BIOMARKERS, 1999, VOL. 4, NO. 4, 263–271

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in human hair as biomarker for dietary exposure

R. REISTAD*[1,2], S. H. NYHOLM[1], L. S. HAUG[1], G. BECHER[1] and J. ALEXANDER[1]

[1] National Institute of Public Health, PO Box 4404 Torshov, N-0403 Oslo, Norway
[2] National Institute for Consumer Research, PO Box 173, N-1324 Lysaker, Norway
e-mail: ragnhild.reistad@folkehelsa.no

Received 16 October 1998, revised form accepted 28 November 1998

Heterocyclic aromatic amines (HAAs), formed during cooking of meat, are multipotent rodent carcinogens and are suspected to cause cancer in humans. In a search for suitable biomarkers for human dietary exposure to HAAs, we have investigated the concentration of a common fried food mutagen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), in human hair. Fourteen volunteers participated in the study, each contributing hair from a regular haircut, and completing a questionnaire about consumption of fried/grilled meat and smoking habits. Hair samples were treated with 1 N NaOH at 100 °C, and the HAAs extracted under alkaline and acidic conditions, derivatized and analysed by gas chromatography–mass spectrometry. PhIP was found in 12 out of 14 hair samples, in amounts from approx. 50 to 5000 pg g^{-1} hair, while two samples were below the detection limit (<50 pg g^{-1} hair). Grey/white hair straws from subjects with a mixture of coloured and grey/white hair had about a 50% reduction in PhIP concentration as compared with the natural hair mixture from the same person. This demonstrates that melanin, responsible for hair colour and spectrophotometrically characterized in the samples, participates in PhIP binding. Thus, when HAA binding components of the hair are taken into account, hair seems like an interesting object of further investigation as a biomarker for human exposure to dietary PhIP.

Keywords: PhIP, biomarker, hair, melanin, diet.

Abbreviations: GC–MS, gas chromatography–mass spectrometry; HAA, heterocyclic aromatic amine; HREI, high resolution electron impact; LREC, low resolution electron capture, PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SIM, selected ion monitoring.

Introduction

Heterocyclic aromatic amines (HAAs) are formed in small quantities during regular cooking of meat. They are potent mutagens, carcinogenic in laboratory animals, and are suspected to play a role in the causation of human cancers. It is of importance to get reliable estimates of the intake of these substances, and extensive work has been devoted to a search for suitable biomarkers. Self-reported dietary intakes are of limited value, as the formation of HAAs is strongly dependent upon cooking conditions. HAA levels (parent compounds or metabolites) in body fluids like blood and urine have been investigated by several groups, giving valuable information (Murray *et al.* 1989, Ushiyama *et al.* 1991, Lynch *et al.* 1992, Ji *et al.* 1994, Turesky *et al.* 1994, Poirier, 1997, Reistad *et al.* 1997). However, the HAAs in these fluids have a quite short half-life, as they are readily metabolized and

* Author for correspondence

Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 1999 Taylor & Francis Ltd
http://www.tandf.co.uk/JNLS/bmk.htm
http://www.taylorandfrancis.com/JNLS/bmk.htm

excreted, thereby reflecting only very recent exposure. Fried meat and fish are commonly consumed throughout life, although the intake may vary considerably from day to day. A biomarker for a longer exposure period would therefore be of great value.

In addition to urine and blood, human hair is considered a promising agent for recording the intake of drugs (DuPont and Baumgartner 1995). *In vitro* binding studies of radiolabelled cocaine to human hairs of different colours revealed that melanin, responsible for hair colour, functions as a major binding site (Joseph *et al.* 1996).

It is well known from autoradiographic studies of HAAs that these compounds also tend to accumulate in tissues rich in melanin, e.g. the retina (Brandt *et al.* 1983, 1989, Bergman 1985, Brittebo *et al.* 1992, Tjötta *et al.* 1992). Based on these properties of HAAs one would expect that they accumulate in hair and that their level would reflect the exposure during the growing period of the hair. Thus, one could obtain an integrated measure of exposure for a period of several months.

Hair melanin exists in two main forms; eumelanin, dominating in dark hair, and pheomelanin, dominating in red/blond hair. It has been shown that PhIP binds to eumelanin *in vitro* and to melanin-rich tissues *in vivo* in the rat (Brittebo *et al.* 1992), and it is therefore reasonable to assume that melanin is also the main binding component for PhIP in human hair. It is, however, not known if PhIP binds mainly to eumelanin, or also to pheomelanin, or even to other hair constituents, such as keratin.

Studies on incorporation of drugs and medications into hair have shown dose–response relationships (Uematsu *et al.* 1992, DuPont and Baumgartner 1995, Nakahara *et al.* 1995, Green and Wilson 1996). However, doses of drugs and medications in those studies were in the range of mg kg^{-1} body weight, while human dietary intake of HAAs are a thousand-fold lower, at the most a few micrograms a day.

Green and Wilson (1996) reported that methadone administered orally to hooded Lister rats resulted in 20 times higher incorporation in black hairs than in white hairs, both in a dose-dependent manner, suggesting that hair colour, and thereby melanin, are important in the binding process. Injection of nicotine in brown and albino rats showed similar colour-dependent dose–response relationships (Gerstenberg *et al.* 1995). A similar approach in humans would be to quantify HAAs in pigmented and unpigmented hair from the same person. Greying of hair is an aging process with a mosaic blending of fully pigmented, grey and white hairs (Ortonne and Prota 1993). The grey hairs are in a transitional stage and are relatively few, while the white ones have reached the end stage of depigmentation. Analysing separately coloured and white hairs from the same person would ensure equal exposure to the two hair types, and differences in HAA concentration would most likely be due to hair colour.

The main objective of the present study was to examine if a common HAA like PhIP, excreted and identified in human urine after intake of a fried meat meal (Reistad *et al.* 1997), accumulated also in hair in concentrations high enough to be quantified by gas chromatography–mass spectrometry (GC–MS). If so, the concentrations of HAAs would be of interest as a biomarker of exposure. Additional questions of interest were whether the concentrations of HAAs in hair were proportional to reported dietary intake of fried meat, and if efficiency of incorporation was dependent on hair colour.

Materials and methods

Chemicals

[$^2H_3$]PhIP, a gift from Dr Spiros Grivas, Swedish University of Agricultural Sciences, Uppsala, