Mol. Nutr. Food Res. 2005, *49*, 263 – 273 DOI 10.1002/mnfr.200400061

Review

Two food-borne heterocyclic amines: Metabolism and DNA adduct formation of amino-α-carbolines

Hanne Frederiksen

Department of Toxiclogy and Risk Assessment, Danish Institute for Food and Veterinary Research, Søborg, Denmark

The amino-α-carbolines 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and 2-amino-3-methyl-9*H*-pyrido-[2,3-*b*]indole (MeAαC) are two mutagenic and carcinogenic heterocyclic amines formed during ordinary cooking. Amino-α-carbolines can be formed in model systems by pyrolyzing tryptophan or proteins of animal or vegetable origin, furthermore they are found in many cooked foods, such as fish, meat, and chicken. The specific mutagenicity of the amino-α-carbolines are lower in the Ames *Salmonella* assay than other heterocyclic amines, but in rodent studies the carcinogenicity of the amino-α-carbolines are comparable to other heterocyclic amines. The metabolic pathways of the amino-α-carbolines have been studied *in vitro* and *in vivo*, and the detoxified phase I and phase II metabolites characterized and quantified. The metabolic activation of the amino-α-carbolines and the formation of DNA-adducts have also been studied. Characteristic for the amino-α-carbolines are that relatively large amounts of these compounds in rat and human hepatic microsomes are activated to potent carcinogenic compounds compared with other heterocyclic amines, but further *in vivo* studies of the amino-α-carbolines are needed to highlight these indications. In this review, the main characteristics with focus on the metabolism and the DNA-adduct formation of the amino-α-carbolines are described and compared with other heterocyclic amines.

Keywords: Amino-α-carbolines / DNA-adducts / Heterocyclic-amines / Metabolism / Review

Received: August 30, 2004; revised: October 8, 2004; accepted: October 11, 2004

Contents

Correspondence: Dr. Hanne Frederiksen, Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark
E-mail: haf@dfvf.dk
Fax: +45-7234-7699

Abbreviations: **AαC**, 2-amino-9*H*-pyrido[2,3-*b*]indole; **CYP**, cytochrome P450; **dG-**, N^2-(2′-deoxyguanosin-8-yl)-; **Glu-P-2**, 2-aminodipyrido[1,2-a:3′,2′-*d*]imidazole; **IQ**, 2-amino-3-methylimidazo[4,5-*f*]-qunoxaline; **MeAαC**, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; **MeIQx**, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; **NAT**, *N*-acetyltransferase; **PCB**, polychlorinated biphenyl (aroclor 1254); **PhIP**, 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine; **SULT**, sulfotransferase; **Trp-P-2**, 3-amino-1-methyl-5*H*-pyrido[3,4-*b*]indole

1 Introduction

Diet is generally accepted as one of the important factors in human cancer development. Heterocyclic amines are a group of mutagens and carcinogens isolated from heated amino acids, proteins, and protein-rich foods. Cigarette smoke contains mutagenic compounds and this inspired Japanese researchers to investigate smoke from grilled fish and meat for mutagenic activity. In 1977, the first studies in this area showed mutagen activity in pyrolyzed proteins and amino acids and in smoke and charred surface of broiled fish and meat [1, 2]. Today more than 20 heterocyclic amines have been discovered and structurally characterized. They are divided into two classes, where the first class contains pyrolyzate mutagens, which are formed during treatment of amino acids or proteins at very high temperatures (>300°C). The pyrolyzate mutagens are derivates of amino-α-carbolines, γ-carbolines, and dipyridoimidazoles [3]. The second class of heterocyclic amines, the aminoimidazoazaarenes, are formed during ordinary cooking conditions (150–200°C) and are characterized as one or two heterocyclic rings fused to an aminoimidazo ring [4]. Figure 1

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

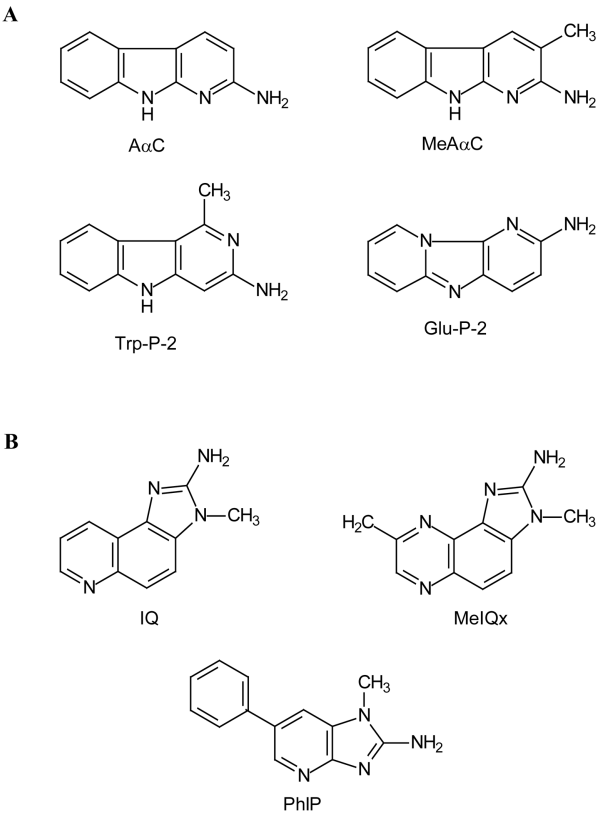

Figure 1. (A) Structures of selected pyrolyzate mutagens: 2-amino-9-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 2-amino-1-methyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-2), and 2-amino-dipyrido[1,2-a:3′,2′-*d*]imidazole (Glu-P-2); (B) selected aminoimidazoazaarene mutagens: 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP).

shows the structures of selected pyrolyzate and aminoimidazoazaarene mutagens.

Since the aminoimidazoazaarenes were discovered in the eighties, there has been a lot of focus on these compounds. 2-Amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and its methyl homologues are well-described as regards their formation and occurrence, mutagenicity and carcinogenicity, metabolism and DNA-adduct formation [5–8]. In comparison, there has been much less attention on the pyrolyzate mutagens, therefore, the aim of this minireview is to describe previous studies of the amino-α-carbolines and particularly recent studies of the metabolism and DNA-adduct formation of amino-α-carbolines.

2 Amino-α-carbolines

Two of the first discovered heterocyclic amines derived from heated food were the amino-α-carbolines. In 1978, a fraction containing mutagenic activity was isolated from a pyrolyzate of soybean globulin. The mutagenic fraction was analyzed by mass spectrometry and crystallized followed by X-ray analysis. Two compounds were characterized as 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and its methyl homologue 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) (Fig. 1) [9]. In model systems amino-α-carbolines can be formed by pyrolyzing either tryptophan or proteins of animal or vegetable origin, *e.g.*, albumin, casein, or soybean globulin [6]. The chemical synthesis of the amino-α-carbolines has been carried out by various attempts. The most successful method was performed by a thermal electrocyclic reaction of 2-azahexa-1,3,5-triene resulting in the formation of AαC with a yield of 40%. The chemical synthesis of MeAαC was carried out in an analogous way [10]. Today the amino-α-carbolines are commercial available compounds.

3 Occurrence

There are sincere problems associated with the determination of heterocyclic amines in cooked food because they occur in low amounts in a complex matrix. For several years, the amino-α-carbolines were thought to be formed exclusively under extreme conditions, such as high-temperature pyrolyzis of protein, but during the past 5–10 years the development of new analytical methods has shown that the amino-α-carbolines are common in the Western diet [11]. The levels of AαC found in fried red meat and fish are generally 0–20 ng/g and 0–10 ng/g, respectively (limit of detection >0.01–0.05 ng/g), but higher amounts of AαC have been reported, too [12]. Only few studies have made comparable investigations of the contents of amino-α-carbolines and other heterocyclic amines in food [13]. Table 1 shows some selected data of AαC, MeAαC, PhIP, and MeIQx found in fish, beef, chicken, and vegetables, such as mushroom and onion [14–22]. Especially in fish AαC was found in concentrations comparable with those of PhIP [15], whereas the level of AαC in beef is comparable with the level of MeIQx [18, 19]. Like other heterocyclic amines it is characteristic for the amino-α-carbolines that the level is increasing in food prepared at high temperature [22]. The average daily intakes of heterocyclic amines in descending order are estimated to PhIP > AαC > MeIQx > 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) > IQ [23]. However, recent studies have shown that the content of amino-α-carbolines are low (<1 ng/g) in ordinary home-cooked dishes with beef, chicken, or fish (content of PhIP and MeIQx of up to 11.1 ng/g and 2.3 ng/g, respectively [14, 17]), but it was also concluded that the amino-α-carbolines were among the frequently found heterocyclic amines in cooked food [17].

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. Occurrence of selected heterocyclic amines (ng/g) in cooked foods

Sample/Cooking method	Temp. (°C)	Time (min)	AαC	MeAαC	PhIP	MeIQx	Ref.
Fish oven-broiled[a)]	200	20	0.1	0.1	1.7	5	[14]
Fish pan-broiled	200	2 × 6	4.6	–	23	5	[15]
Fish pan-broiled	200	2 × 12	9	–	17	3.7	[15]
Fish barbecued	200	2 × 6	6.9	–	6.2	< 1	[15]
Fish barbecued	200	2 × 12	109	–	73	< 1	[15]
Meat extract[b)]	–	–	8.1	4.0	–	–	[16]
Beef hamburger fried[a)]	175 – 200	2 × 5.6	< 0.1	< 0.04	0.6	0.7	[17]
Beef broiled	–	–	1.2	–	15.7	2.11	[18]
Beef steak fried	190	6	3.2	–	23.5	5.1	[19]
Beef steak fried	190	13	8.9	–	48.5	8.3	[19]
Beef steak grilled[a)]	180 – 210	2 × 2	0.5	0.4	4.8	2.9	[17]
Beef steak grilled	–	–	650	63	–	–	[20]
Chicken pan-fried (wok)[a)]	–	3	0.1	0.05	11.1	–	[14]
Chicken broiled	–	–	0.21	nd	2.33	38.1	[21]
Chicken grilled breast[a)]	175 – 200	2 × 6.5	0.2	< 0.02	2.3	0.3	[17]
Chicken grilled breast	350	–	170	–	21	nd	[22]
Chicken grilled sliced	–	–	180	15	–	–	[20]
Mushroom grilled	–	–	47.2	5.4	–	–	[20]
Onion grilled	–	–	1.5	nd	–	–	[20]

– not reported; nd, not detected; [a)] content in home-cooked dishes; [b)] ng/g dry product

MeAαC was also found in wine in a study where 25 wines from various regions were analyzed for heterocyclic amines. MeAαC was found to be the most widely distributed one, ranging from < 7.5 to 107 ng/L, while the amounts of PhIP and IQ ranged from nd – 83 ng/L and nd – 10 ng/L, respectively (limit of quantification > 0.1 ng/L; nd, not detected). Data on AαC content in wine were not reported [24]. Contents of amino-α-carbolines in other beverages have not been reported. Finally, both AαC and MeAαC are found in cigarette smoke condensates ranging from 2 to 260 ng/cigarette and 0.4 – 40 ng/cigarette, respectively [20, 25 – 27]. From the above data it appears that AαC is formed in amounts about tenfold higher than MeAαC in foods and cigarettes.

4 Mutagenicity and carcinogenicity

Metabolic activated amino-α-carbolines have shown mutagenicity in the *Salmonella* assay. Compared with other heterocyclic amines, the specific mutagenic activity of the amino-α-carbolines was very low. Table 2 shows the mutagenicities of selected heterocyclic amines in two *Salmonella typhimurium* strains. Generally the specific mutagenic activity of heterocyclic amines was much higher in *Salmonella typhimurium* TA98 than in *Salmonella typhimurium* TA100, indicating that heterocyclic amines induced frameshift mutations [28]. The mutagenic activity of the amino-α-carbolines compared with other heterocyclic amines such as MeIQx, DiMeIQx, IQ and PhIP was also low in the *Salmonella* strains, TA1538 and YG1019 [29]. Metabolic activated amino-α-carbolines were also mutagenic in cultivated lung cells of Chinese hamsters and in *Drosophila melanogaster* [30].

Table 2. Mutagenicities of selected heterocyclic amines in *Salmonella typhimurium* TA98 and TA100

	Revertants/μg	
Compound	TA98	TA100
AαC	300	20
MeAαC	200	120
Trp-P-2	104 200	1 800
Glu-P-2	1 900	1 200
IQ	433 000	7 000
MeIQx	145 000	14 000
PhIP	1 800	120

Data were reviewed in [28]

The International Agency for Research on Cancer (IARC) regards the amino-α-carbolines as possible carcinogens to humans (IARC, 1987, Class 2B, vol. 40, Suppl. 7). Table 3 shows the carcinogenicities of selected heterocyclic amines in rats and mice reviewed by Sugimura [2]. Furthermore, Ohgaki *et al.* [31] have shown that dietary administration of amino-α-carbolines to CDF_1 mice of both sexes induced tumors in liver and blood vessels. In this study, it was shown that the level of tumors induced by amino-α-carbolines was similar to the level of tumors induced by 2-amino-6-methyl-dipyrido[1,2-a:3′,2′-*d*]imidazole (Glu-P-1) and Glu-P-2 [31]. One other study has shown that MeAαC caused a dose-related response in development of GST-P^+ foci in the liver of male F344 rats. In contrast, PhIP only increased foci development in the highest dose group when

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 3. Carcinogenicities of selected heterocyclic amines in rats and mice

Compound	Species	Concentration (%)[a)]	Target organs
AαC	Mice	0.08	Liver, blood vessels
MeAαC	Mice	0.08	Liver, blood vessels
	Rats	0.02, 0.01	Liver
Trp-P-2	Rats	0.02	Liver
	Mice	0.01	Liver, urinary bladder
Glu-P-2	Mice	0.05	Liver, blood vessels
	Rats	0.05	Liver, small and large intestines, Zymbal gland, clitoral gland
IQ	Mice	0.03	Liver, forestomach, lung
	Rats	0.03	Liver, small and large intestines, Zymbal gland, clitoral gland, skin
MeIQx	Mice	0.06	Liver, lung, hematopoietic system
	Rats	0.04	Liver, Zymbal gland, clitoral gland, skin
PhIP	Mice	0.04	Lymphoid tissue
	Rats	0.04	Large intestines, mammary gland

Data were reviewed in [2]
[a)] The dose was given in the daily food

given at the same dosage [32]. Other studies have shown that AαC induced preneoplastic colonic aberrant crypt foci (ACF) in colon and small intestine of C57BL/N6 mice [33], DNA adducts in colon and small intestine of Sprague-Dawley rats [34], and mutations in the colon of Big Blue™ mice [35]. Recently, it was shown that AαC, but not MeAαC, increased the number and diameter of small intestinal tumors in C57BL/6J-*Min*/+ mice [36].

