhIP after conversion into an electron-capturing pentafluorobenzyl derivative. Quantification is carried out by selected-ion monitoring of the $[M-C_6F_5CH_2]^-$ ions of the derivatives of PhIP (m/z 403) and d_5 -PhIP (m/z 408) as an internal standard.

2.5. Capillary zone electrophoresis

CZE is capable of achieving higher separation efficiency, uses less organic solvents, and requires small amounts of samples in comparison with HPLC. The migration behavior of ionized compounds are dependent on the various factors such as buffer pH, organic modifier, concentration of buffer solution, capillary tubing temperature and electric field strength. A CZE method for the determination of heterocyclic amines was recently developed by Wu et

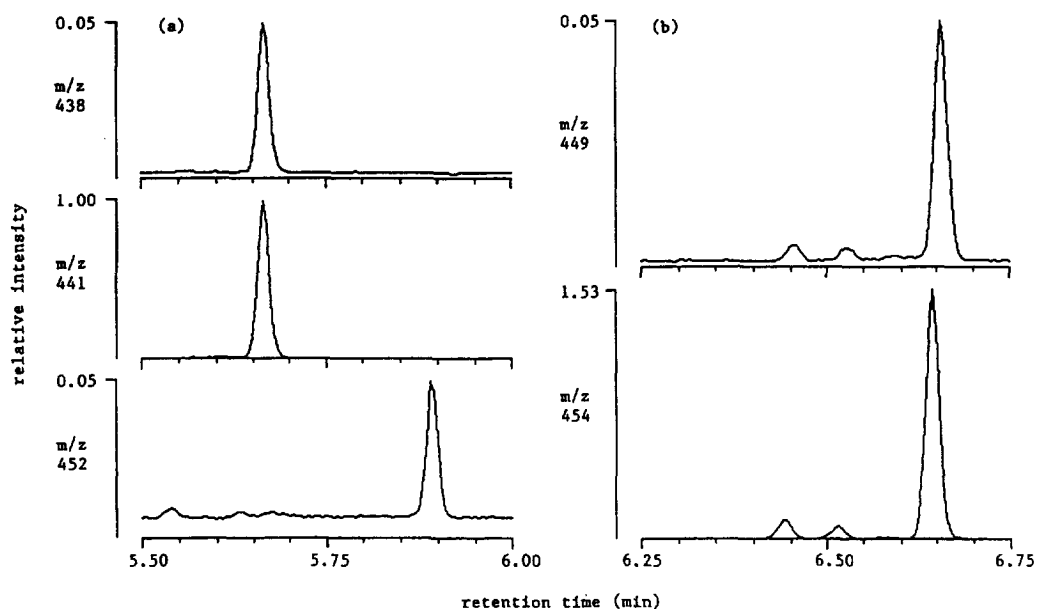


Fig. 4. SIM traces of a derivatized standard containing [^{13}C , $^{15}\text{N}_2$]MeIQx (5 ng), [$^2\text{H}_5$]PhIP (25 ng), MeIQx (0.2 ng), 4,8-DiMeIQx (0.2 ng) and PhIP (1 ng). Instrument: Finnigan-MAT 4500 combined gas chromatograph–quadrupole mass spectrometer. GC conditions: column, DB-5 (15 m \times 0.25 mm I.D., 0.25 μm film thickness); oven temperature, held at 200°C for 1 min, programmed at 20°C/min from 200 to 320°C and held at 320°C for 1 min; injection temperature, 270°C; helium head pressure, 69 kPa. MS conditions: operation, negative ion chemical ionization mode with an electron energy of 100 eV; admitted ammonia gas pressure, 53 Pa; ion source temperature, 150°C. Retention times of the di-bis(trifluoromethyl)benzyl derivatives of MeIQx (m/z 438) and [^{13}C , $^{15}\text{N}_2$]MeIQx (m/z 441) are 5.65 min, of 4,8-DiMeIQx (m/z 452) 5.90 min and of PhIP (m/z 449) and [$^2\text{H}_5$]PhIP (m/z 454) 6.65 min. Reproduced from Ref. [126].

al. [28,128,129]. As shown in Fig. 5, thirteen heterocyclic amines were successfully separated using an uncoated silica capillary (46.4 cm effective length) within 12 min. UV absorbance was monitored at 190, 220, 240 and 263 nm using a diode array detection system. The detection limits of these compounds were 0.05–1.3 ng/ μl . Although systematic optimization is conditional and the optimal conditions are restricted by the experimental conditions available, the most influential factors can be established using the orthogonal array design technique.

2.6. Enzyme-linked immunosorbent assay

Immunoassays based on both monoclonal and polyclonal antibodies have been developed for small organic carcinogens such as pesticides, toxic chemicals and DNA adduct. To produce antibodies for these molecules, the compound of interest must first

be conjugated to carrier proteins such as bovine serum albumin and keyhole limpet hemocyanine. The site and type of conjugation are important because they influence the specificity of the antibodies produced. By selecting different linkage-sites, antibodies binding preferentially to various sites on the haptens can be elicited. Vanderlaan et al. [130–132] developed two general types of antibodies: those that are compound specific (e.g. differentiating IQ from all other aminoimidazoazaarenes), and those that are class-specific (e.g. recognizing all or most of the aminoimidazoazaarenes), on the basis of the aminoimidazo ring and unique quinoline, quinoxaline or pyridine rings. By using these antibodies, they established a sensitive and selective ELISA assay of IQ, MeIQ, 4,8-DiMeIQx and PhIP. Although this technique was used to the direct analysis of these heterocyclic amines, cross-reacting interfering substances made quantification of these compounds in

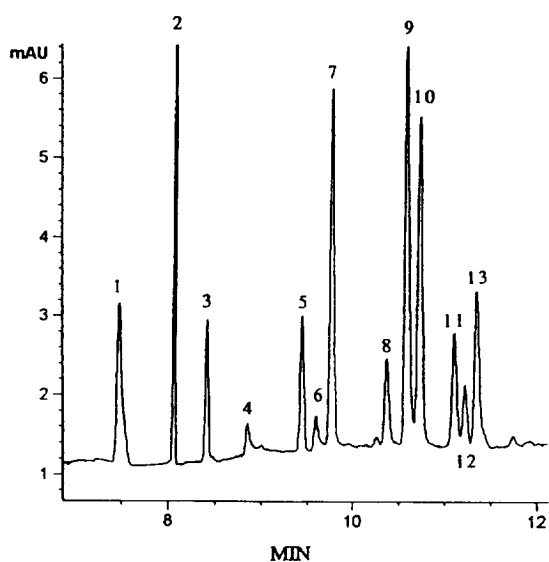


Fig. 5. Optimized electropherogram of the separation of the thirteen heterocyclic amines at point 1 ($x_1=0\%$, $x_2=80\%$ and $x_3=0\%$) in overlapping resolution mapping scheme. Instrument: BioFocus 3000 automatic capillary electrophoresis system with a multi-wavelength UV detector. Electrophoretic conditions: capillary, uncoated silica, 46.4 cm effective length; buffer, 20 mM Na_2HPO_4 , 31% MeOH, pH 2.0; constant applied voltage, 18 kV; capillary temperature, 25°C; autosampler temperature, 15°C. Detection: UV at 190, 220, 240 and 263 nm. Injection: pressure mode, 1 p.s.i. of a standard mixture solution (10 ng/ μl). Peak identifications: 1=Iso-IQ; 2=IQ; 3=norharman; 4=Glu-P-2; 5=harman; 6=Glu-P-1; 7=Trp-P-2; 8=AaC; 9=Trp-P-1; 10=MeIQx; 11=4,8-DiMeIQx; 12=PhIP; 13=4,7,8-TriMeIQx. Reproduced from Ref. [128].

the complex sample impractical. Recently, Dragsted et al. [133] developed a sensitive fluorescence-based ELISA assay of PhIP. The competitive fluorescence ELISA was found to be 5–10 times more sensitive than the photometric assay, showing 50% inhibition with only 150 fmol (33.6 pg) PhIP per well.

3. Applications in environmental analysis

3.1. General techniques for sample preparation

Heterocyclic amines are present in the environment at low parts per billion or less. When the environmental samples are analyzed by various methods described in above section, many peaks with the same retention times as those of

heterocyclic amines are often present in the chromatograms. Therefore it is not too much to say that the clean-up procedures for the complex sample matrix greatly influence the reliable and accurate analysis of these compounds. In order to remove co-eluting interferences and to preconcentrate heterocyclic amines, the extraction and clean-up of the sample has been performed using a number of different purification techniques, such as liquid-liquid extraction with dichloromethane [16,21,23,27,116,135], ethyl acetate [25] or ether [19], column chromatography on CM-Sephadex C-25 [19], Sephadex LH-20 [19,20] or XAD-2 [25,27], solid-phase extraction with Bond-Elut C_{18} [27,28,94], PRS [26–28] or SI [16,23,24,116] cartridges, Sep-Pak C_{18} cartridge [95], Extrelut-20 [26] or TSK CM gel cartridge [27], adsorption on Blue-Cotton [21,70,89,90] or Blue-Rayon [14,15,28,88,92,94,95,135] and preparative HPLC [14–18,22,23]. Heterocyclic amines can be separated by acid–base partition, because these compounds can be extracted with the above organic solvents not at low pH (pH<1), but at high pH (pH>10). Column chromatography is effective for the separation of heterocyclic amines, but it is time-consuming. Solid-phase extraction is simple and rapid, and good recoveries of heterocyclic amines are obtained ranging 50–110%, 79–100% and 43–107% with Bond-Elut C_{18} , PRS and Extrelut cartridges, respectively [114]. Gross et al. [100–102] separated a series of heterocyclic amines into two well-defined groups: (A) polar group and (B) apolar group by the optimization of the PRS step in the solid-phase extraction with Extrelut-PRS- C_{18} coupled cartridges. One of the main advantages of this technique is that all the fluorescent compounds are eluted in the same fraction. By using this technique, Galceran et al. [114] ‘selectively and sensitively’ analyzed the heterocyclic amines in polar and apolar groups using electrochemical and fluorescence detection, respectively. Blue-Cotton or Blue-Rayon, cotton or rayon bearing covalently linked copper phthalocyanine 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 mutagens and carcinogens in aqueous solution [136]. Overall recoveries of heterocyclic amines by

this technique are 45–93%. Furthermore, Hayatsu et al. [137] recently developed a more effective powdered form, Blue-chitin, which is suitable for preparing a packed column. Normal or reversed-phase HPLC fractionations are also useful for preparation of the sample. The sample preparation for the heterocyclic amine analysis has been carried out by a combination of the techniques described above. The contents of heterocyclic amines in various environments are listed in Table 4.

3.2. Outdoor and indoor air

Air pollution has now been recognized as a potentially important problem for public health, and the mutagenic activity of airborne particles has been demonstrated [81,86]. Manabe et al. [14–17] detected some pyrolytic mutagens, Trp-P-1, Trp-P-2, A α C, MeA α C, and PhIP in indoor and outdoor airs. These heterocyclic amines in the airborne particles were determined by HPLC with fluorescence detection using a Kaseisorb LC ODS-300-5 column (25 cm \times 7.5 mm I.D., 5 μ m particle size) after purification by two step preparative HPLC fractionations. The mean levels of Trp-P-1 in outdoor air (pg/m³) were slightly higher than those of Trp-P-2, and these levels in the city air were approximately twice those of the country air [14]. When these heterocyclic amine levels were expressed on the basis of total suspended particles (TPS), the mean amounts of Trp-P-1 and Trp-P-2 were 2.72–5.16 and 1.52–4.60 pg/mg TPS, respectively. A α C levels in the outdoor air were remarkably higher than MeA α C levels during a one-year observation period [15]. Moreover, the levels of A α C, MeA α C and PhIP in outdoor air during winter were apparently higher than those during summer [15,16]. These observations suggest that these mutagenic heterocyclic amines are partly formed from various combustion systems in heating during the winter season. When these heterocyclic amine levels were expressed on the basis of TPS, the mean amounts of A α C, MeA α C and PhIP were 10, 0.95 and 27.8 pg/mg TPS, respectively. Moreover, the levels of Trp-P-1, Trp-P-2, A α C, MeA α C and PhIP in indoor air were much higher than those in outdoor air. This might be supported by the fact that these heterocyclic amines were present in cigarette smoke and cooking fumes, and the levels of Trp-P-1

and Trp-P-2 in indoor air polluted with cigarette smoke were much higher than those in indoor air before cigarette smoking [17].

3.3. Diesel-exhaust particles

Diesel-exhaust particles are known to contain mutagens and carcinogens. There is strong experimental evidence that diesel emissions produce cancers in experimental animals [138–140]. Some epidemiological studies have shown that workers in motor exhaust-related occupations are at excess risk of lung cancer [141–143]. Manabe et al. [16,18] demonstrated that mutagenic and carcinogenic heterocyclic amines, Trp-P-1, Trp-P-2, A α C, MeA α C and PhIP, are present in diesel-exhaust particles. The heterocyclic amines in these particles were determined by HPLC with fluorescence detection using a Kaseisorb LC ODS-300-5 column (25 cm \times 7.5 mm I.D., 5 μ m particle size) after purification by two-step preparative HPLC fractionations. Typical chromatograms obtained from preparative HPLC and final HPLC of diesel-exhaust particles are shown in Figs. 6 and 7. The levels of Trp-P-1, Trp-P-2, A α C and MeA α C in the diesel-exhaust particles obtained at high-speed cruise conditions were much higher than those in the diesel-exhaust particles obtained at low-speed cruise conditions. On the other hand, the levels of PhIP in these samples were higher not at high-speed cruise conditions but at low-speed cruise conditions. These results suggest that these heterocyclic amines are likely to be environmental pollutants and that diesel-exhaust is one of the sources of these mutagens/carcinogens in the outdoor environment.

3.4. Cigarette smoke

Epidemiological studies have shown that cigarette smoking is associated with the development of human cancers [144–146], and cigarette smoke condensate has been known to be mutagenic in bacteria [147,148] and carcinogenic to experimental animals [149]. Cigarette smoke contains various pyrolysis products and combustion products emitted by heating at 100–950°C. Among them, known potent mutagens and carcinogens such as polynuclear aromatic hydrocarbons, volatile N-nitrosamines,

Table 4
Contents of heterocyclic amines in various environments

Environmental sample	Method ^a	Heterocyclic amine content											Ref.
		Trp-P-1	Trp-P-2	AαC	MeAαC	Glu-P-1	Glu-P-2	IQ	MeIQ	MeIQx	DiMeIQx	PhP	
Outdoor air	HPLC-FL	0.15–0.34 pg/m ³	0.09–0.29 pg/m ³	–	–	–	–	–	–	–	–	–	[14]
Outdoor air	HPLC-FL	–	–	0.70 pg/m ³	0.07 pg/m ³	–	–	–	–	–	–	–	[15]
Outdoor air	HPLC-FL	–	–	–	–	–	–	–	–	–	–	2.54 pg/m ³	[16]
Indoor air	HPLC-FL	0.06 pg/m ³	0.05 pg/m ³	–	–	–	–	–	–	–	–	–	[17]
Cigarette smoke— polluted indoor air	HPLC-FL	0.42–0.78 pg/m ³	0.61–1.67 pg/m ³	–	–	–	–	–	–	–	–	–	[17]
Diesel-exhaust particles	HPLC-FL	ND ^b –0.96 pg/mg	0.07–5.69 pg/mg	0.7–50.6 pg/mg	ND–2.10 pg/mg	–	–	–	–	–	–	–	[18]
Diesel-exhaust particles	HPLC-FL	–	–	–	–	–	–	–	–	–	–	14.1 ng/g	[16]
Cigarette smoke	HPLC-FL	–	–	25–258 ng/cig.	9–37 ng/cig.	–	–	–	–	–	–	–	[19]
Cigarette smoke	HPLC-ED	–	–	–	–	–	–	0.26 ng/cig.	–	–	–	–	[20]
Cigarette smoke (mainstream)	HPLC-FL	0.29–0.48 ng/cig.	0.82–1.10 ng/cig.	27.7–47.8 ng/cig.	2.0–2.9 ng/cig.	–	–	–	–	–	–	–	[22]
Cigarette smoke (mainstream)	HPLC-FL	0.19–0.31 ng/cig.	0.51–0.66 ng/cig.	–	–	–	–	–	–	–	–	–	[17]
Cigarette smoke (mainstream)	HPLC-FL	–	–	–	–	0.37–0.89 ng/cig.	0.25–0.88 ng/cig.	–	–	–	–	–	[23]
Cigarette smoke (mainstream)	HPLC-FL	–	–	–	–	–	–	–	–	–	–	10.9–22.9 ng/cig.	[24]
Cigarette smoke (mainstream)	HPLC-FL	0.02–0.51 ng/cig.	0.63–1.54 ng/cig.	3.7–13.5 ng/cig.	0.37–1.92 ng/cig.	–	–	–	–	–	–	<0.01 ng/cig.	[70]
Cigarette smoke (sidestream)	HPLC-FL	0.14–0.28 ng/cig.	0.22–0.30 ng/cig.	2.13–2.72 ng/cig.	0.38–0.39 ng/cig.	–	–	–	–	–	<0.01	ng/cig.	[70]
Cigarette smoke (mainstream)	GC-NPD	0.33–0.74 ng/cig.	ND	ND–1.96 ng/cig.	–	ND	–	0.33–0.49 ng/cig.	0.28–0.75 ng/cig.	ND	ND	ND	[135]
Cigarette smoke (sidestream)	GC-NPD	0.27–0.37 ng/g	ND	5.00–6.51 ng/g	–	ND	–	0.16–0.61 ng/g	0.25–0.45 ng/g	ND	ND	1.48–2.09 ng/g	[135]
Cooking smoke	GC-NPD	0.51–0.79 ng/g	0.66–3.70 ng/g	1.60–3.86 ng/g	–	ND–4.06 ng/g	–0.22–0.35 ng/g	ND–0.19 ng/g	ND	ND	ND	2.24–3.92 ng/g	[135]
Cooking fumes	GC-MS- EI-SIM	–	–	–	–	–	–	–	–	14 pg/g	7 pg/g	–	[25]
Cooking fumes	HPLC- UV/FL	–	–	3.48 ng/g	–	–	–	–	–	0.97 ng/g	–	1.47 ng/g	[26]
Cooking fumes	HPLC- UV/FL	–	–	0.08–4.0 ng/g	–	–	–	–	–	ND–1.1 ng/g	ND–0.25 ng/g	0.007–1.8 ng/g	[27]
Rain water	HPLC-FL	–	–	81 pg/l	13 pg/l	–	–	–	–	–	–	–	[15]
Rain water	CZE-UV	–	ND–34.5 ng/l	–	–	–	–	–	–	–	ND–16.8 ng/l	ND–84.7 ng/l	[28]
Incineration-ash	HPLC-FL	–	–	–	–	–	–	–	–	–	–	173 pg/g	[16]
Soil	HPLC-FL	–	–	6.5 pg/g	ND	–	–	–	–	–	–	–	[15]

^a Method abbreviations correspond to those in Table 3.

^b Not detectable.