While the mutagenicities of the amino-α-carbolines were very low compared with other heterocyclic amines, the carcinogenicities of the amino-α-carbolines were on the same order as those of, *e.g.*, IQ and MeIQx and even higher than the carcinogenicity of PhIP in some studies. However, most of the rodent carcinogenicity studies were performed with dietary administrated doses close to the maximum tolerable dose of the rodents.

5 In vitro metabolism

Heterocyclic amines including amino-α-carbolines follow two metabolic pathways, detoxification or activation (Fig. 2). The first step in the metabolism of the heterocyclic amines is a phase I hydroxylation catalyzed by cytochrome P450 enzymes. Detoxified compounds are often ring-hydroxylated followed by phase II conjugation with glucuronic or sulfonic acid. However, conjugation of the exocyclic amino group with glucuronic or sulfuric acid is also a usual detoxification pathway. Activated compounds are

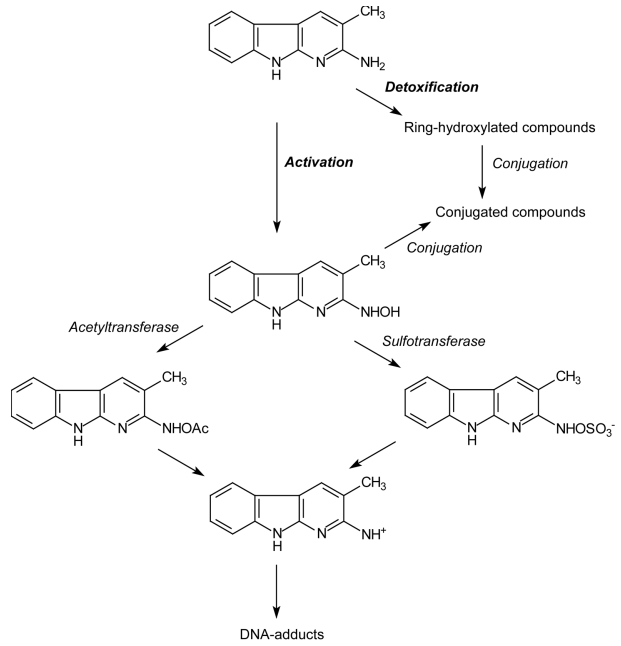

Figure 2. Metabolic pathway for amino-α-carbolines (MeAαC).

hydroxylated in their characteristic exocyclic amino group, usually followed by *O*-esterification catalyzed by, *e.g.*, acetyltransferase or sulfotransferase. This results in formation of reactive derivates, which spontaneously undergo heterolytic fission to electrophilic arylnitrenium ion intermediates, which are able to form adducts with macromolecules, such as proteins and DNA [5, 30].

In vitro studies have shown that amino-α-carbolines were activated to mutagenic N^2-hydroxy derivates by hepatic microsomes from various species, such as rats, mice, hamsters, guinea pigs, and humans [9, 37–39]. Previously, the major metabolites of AαC in human and rodent hepatic microsomes and the major metabolites of MeAαC in hepatic microsomes from polychlorinated biphenyl (PCB)-induced rat were characterized [39, 40], and recently the metabolisms of both AαC and MeAαC were compared in hepatic microsomes from PCB-induced rat, untreated rat, and human [41].

In the latter study the phase I metabolites were characterized and the distributions between detoxification and activation were determined. In addition to three minor detoxified metabolites, two major detoxified metabolites of AαC were characterized as 3-OH-AαC and 6-OH-AαC. MeAαC was metabolized to one minor and three major detoxified metabolites characterized as 3-CH_2OH-MeAαC, 6-OH-MeAαC, and 7-OH-MeAαC (Fig. 3). The minor detoxified metabolites of both AαC and MeAαC were proposed to be other ring-hydroxylated metabolites based on MS data and comparison of UV data with the major metabolites. Both

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

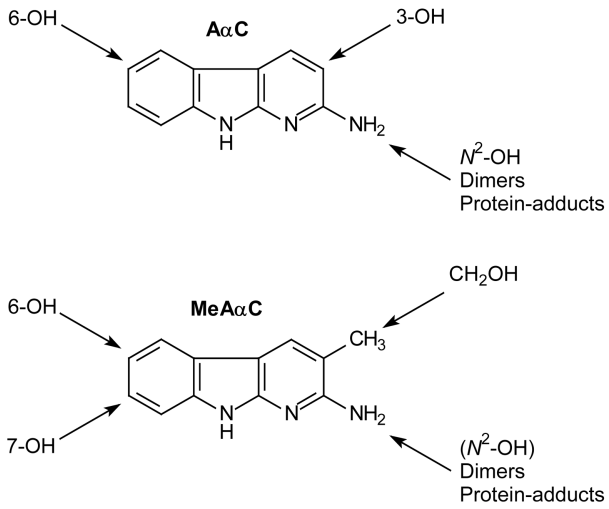

Figure 3. *In vitro* metabolites of amino-α-carbolines catalyzed by phase I enzymes (–OH, hydroxylation). Furthermore three minor hydroxylated AαC metabolites and one minor hydroxylated MeAαC metabolite were identified and proposed to be other ring-hydroxylated metabolites.

AαC and MeAαC were activated to their respective N^2-hydroxy derivates. In the microsomal incubations of AαC, the N^2-OH-derivate was detected, but not in incubations of MeAαC. Incubations of both AαC and MeAαC contained products, proposed to be dimers formed from the reactive N^2-OH-derivates and their parent compounds. Also, relatively large amounts of AαC and MeAαC were bound to the microsomal proteins, indicating an adduct formation of reactive metabolites with proteins (Fig. 3) [41].

The percentage distribution between detoxification and activation of the amino-α-carboline metabolites in the different types of hepatic microsomes showed similar patterns for both AαC and MeAαC. In PCB-induced rat microsomes, the major part of the metabolites was detoxified; only a small amount (~10%) was activated. In the untreated rat microsomes there was a 50:50% distribution between detoxification and activation, while the major part of the metabolites from the human microsomes (~60%) was activated and reacted further to dimers and protein adducts [41]. The distribution between detoxification and activation of MeAαC in PCB-induced hepatic rat microsomes was comparable with a previous study of the MeAαC metabolism, where about 13% of MeAαC was activated [40]. On the contrary, an other study has shown that the activation of AαC by PCB-induced hepatic rat microsomes increased the amount of revertants in *Salmonella typhimurium* TA98 assays sixteenfold compared with activation of AαC by untreated hepatic rat microsomes [37]. Raza *et al.* [39], found a lower degree of activation of AαC by human hepatic microsomes, only 15% of AαC was activated and 85% was detoxified. In this study, however, binding of the activated metabolite to protein was not taken into consideration [39]. However, *in vitro* studies of PhIP have shown species variation in metabolic distribution similar to the present results on AαC and MeAαC. The ratios of *N*-hydroxylation to 4′-hydroxylation of PhIP was 97:1, 3.3:1, and 1.7:1 for humans, rats, and mice, respectively [42].