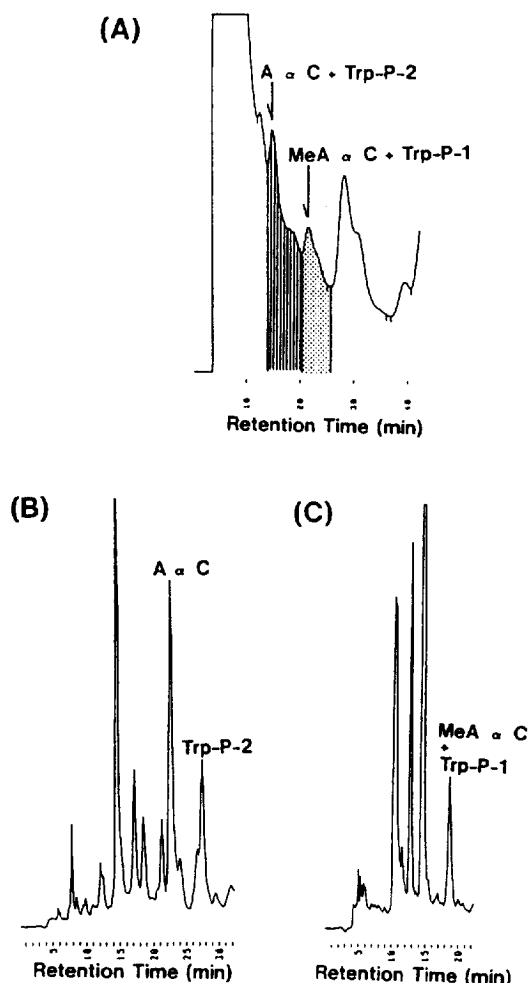


Fig. 6. HPLC chromatograms of preparative and second-step purification of diesel-exhaust particles. (A) Preparative chromatogram on an ES-502C column (100 mm \times 7.6 mm I.D., 9.0 mm particle size). Chromatographic conditions: mobile phase, 20 mM H_3PO_4 (pH 2.0)–acetonitrile (90:10, v/v); flow-rate, 1.0 ml/min at 40°C; fluorescence detection (λ_{ex} = 266 nm, λ_{em} = 399 nm). The fractions that appeared to correspond to A α C/Trp-P-2 and MeA α C/Trp-P-1 were collected separately. (B and C) Chromatograms of the second-step purification on a Nucleosil 5C₈ column (150 mm \times 4 mm I.D., 10 μ m particle size). Chromatographic conditions: (B) mobile phase, 20 mM H_3PO_4 (pH 2.0)–acetonitrile (90:10, v/v) and (C) 20 mM H_3PO_4 (pH 2.0)–acetonitrile (85:15, v/v); flow-rate, 1.0 ml/min at 50°C; fluorescence detection (λ_{ex} = 266 nm, λ_{em} = 399 nm). Reproduced from Ref. [18].

tobacco-specific nitrosamines, polycyclic aza-arenes and miscellaneous nitrogen compounds. Mutagenic and carcinogenic heterocyclic amines have also been isolated and determined in cigarette smoke conden-

sates [17,19–24,70]. Yoshida et al. [19,20] isolated pyrolytic mutagens A α C and MeA α C from the smoke of 1000 cigarettes after purification by several chromatographic techniques. Yamashita et al. [21] detected IQ from the smoke of 20 cigarettes by HPLC with electrochemical detection using TSK gel ODS-120A column (30 cm \times 7.8 mm I.D., 10 μ m particle size) after purification by acid–base partition with dichloromethane and Blue-Cotton extraction. Manabe et al. [17,22] detected Trp-P-1, Trp-P-2, A α C and MeA α C in the mainstream cigarette smoke by HPLC with fluorescence detection using a Kaseisorb LC ODS-300-5 column (25 cm \times 7.5 mm I.D., 5 μ m particle size) after purification by two-step preparative HPLC fractionations. Moreover, Manabe et al. [23,24] detected Glu-P-1, Glu-P-2 and PhIP in the mainstream cigarette smoke by HPLC with fluorescence detection using the same column after purification by acid–base partition with dichloromethane, solid-phase extraction with Bond-Elut SI cartridge and two-step preparative HPLC fractionations. Wakabayashi et al. [70] reported that Trp-P-1, Trp-P-2, A α C and MeA α C were present in cigarette smoke condensates and the levels of these compounds in the mainstream smoke of some cigarettes were relatively higher than those in sidestream smoke. Recently, Kataoka et al. [135] detected Trp-P-1, A α C, IQ, MeIQ and PhIP in mainstream and sidestream smokes by GC–NPD using two-connected fused-silica capillary columns containing DB-1 (10-m \times 0.25-mm I.D., 0.25- μ m film thickness) and DB-17ht (10-m \times 0.25-mm I.D., 0.15- μ m film thickness) after purification by acid–base partition with dichloromethane, Blue-Rayon extraction and derivatization with N,N-dimethylformamide dimethyl acetal. Typical chromatogram obtained from sidestream cigarette smoke is shown in Fig. 8 (A). On the other hand, higher levels of mutagens were detected in the urine during cigarette smoking [150–153], although the heterocyclic amine levels in these urine samples were not determined. These facts suggest that these heterocyclic amines are likely to be environmental pollutants and that cigarette smoke is one of the sources of these mutagens/carcinogens in the indoor environment.