Species differences between rats, mice, hamsters, and guinea pigs in the activity of hepatic cytochrome P450 enzymes responsible for the activation of MeAαC have been reported [38]. *In vitro* studies of the activation of AαC catalyzed by recombinant cytochrome P450 enzymes showed that rat CYP1A1 activated AαC more efficiently than rat CYP1A2 [43]. In accordance with that, the activation of AαC was shown to be about 56% catalyzed by rat CYP1A1 and 23% catalyzed by rat CYP1A2. Also, the total amount of AαC metabolized by rat CYP1A1 was about threefold higher than AαC metabolized by rat CYP1A2 [44]. For other heterocyclic amines of the aminoimidazoazaarene type, such as PhIP, IQ, MeIQ, and MeIQx, rat CYP1A2 was the major activating enzyme [43, 45]. Also, MeAαC was primarily metabolized by rat CYP1A1 (~57%), while only a small amount (~7%) was metabolized by rat CYP1A2. The degree of activation was low, about 10% and 2% catalyzed by rat CYP1A1 and rat CYP1A2, respectively [44].

The metabolism of amino-α-carbolines catalyzed by recombinant human cytochrome P450 enzymes showed that human CYP1A2 was the major activating enzyme, while human CYP1A1 and human CYP2C10 showed low catalytic activities and human CYP3A4 showed no catalytic activity towards AαC or MeAαC [39, 44]. These data were in accordance with *in vitro* studies of other heterocyclic amines, where enzymes from the CYP1A-family and especially human CYP1A2 were shown to be involved in the phase I metabolism and activation of heterocyclic amines [7, 46, 47]. Only 2% of both AαC and MeAαC were metabolized without activation by human CYP1A1, while 64% and 13% of AαC and MeAαC, respectively were metabolized by human CYP1A2. The activation catalyzed by human CYP1A2 was about 27% and 1% of total metabolized AαC and MeAαC, respectively [44].

The activation of AαC and MeAαC was about 50% in hepatic microsomes from untreated rats and about 60% in human hepatic microsomes [41]. The catalytic activity of human CYP1A2 and rat CYP1A1 were very low towards MeAαC compared with AαC. Therefore, it was suggested that the methyl group sterically hinders activation of MeAαC and that other microsomal enzymes than CYP1A may be involved in the activation of AαC and particularly MeAαC [44].

The *in vitro* phase II metabolism of the amino-α-carbolines has been described in few studies. Correlations and inhibi-

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

tion studies suggested that the isoform of sulfotransferase primarily responsible for bioactivation of N^2-OH-AαC in human liver cytosol was SULT1A1, but also the *N*-acetyltransferases, NAT1 and NAT2, catalyzed the bioactivation of N^2-OH-AαC. The rate of bioactivation of N^2-OH-AαC catalyzed by SULT1A1, NAT1, and NAT2 was significantly higher than the bioactivation of N^2-OH-PhIP [48]. A recent study has shown similar results for the bioactivation of MeAαC in recombinant test systems expressing human xenobiotic-metabolizing enzymes. MeAαC induced gene mutations in Chinese hamster V79-derived cells co-expressing human CYP1A2 and human SULT1A1, but was inactive in cells co-expressing CYP1A2 and NAT1 or NAT2. MeAαC, tested in the presence of rat liver postmitochondrial fraction, showed strongly enhanced mutagenicity in a strain expressing SULT1A1 compared to control strains, *Salmonella typhimurium* TA1538 and TA1538/1,8-DNP (deficient in endogenous acetyltransferase). N^2-OH-MeAαC was a direct mutagen to *Salmonella typhimurium* strains TA1538 and TA1538/1,8-DNP, and the mutagenicity was strongly enhanced in corresponding strains expressing SULT1A1. A moderate enhancement was observed when SULT1A2, SULT1B1, SULT1C2, or NAT2 were expressed, while several other enzymes tested (SULT1A3, 1C1, 1E1, 2A1, 2B1a, 2B1b, 4A1, and NAT1) did not indicate any activation of N^2-OH-MeAαC. Finally, 3-CH_2OH-AαC showed a week mutagenic activity in *Salmonella typhimurium* TA1538 expressing SULT1A1, while the other major detoxified metabolites, 6-OH-MeAαC and 7-OH-MeAαC, were inactive under the conditions studied [49].

6 In vivo metabolism

Compared with other heterocyclic amines, little is known about the *in vivo* metabolism of the amino-α-carbolines. While the *in vitro* metabolism of amino-α-carbolines has been studied [50, 51], the *in vivo* metabolism of amino-α-carbolines has only been described in rats in two recent studies [52, 53].

For both AαC and MeAαC the superior excretion in urine and faeces was similar in the rats dosed with each of the compounds, but large individual differences were found between the metabolic pattern of AαC and MeAαC (Fig. 4). AαC showed a very simple metabolic pattern compared with MeAαC. In 24 h urine collections unmetabolized AαC and seven conjugated AαC metabolites were characterized. Three minor metabolites were characterized as AαC-N^2-glucuronide and glucuronic acid conjugates of 3-OH-AαC and 6-OH-AαC. Four metabolites were all characterized as sulfuric acid conjugates and accounted for the largest amount of metabolites excreted in urine. The two major sulfuric acid conjugates were identified as AαC-3-*O*-sulfate and AαC-6-*O*-sulfate, while the minor sulphuric acid conjugates were proposed to be other O-sulfonated metabolites [52].

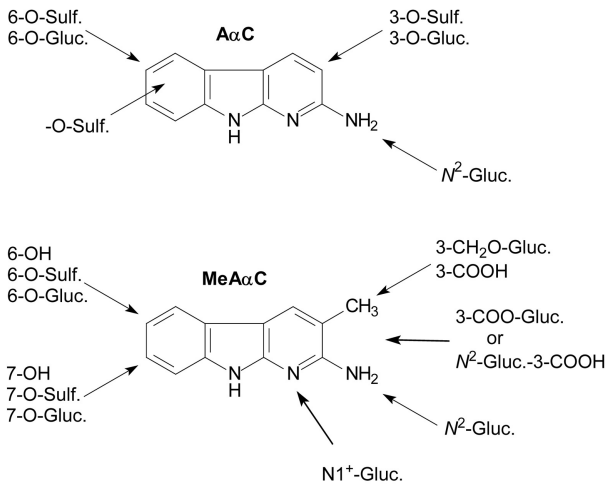

Figure 4. *In vivo* metabolites of amino-α-carbolines catalyzed by phase I and phase II enzymes.

MeAαC showed a more complex metabolic pattern compared with AαC (Fig. 4). In 24 h urine collections both unmetabolized MeAαC and the following phase I and phase II metabolites were identified: 6-OH-MeAαC and 7-OH-MeAαC, MeAαC-N^2-glucuronide, AαC-3-CH_2O-glucuronide, 3-carboxy-AαC and 3-carboxy-AαC-glucuronide, sulfate and glucuronide conjugates of 6-OH-MeAαC and 7-OH-MeAαC. The major metabolite was a very unstable compound, proposed to be MeAαC-N^1-glucuronide [53]. It was remarkable that AαC primarily was metabolized to sulfate conjugates, while MeAαC primarily was metabolized to glucuronide conjugates.

The major amounts of both the AαC and MeAαC doses were excreted in the urine samples collected in 24 h, 34% and 31%, respectively. Only a few percent of the doses were excreted in the urine samples collected in 48 and 72 h. In the 24 h faeces samples a large amount of unmetabolized AαC and MeAαC was excreted, 26% and 22%, respectively. In 48 h urine samples 8% and 7% of the doses were excreted in faeces as unmetabolized AαC and MeAαC, respectively. The only metabolite found in the faeces samples was a few percent of the phase I metabolite, 7-OH-MeAαC [52, 53].

Hydroxylation of the exocyclic amino group followed by glucuronic acid conjugation is a usual route for detoxification of phase I activated heterocyclic amines [7, 8], but activated metabolites excreted in urine or faeces were not detected *in vivo* [52, 53]. All the identified AαC and MeAαC metabolites were conjugated detoxification products. Less than 2% of the excreted AαC and MeAαC was bound to urine precipitate and it is possible that activated

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

N^2-OH-AαC or N^2-OH-MeAαC could be bound to proteins or other macromolecules in these precipitates [52, 53].

The total recoveries of the AαC and MeAαC doses in rat urine and faeces collected during three days were about 55% and 65%, respectively [52, 53]. These data were in accordance with the previous *in vitro* studies on AαC or MeAαC, where a 50 : 50 distribution between detoxification and activation in rat liver microsomes was found [41]. Compared with other *in vivo* studies of heterocyclic amines, a 55 – 65% recovery of the dose was low, *e. g.*, the total recovery of urinary metabolites in rats was about 80 – 90% after dosing with PhIP, IQ, and MeIQx [54, 55]. However, many studies have shown that the metabolism of heterocyclic amines was highly species-dependent [8]. The phase I metabolism of AαC and MeAαC in hepatic microsomes from PCB-induces Wistar rats and control Wistar rat showed remarkable differences, about 90% and 50% detoxification, respectively. In human hepatic microsomes only about 40% of both AαC and MeAαC were detoxified [41]. The *in vivo* metabolism of the amino-α-carbolines indicates according to the *in vitro* metabolism, that a considerable amount of the amino-α-carbolines probably was activated in rats and maybe the amino-α-carbolines differ from other heterocyclic amines by a metabolism dominated of activation.

7 DNA adduct formation

The adduct formation is the final step in the bioactivation of heterocyclic amines (Fig. 2). Heterocyclic amines, like other chemical carcinogens, have been shown to form adducts with cellular macromolecules, such as DNA, RNA, and proteins. DNA-adducts are generally regarded as a cellular event which can result in genetic mutations; several studies support the role of DNA-adducts in carcinogenesis. Generally, it is proposed that the arylnitrenium ions of heterocyclic amines form adducts by covalent modification of bases in deoxyribonucleic acid. To date, the guanine adducts of MeAαC, AαC, Trp-P-2, Glu-P-1, MeIQ, DiMeIQx and PhIP have been characterized [5].

The chemical syntheses of DNA adducts of heterocyclic amines have been carried out by several methods. A well-described method with a high yield of DNA-adducts involved *O*-acetylation of the *N*-hydroxylamine derivates of the heterocyclic amines by acetic anhydride, followed by incubation with nucleotides or DNA [56, 57]. Previously DNA-adducts of the amino-α-carbolines were synthesized by an alternative route, where nitro derivates of the amino-α-carbolines were chemically reduced in the presence of DNA. The major adducts synthesized by this method were characterized as N^2-(deoxyguanosin-8-yl)-MeAαC (dG-MeAαC) and N^2-(deoxyguanosin-8-yl)-AαC (dG-AαC) [50, 58]. Recently, the chemical syntheses of dG-MeAαC and dG-AαC succeeded by using the *O*-acetylation method (Fig. 5) [59].

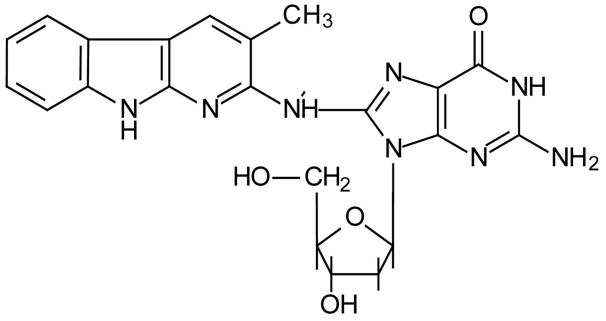

Figure 5. Structure of N^2-(deoxyguanosin-8-yl)-MeAαC (dG-MeAαC).

The yields of synthetic dG-MeAαC and dG-AαC were very dependent on parameters, such as absence of atmospheric oxygen, reaction time, and temperature. The highest yields of dG-MeAαC and dG-AαC were obtained by acetylation at -20°C in argon atmosphere for a short time, followed by reaction with a large amount of 2′-deoxyguanosine at room temperature [59]. The reaction temperatures used were different from the temperature used for the synthesis of dG-PhIP and dG-4,8-DiMeIQx where the acetylation were carried out 0°C and -50°C, respectively [56], this indicates that different heterocyclic amines require different reaction conditions for optimum yield [57]**.** Surprisingly, it was found that dG-amino-α-carbolines and 3′-phospho-dG-amino-α-carbolines were unstable compounds at pH-values <7 and easily decomposed to the N^2-(guanin-8-yl)-adducts (G-MeAαC and G-AαC) and the parent compounds (MeAαC and AαC), respectively [59]. Such pH-dependent instability of the dG-adduct of heterocyclic amines has not been reported before.

Under the above-mentioned condition, N^2-acetoxy-derivates did not form DNA-adducts with the other deoxynucleosides; 2′-deoxyadenosine, 2′-deoxycytidine, or 2′-deoxythymidine [59]. Other studies of heterocyclic amines showed similar results: AαC, IQ, MeIQx, 4,8-DiMeIQx, Glu-P-1, Trp-P-2, and PhIP formed major adducts by covalently binding to the C8-carbon atom on the guanine base in DNA. IQ and MeIQx in addition formed minor adduct where the C5 atom was attached to N2 of deoxyguanosine [34, 56, 57, 60].