3.5. Cooking fumes

Epidemiological studies have shown that cooks

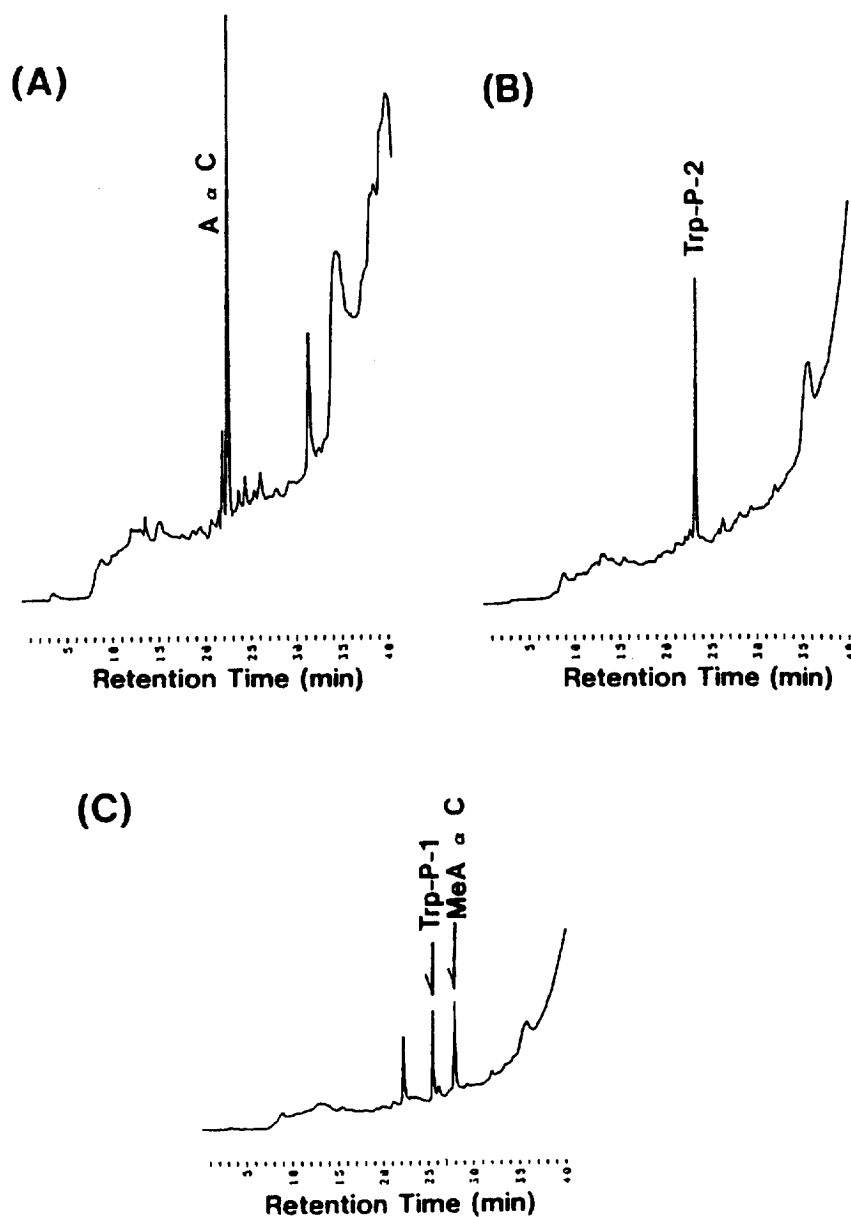


Fig. 7. Final HPLC chromatograms of diesel-exhaust particles on an ODS-300-5 column (250 mm \times 7.5 mm I.D., 5 μ m particle size). The fraction corresponding to (A) A α C, (B) Trp-P-2 and (C) MeA α C/Trp-P-1 in the second purification step (Fig. 6) were analyzed separately. Chromatographic conditions: mobile phase, a linear gradient (0–30%, v/v) of acetonitrile in 10 mM H₃PO₄ over 35 min; flow-rate, 3.0 ml/min at 50°C; fluorescence detection (λ_{ex} = 266 nm, λ_{em} = 399 nm). Reproduced from Ref. [18].

have an increased risk of lung cancer [154,155]. The mutagens have been found not only in cooked foods but also in the fumes [27,80,85,156–158] emitted during cooking, and the mutagenic compounds in the

fumes have been the same as those found in cooked foods [27,159]. Several mutagenic heterocyclic amines such as A α C, MeIQx, 4,8-DiMeIQx and PhIP have been found in cooking fumes. Vainiotalo

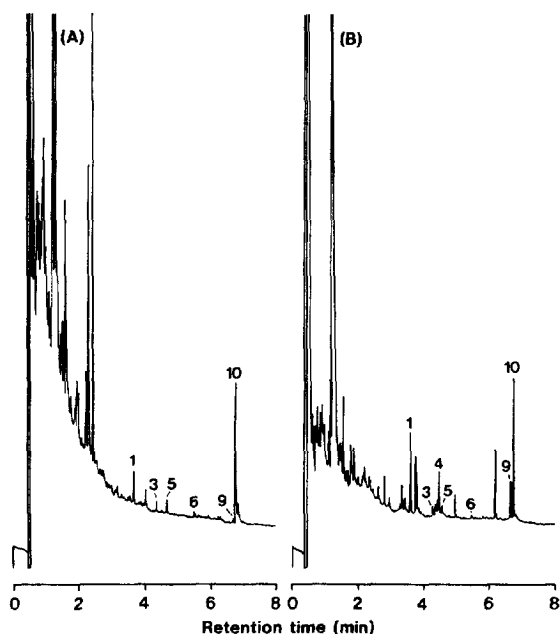


Fig. 8. Gas chromatograms obtained from (A) sidestream cigarette smoke and (B) combustion smoke of semi-dried fish. GC conditions and peak numbers as in Fig. 3.

et al. [25] detected MeIQx and 4,8-DiMeIQx in the fumes from frying meat by GC-MS using electron ionization in SIM mode after purification by XAD-2 treatment, acid-base partition with dichloromethane and derivatization with 3,5-bistrifluoromethylbenzyl bromide. Thiebaut et al. [26,27] detected A α C, MeIQx, 4,8-DiMeIQx and PhIP in the fumes from cooking meats and soy-based food by HPLC with UV and fluorescence detection after purification by dichloromethane extraction, XAD-2 column chromatography and extraction with TSK gel CM-650 cartridge. Recently, Kataoka et al. [135] detected Trp-P-1, Trp-P-2, Glu-P-1, A α C, IQ, MeIQ and PhIP in combustion smoke of some cooking foods by GC-NPD after purification by acid-base partition with dichloromethane, Blue-Rayon extraction and derivatization with *N,N*-dimethylformamide dimethyl acetal. A typical chromatogram obtained from combustion smoke of semi-dried fish is shown in Fig. 8 (B). These results suggest that mutagenic and carcinogenic heterocyclic amines are emitted into the air during cooking. Therefore, long-term air sampling inside restaurants, kitchens and the food

industry is necessary to assess the human health risks, especially to food processing workers, due to prolonged or occupational exposure to airborne mutagens and carcinogens.

3.6. Rain water

Water pollution has now been recognized as a potentially important problem for public health, and the mutagenic activities in several water samples such as river water, rain water and sewage water have been demonstrated [86–94]. Manabe et al. [15] detected A α C and MeA α C from rain water (ca. 2 l) collected during the summer season by HPLC with fluorescence detection using a Kaseisorb LC ODS-300-5 column (25 cm \times 7.5 mm I.D., 5 μ m particle size) after purification by Blue-Rayon extraction and two steps preparative HPLC fractionations. Wu et al. [28] detected Trp-P-2, 4,8-DiMeIQx and PhIP in rain water (ca. 2 l) collected during the forest fires in Southeast Asia by CZE with UV diode array detection after purification by Blue-Rayon extraction and solid-phase extraction with Bond-Elut PRS and C₁₈ cartridges. Electropherograms obtained from rain water are shown in Fig. 9. These heterocyclic amines were present at relatively high concentrations in the rain samples collected during the first rainfall since the beginning of the forest fires, but these compounds were not detected in the samples collected during the annual rainy season. These facts suggest that heterocyclic amines can be formed not only from proteinaceous foods but also from a large variety of combustible materials such as grass, wood and petroleum. Therefore the contribution to the overall environmental pollution through the traditional slash-and-burn agricultural techniques (residents in certain Southeast Asia countries still use) using fire for land cleaning purposes should be considered as seriously as that attributed to modern industrial activities [28]. On the other hand, Sasaki et al. [95] and Ono et al. [94] detected Trp-P-1 and Trp-P-2 in river water and biologically treated human faeces and urine, but their contents were not determined. These results suggest that mutagenic heterocyclic amines may be discharged into the river water through domestic waste and human waste.