The amount of DNA-adducts formed *in vivo* is often very low and therefore the ^{32}P-postlabeling analysis is a common method to analyze DNA modified with heterocyclic amines. The detection limits for quantitative assays are typically in the range of one adduct/10^9 nucleotides [61 – 64]. Only few *in vivo* studies have focused on DNA-adduct formation of the amino-α-carbolines. The adduct level in

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 4. Total adduct levels in liver from rats dosed with amino-α-carbolines

Species	Compound	Dose (mg/kg)	Adduct/10^7 nucleotides	Ref.
Male Wistar rats	MeAαC	8.6[a)]	0.5	[59]
Male F344 rats	MeAαC	100–800[b)]	1–5	[32]
Female F344 rats	MeAαC acetate	800[c)]	128	[51]
Female F344 rats	AαC acetate	800[c)]	27	[51]
Male F344 rats	AαC	160–800[d)]	0.1–2	[50]
Male Spague Dawley rats	AαC	75[a)]	15	[34]
Female Spague Dawley rats	AαC	75[a)]	17	[34]
Female Spague Dawley rats	AαC	75[e)]	51	[34]

a) single dose
b) six weeks in food
c) one week in food
d) two weeks in food
e) multi dose × 12

the liver of male Wistar rats dosed with a single dose of MeAαC was found to be about 50 adducts/10^9 nucleotides three days after dosing. A four fold lower adduct level was found in the DNA from colon and heart and a twelvefold lower adduct level in the kidney [59]. These adduct levels were low as expected because the adduct level decrease over time, *e.g.*, the level of tritium labeled PhIP in liver of rats decreased fourteenfold from 2 to 72 h after single dosing [65]. In comparison female F344 rats fed MeAαC acetate showed higher adduct levels: 128 adducts/10^7 nucleotides in the liver and two- to threefold lower adduct level in other organs, such as kidney, lung, spleen, and salivary glands [51]. In another study of male F344 rats fed 100–800 ppm of MeAαC showed a dose-response dependent adduct formation of 1–5 adducts/10^7 nucleotides in the liver. This adduct level was up to tenfold higher than the PhIP-DNA adduct level in similar studies [32]. Studies of AαC-DNA adduct formation in tissues of male and female Spague Dawley rats showed an adduct level of 15–17 adducts/10^7 nucleotides in the liver. A 12- to 34-fold lower adduct level was found in the DNA from stomach, small intestine, colon, kidney, and mammary gland of female Spague Dawley rats [34]. In all studies of amino-α-carboline-DNA-adduct formation it was found that the adduct level was highest in the liver of rats, compared with other investigated organs. Table 4 shows the adduct levels found in rat liver in different investigations [32, 34, 50, 51, 59]. The liver of rats seems to be the major target tissues for activated amino-α-carbolines, like many of the aminoimidazoazaarenes that form the highest adduct level in the liver of different species. In contrast, PhIP forms low adduct levels in the liver relative to many extrahepatic tissues *e.g.*, the colon and kidney [5]. One explanation for the relatively low level of amino-α-carboline-adduct formation in extrahepatic tissues could be that activated amino-α-carbolines are extremely unstable and reactive substances compared with other activated heterocyclic amines [41]. The *in vivo* phase I activation of amino-α-carbolines primarily takes place in the liver and the metabolites may be too unstable to be transported to other organs via the bloodstream.

8 Conclusions

The amino-α-carbolines were among the first food-borne heterocyclic amines discovered, but compared with other heterocyclic amines there has not been much attention to these compounds and relatively little is known about the metabolism and bioactivation of the amino-α-carbolines. They are found in various proteinouceous food, wine, and cigarette smoke. Modern analysis methods have recently shown that the amino-α-carbolines are common in the Western food and the contents in food are comparable with that of, *e.g.*, PhIP and MeIQx.

The amino-α-carbolines have been shown to be mutagenic in bacterial and mammalian tests and carcinogenic in rat and mice. The mutagenicity of the amino-α-carbolines are low in the Ames test compared to other heterocyclic amines, but the carcinogenicity is moderate to high compared to other heterocyclic amines. The amino-α-carbolines form a high level of DNA adduct in the liver compared with other organs in rats.

The phase I and phase II metabolites of amino-α-carbolines have been characterized and quantified. Both *in vitro* and *in vivo* metabolism studies in rats showed that the distribution between detoxification and activation differs from other heterocyclic amines by the low degree of detoxification, indicating that high amounts of the amino-α-carbolines were activated. Metabolism studies in rats showed that AαC is excreted in urine mainly as metabolites conjugated with sulfates, while MeAαC is excreted in urine mainly as metabolites conjugated with glucuronic acid. The parent com-

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

pounds of both amino-α-carbolines are excreted unmetabolized in faeces. The total excretion of the amino-α-carbolines is low compared with other heterocyclic amines.

Amino-α-carbolines are carcinogenic in rodents when exposed to high doses. Humans are exposed to low doses of these compounds through the diet. Risk extrapolation from high-dose animal studies to low-dose human exposure is associated with uncertainty. Furthermore, differences in the metabolism between man and rodents may influence the relative risk. Also, differences in metabolism within the human population may affect the individual cancer risk from exposure to these substances.

In future, further investigations, on the metabolism, bioactivation, and DNA binding of the amino-α-carbolines are needed particularly in applying low doses. The distribution of activated amino-α-carbolines in various organs of rats may be studied to establish whether the liver is the major target organ of activated amino-α-carbolines. Furthermore, the binding of activated amino-α-carbolines to proteins has to be studied. The ultimate advance in the study of the amino-α-carbolines will be to find and develop a human biomarker, which reflects the human intake and the amount of compounds activated in human, like the recently discovered human biomarker of PhIP [66].

9 References

[1] Matsumoto, T., Yoshida, D., Mizusaki, S., Okamoto, H., Mutagenic activity of amino acid pyrolyzates in *Salmonella typhimurium* TA 98. *Mutat. Res.* 1977, *48*, 279 – 286.

[2] Sugimura, T., Overview of carcinogenic heterocyclic amines. *Mutat. Res.* 1997, *376*, 211 – 219.

[3] Sugimura, T., Adamson, R. H., Introduction. In: Nagao, M., Sugimura, T. (Eds.), *Food borne carcinogegens; Heterocyclic amines*, John Wiley & Sons, West Sussex, England 2002, pp. 1 – 4.

[4] Felton, J. S., Knize, M. G., Shen, N. H., Lewis, P. R., *et al.*, The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Carcinogenesis* 1986, *7*, 1081 – 1086.

[5] Snyderwine, E. G., Turteltaub, K. W., Interactions with cellular macromolecules. In: Nagao, M., Sugimura, T. (Eds.), *Food borne carcinogegens; Heterocyclic amines*, John Wiley & Sons, West Sussex, England 2002, pp. 131 – 161.

[6] Felton, J. S., Jägerstad, M., Knize, M. G., Skog, K., Wakabayashi, K., Contents in Foods, Beverages and Tobacco. In: Nagao, M., Sugimura, T., (Eds.) *Food borne carcinogegens; Heterocyclic amines*, John Wiley & Sons, West Sussex, England 2002, pp. 31 – 72.

[7] Yanazoe, Y., Nagata, K., *In vitro* metabolism. In: Nagao, M., Sugimura, T. (Eds.), *Food borne carcinogegens; Heterocyclic amines*, John Wiley & Sons, West Sussex, England 2002, pp. 74 – 89.

[8] King, R. S., Kadlubar, F. F., Turesky, R., *In vivo* metabolism. In: Nagao, M., Sugimura, T. (Eds.), *Food borne carcinogegens; Heterocyclic amines*, John Wiley & Sons, West Sussex, England 2002, pp. 90 – 111.

[9] Yoshida, D., Matsumoto, T., Yoshimura, R., Matsuzaki, T., Mutagenicity of amino-α-carbolines in pyrolysis products of soybean globulin. *Biochem. Biophys. Res. Commun.* 1978, *83*, 915 – 920.