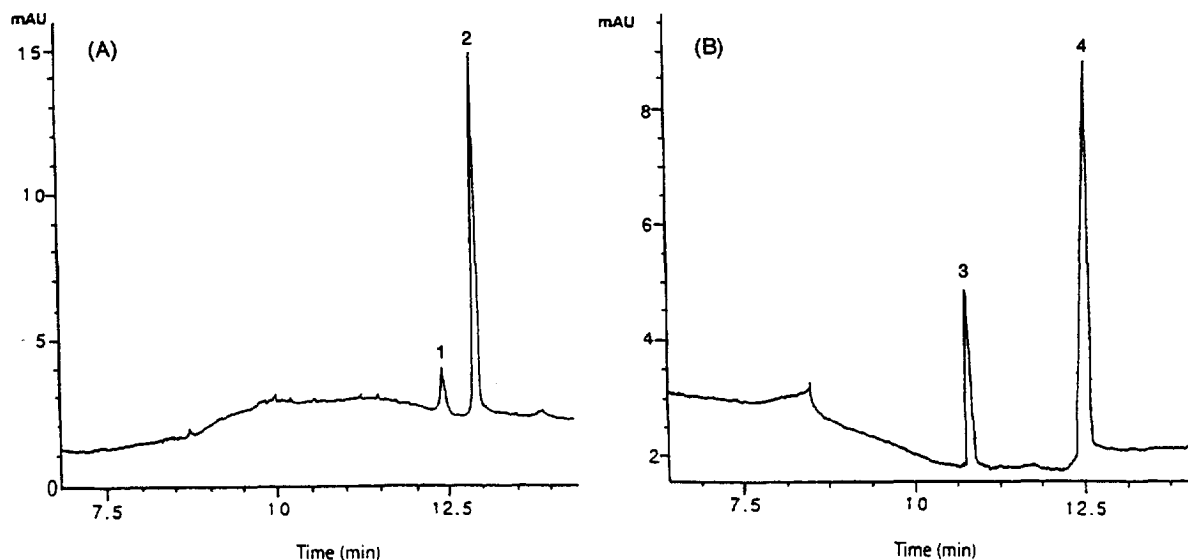


Fig. 9. Electropherograms obtained from (A) rain water extract and (B) extract of particulates present in rain water. Instrument: Hewlett–Packard HP^{3D} capillary electrophoresis system with UV diode-array detector. Electrophoretic conditions: capillary, uncoated silica, 42.5 cm effective length; buffer, 50 mM Na₂HPO₄, 20 mM citric acid, 30 mM NaCl, 26% methanol, pH 2.1; voltage, 20 kV; capillary temperature, 25°C. Detection: UV at 190, 240 and 263 nm. Injection: pressure mode, 45 mbar/s. Peak identifications: 1=4,8-DiMeIQx, 2=4,7,8-TriMeIQx (internal standard), 3=Trp-P-2, 4=PhIP. Reproduced from Ref. [28].

3.7. Incineration-ash and soil

Manabe et al. [16] detected PhIP in incineration-ash collected from garbage-burning plants in a metropolitan area by HPLC with fluorescence detection using a Kaseisorb LC ODS-300-5 column (25 cm×7.5 mm I.D., 5 μm particle size) after purification by reflux, dichloromethane extraction, solid-phase extraction with Bond-Elut SI cartridge and two-step preparative HPLC fractionations. Moreover, they [15] detected AαC in soil collected from different districts in urban and industrial areas by HPLC with fluorescence detection using the same column after purification by reflux, dichloromethane extraction and two-step preparative HPLC fractionations. However, the origins of these heterocyclic amines in these samples are unknown.

4. Conclusions

Monitoring of mutagenic and carcinogenic heterocyclic amines in the ambient environment is very important for human health risk assessment.

Practical and reliable methods for determining accurate exposure levels of these compounds should be established. For this purpose, various analytical methods have been developed. HPLC with UV detection is the most useful technique for the simultaneous determination of most heterocyclic amines and it can confirm the identities of suspected peaks in complex matrix samples by a photodiode-array UV detection system, but sensitivity is not so high. HPLC with electrochemical or fluorescence detection is an option to be considered for their high selectivity and sensitivity, although these techniques are restricted to the determination of a selected group. LC-MS and GC-MS, capable of simultaneously measuring retention time and molecular mass, are powerful techniques for the identification and quantification of heterocyclic amines in complex matrix samples. Although these techniques are selective and sensitive, they require sophisticated and expensive equipment that is beyond the reach of many laboratories. GC with nitrogen–phosphorus selective detection, available in most laboratories, is highly sensitive and selective, but this technique requires conversion of heterocyclic amines into

volatile derivatives and is not yet well applied to the determination of the sample. CZE using a photo-diode-array UV detection system is capable of achieving higher separation efficiency, but its sensitivity is not so high. ELISA is available for the direct analysis of heterocyclic amines in sample, but this technique requires the production of monoclonal antibodies and is restricted to the determination of a single compound. The methods described above have advantages and disadvantages, and so far there are no all-powerful analytical methods.

The choice of an analytical method depends on the presence of heterocyclic amines in the environment at low parts per billion or less and the variety and complexity of sample. Therefore it is not an exaggeration to say that the clean-up procedures for the complex sample matrix greatly influence the reliable and accurate analysis of these compounds. In order to achieve an efficient isolation and preconcentration of heterocyclic amines, several methods of sample preparation for the analysis of various environmental samples have been developed using a number of different purification techniques, such as liquid–liquid extraction, column chromatography, solid-phase extraction, extraction with mutagen-specific adsorbent and preparative HPLC. The combination of these techniques is used successfully for the extraction and purification of heterocyclic amines. However, many of these methods are time-consuming, laborious and give low recoveries for some specific heterocyclic amines. Therefore, further investigation is necessary to improve the simplicity, rapidity, recovery and reproducibility for the sample preparation.

Environmental pollution is now recognized as a potentially important problem for public health, and mutagenic activity has been detected in various environmental samples. Mutagenic and carcinogenic heterocyclic amines are widely distributed in a number of ambient environmental components such as airborne particles, diesel-exhaust particles, cigarette smoke, cooking fumes, rain water, sewage water, incineration-ash and soil. These 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, although there are little data

on the emission and discharge of these compounds into various environments. Therefore, the presence of heterocyclic amines in the environment may be more extensive than previously thought. These facts suggest that mutagenic and carcinogenic heterocyclic amines are likely to be ubiquitous environmental pollutants and humans are continually exposed to these compounds in normal daily life.

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