[10] Hibino, S., Sugino, E., Kuwada, T., Ogura, N., *et al.*, Synthesis of mutagenic amino-α-carbolines AαC and MeAαC by the thermal electrocyclic reaction of 2-azahexa-1,3,5-triene intermediates. *Chem. Pharmaceut. Bull.* 1991, *39*, 79 – 80.

[11] Skog, K., Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. *Food Chem. Toxicol.* 2002, *40*, 1197 – 1203.

[12] Jägerstad, M., Skog, K., Arvidsson, P., Solyakov, A., Chemistry, formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. *Z. Lebensm.-Unters. Forsch. A* 1998, *207*, 419 – 427.

[13] Pais, P., Knize, M. G., Chromatographic and related techniques for the determination of aromatic heterocyclic amines in foods. *J. Chromatogr. B* 2000, *747*, 139 – 169.

[14] Ristic, A., Cichna, M., Sontag, G., Determination of less polar heterocyclic aromatic amines in standardised beef extracts and cooked meat consumed in Austria by liquid chromatography and fluorescence detection. *J. Chromatogr. B* 2004, *802*, 87 – 94.

[15] Gross, G. A., Gruter, A., Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. *J. Chromatogr.* 1992, *592*, 271 – 278.

[16] Skog, K., Solyakov, A., Arvidsson, P., Jägerstad, M., Analysis of nonpolar heterocyclic amines in cooked foods and meat extracts using gas chromatography-mass spectrometry. *J. Chromatogr. A* 1998, *803*, 227 – 233.

[17] Busquets, R., Bordas, M., Toribio, F., Puignou, L., Galceran, M. T., Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. *J. Chromatogr. B* 2004, *802*, 79 – 86.

[18] Wakabayashi, K., Nagao, M., Esumi, H., Sugimura, T., Food-derived mutagens and carcinogens. *Cancer Res.* 1992, *52*, 2092s – 2098s.

[19] Gross, G. A., Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. *Carcinogenesis* 1990, *11*, 1597 – 1603.

[20] Matsumoto, T., Yoshida, D., Tomita, H., Determination of mutagens, amino-α-carbolines in grilled foods and cigarette smoke condensate. *Cancer Lett.* 1981, *12*, 105 – 110.

[21] Wakabayashi, K., Ushiyama, H., Takahashi, M., Nukaya, H., *et al.*, Exposure to heterocyclic amines. *Environ. Health Perspect.* 1993, *99*, 129 – 134.

[22] Knize, M. G., Sinha, R., Salmon, C. P., Mehta, S. S., *et al.*, Formation of heterocyclic amine mutagens/carcinogens during home and commercial cooking of muscle foods. *J. Muscle Foods* 1996, *7*, 271 – 279.

[23] Layton, D. W., Bogen, K. T., Knize, M. G., Hatch, F. T., *et al.*, Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. *Carcinogenesis* 1995, *16*, 39 – 52.

[24] Richling, E., Decker, C., Haring, D., Herderich, M., Schreier, P., Analysis of heterocyclic aromatic amines in wine by high-performance liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. A* 1997, *791*, 71 – 77.

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[25] Manabe, S., Wada, O., Kanai, Y., Simultaneous determination of amino-alpha-carbolines and amino-γ-carbolines in cigarette smoke condensate by high-performance liquid chromatography. *J. Chromatogr.* 1990, *529*, 125 – 133.

[26] Wakabayashi, K., Kim, I. S., Kurosaka, R., Yamaizumi, Z., *et al.*, Identification of new mutagenic heterocyclic amines and quantification of known heterocyclic amines. *Princess Takamatsu Symp.* 1995, *23*, 39 – 49.

[27] Yoshida, D., Matsumoto, T., Amino-α-carbolines as mutagenic agents in cigarette smoke condensate. *Cancer Lett.* 1980, *10*, 141 – 149.

[28] Sugimura, T., Wakabayashi, K., Mutagens and carcinogens in food. *Prog. Clin. Biol. Res.* 1990, *347*, 1 – 18.

[29] Pfau, W., Martin, F. L., Cole, K. J., Venitt, S., *et al.*, Heterocyclic aromatic amines induce DNA strand breaks and cell transformation. *Carcinogenesis* 1999, *20*, 545 – 551.

[30] Eisenbrand, G., Tang, W., Food-borne heterocyclic amines. Chemistry, formation, occurrence and biological activities. A literature review. *Toxicology* 1993, *84*, 1 – 82.

[31] Ohgaki, H., Matsukura, N., Morino, K., Kawachi, T., *et al.*, Carcinogenicity in mice of mutagenic compounds from glutamic acid and soybean globulin pyrolysates. *Carcinogenesis* 1984, *5*, 815 – 819.

[32] Hasegawa, R., Takahashi, S., Shirai, T., Iwasaki, S., *et al.*, Dose-dependent formation of preneoplastic foci and DNA adducts in rat liver with 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Carcinogenesis* 1992, *13*, 1427 – 1431.

[33] Okonogi, H., Ushijima, T., Shimizu, H., Sugimura, T., Nagao, M., Induction of aberrant crypt foci in C57BL/6N mice by 2-amino-9*H*- pyrido[2,3-*b*]indole (AαC) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx). *Cancer Lett.* 1997, *111*, 105 – 109.

[34] Snyderwine, E. G., Sadrieh, N., King, R. S., Schut, H. A., Formation of DNA adducts of the food-derived mutagen 2-amino-9*H*-pyrido- [2,3-*b*]indole (AαC) and bioassay of mammary gland carcinogenicity in Sprague-Dawley rats. *Food Chem. Toxicol.* 1998, *36*, 1033 – 1041.

[35] Zhang, X. B., Felton, J. S., Tucker, J. D., Urlando, C., Heddle, J. A., Intestinal mutagenicity of two carcinogenic food mutagens in transgenic mice: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and amino(α)carboline. *Carcinogenesis* 1996, *17*, 2259 – 2265.

[36] Steffensen, I. L., Paulsen, J. E., Alexander, J., The food mutagen 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) but not its methylated form (MeAαC) increases intestinal tumorigenesis in neonatally exposed multiple intestinal neoplasia mice. *Carcinogenesis* 2002, *23*, 1373 – 1378.

[37] Niwa, T., Yamazoe, Y., Kato, R., Metabolic activation of 2-amino-9*H*-pyrido[2,3-*b*]indole by rat-liver microsomes. *Mutat. Res.* 1982, *95*, 159 – 170.

[38] Degawa, M., Agatsuma, T., Hashimoto, Y., Species difference among experimental rodents in the activity and induction of cytochrome P-450 isozymes for mutagenic activation of carcinogenic aromatic amines. *Jpn. J. Cancer Res.* 1990, *81*, 1253 – 1258.

[39] Raza, H., King, R. S., Squires, R. B., Guengerich, F. P., *et al.*, Metabolism of 2-amino-α-carboline. A food-borne heterocyclic amine mutagen and carcinogen by human and rodent liver microsomes and by human cytochrome P4501A2. *Drug. Metab. Dispos.* 1996, *24*, 395 – 400.

[40] Frandsen, H., Damkjaer, S., Grivas, S., Andersson, R., *et al.*, Microsomal metabolism and activation of the environmental carcinogen 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole. *Mutagenesis* 1998, *13*, 181 – 185.

[41] Frederiksen, H., Frandsen, H., *In vitro* metabolism of two heterocyclic amines, 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and 2-amino-3-methyl-9*H*-pyridol2,3-*b*]indole (MeAαC) in human and rat hepatic microsomes. *Pharmacol. Toxicol.* 2002, *90*, 127 – 134.

[42] Lin, D. X., Lang, N. P., Kadlubar, F. F., Species differences in the biotransformation of the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by hepatic microsomes and cytosols from humans, rats, and mice. *Drug. Metab. Dispos.* 1995, *23*, 518 – 524.

[43] Shimada, T., Guengerich, F. P., Activation of amino-α-carboline, 2-amino-1-methyl-6- phenylimidazo[4,5-*b*]pyridine and a copper phthalocyanine cellulose extract of cigarette smoke condensate by cytochrome P-450 enzymes in rat and human liver microsomes 1. *Cancer Res.* 1991, *51*, 5284 – 5291.

[44] Frederiksen, H., Frandsen, H., Impact of Five Cytochrome P450 Enzymes on the Metabolism of two Heterocyclic Aromatic Amines, 2-Amino-9*H*-pyrido[2,3-*b*]indole (AαC) and 2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC). *Pharmacol. Toxicol.* 2003, *92*, 246 – 248.

[45] McManus, M. E., Burgess, W. M., Veronese, M. E., Huggett, A., *et al.*, Metabolism of 2-acetylaminofluorene and benzo(a)pyrene and activation of food-derived heterocyclic amine mutagens by human cytochromes P-450. *Cancer Res.* 1990, *50*, 3367 – 3376.

[46] Sugimura, T., Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. *Mutat. Res.* 1985, *150*, 33 – 41.

[47] Knize, M. G., Shen, N. H., Healy, S. K., Hatch, F. T., Felton, J. S., The use of bacterial mutation tests to monitor chemical isolation of the mutagens in cooked food. *Dev. Ind. Microbiol.* 1987, *28*, 171 – 180.

[48] King, R. S., Teitel, C. H., Kadlubar, F. F., *In vitro* bioactivation of *N*-hydroxy-2-amino-α-carboline. *Carcinogenesis* 2000, *21*, 1347 – 1354.

[49] Glatt, H., Pabel, U., Meinl, W., Frederiksen, H., Frandsen, H., Muckel, E., Bioactivation of the heterocyclic aromatic amine 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) in recombinant test systems expressing human xenobiotic-metabolizing enzymes. *Carcinogenesis* 2004, *25*, 801 – 807.

[50] Pfau, W., Schulze, C., Shirai, T., Hasegawa, R., Brockstedt, U., Identification of the major hepatic DNA adduct formed by the food mutagen 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC). *Chem. Res. Toxicol.* 1997, *10*, 1192 – 1197.

[51] Yamashita, K., Takayama, S., Nagao, M., Sato, S., Sugimura, T., Aminomethyl-α-carboline-induced dna modification in rat salivary glands and pancreas detected by phosphorus-31 postlabeling method. *Proceedings of the Japan Academy Series B, Physical and Biological Sciences* 1986, *62*, 45 – 48.

[52] Frederiksen, H., Frandsen, H., Excretion of metabolites in urine and faeces from rats dosed with the heterocyclic amine, 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC). *Food Chem. Toxicol.* 2004, *42*, 879 – 885.

[53] Frederiksen, H., Frandsen, H., Identification of metabolites in urine and feces from rats dosed with the heterocyclic amine, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC). *Drug. Metab. Dispos.* 2004, *32*, 661 – 665.

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[54] Kadlubar, F., Kaderlik, R. K., Mulder, G. J., Lin, D., *et al.*, Metabolic activation and DNA adduct detection of PhIP in dogs, rats, and humans in relation to urinary bladder and colon carcinogenesis. *Princess Takamatsu Symp.* 1995, *23*, 207 – 213.

[55] Turesky, R. J., Gross, G. A., Stillwell, W. G., Skipper, P. L., Tannenbaum, S. R., Species differences in metabolism of heterocyclic aromatic amines, human exposure, and biomonitoring. *Environ Health Perspect* 1994, *102*, Suppl. 6, 47 – 51.

[56] Frandsen, H., Grivas, S., Andersson, R., Dragsted, L., Larsen, J. C., Reaction of the N2-acetoxy derivative of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) with 2′-deoxyguanosine and DNA. Synthesis and identification of N2-(2′-deoxyguanosin-8-yl)-PhIP. *Carcinogenesis* 1992, *13*, 629 – 635.

[57] Frandsen, H., Grivas, S., Turesky, R. J., Andersson, R., *et al.*, Formation of DNA adducts by the food mutagen 2-amino-3,4,8-trimethyl-3*H*-imidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) in vitro and in vivo. Identification of a N2-(2′-deoxyguanosin-8-yl)-4,8-DiMeIQx adduct. *Carcinogenesis* 1994, *15*, 2553 – 2558.

[58] Pfau, W., Brockstedt, U., Schulze, C., Neurath, G., Marquardt, H., Characterization of the major DNA adduct formed by the food mutagen 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) in primary rat hepatocytes. *Carcinogenesis* 1996, *17*, 2727 – 2732.

[59] Frederiksen, H., Frandsen, H., Pfau, W., Syntheses of DNA adducts of two heterocyclic amines, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) and 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and identification of DNA adducts in organs from rats dosed with MeAαC. *Carcinogenesis* 2004, *25*, 1525 – 1533.

[60] Hashimoto, Y., Shudo, K., Okamoto, T., Activation of a mutagen, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole. Identification of 3-hydroxyamino-1-methyl-5*H*-pyrido[4,3-b]indole and its reacion with DNA. *Biochem. Biophys. Res. Commun.* 1980, *96*, 355 – 362.

[61] Poirier, M. C., Santella, R. M., Weston, A., Carcinogen macromolecular adducts and their measurement 2. *Carcinogenesis* 2000, *21*, 353 – 359.

[62] Gupta, R. C., Reddy, M. V., Randerath, K., 32P-postlabeling analysis of non-radioactive aromatic carcinogen–DNA adducts. *Carcinogenesis* 1982, *3*, 1081 – 1092.

[63] Gupta, R. C., Enhanced sensitivity of 32P-postlabeling analysis of aromatic carcinogen:DNA adducts. *Cancer Res.* 1985, *45*, 5656 – 5662.

[64] Randerath, E., Agrawal, H. P., Weaver, J. A., Bordelon, C. B., Randerath, K., 32P-postlabeling analysis of DNA adducts persisting for up to 42 weeks in the skin, epidermis and dermis of mice treated topically with 7,12-dimethylbenz[a]anthracene. *Carcinogenesis* 1985, *6*, 1117 – 1126.

[65] Dragsted, L. O., Frandsen, H., Reistad, R., Alexander, J., Larsen, J. C., DNA-binding and disposition of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) in the rat. *Carcinogenesis* 1995, *16*, 2785 – 2793.

[66] Frandsen, H., Frederiksen, H., Alexander, J., 2-Amino-1-methyl-6-(5-hydroxy-)phenylimidazo[4,5-*b*]pyridine (5-OH-PhIP), a biomarker for the genotoxic dose of the heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Food Chem. Toxicol.* 2002, *40*, 1125 – 1130.

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim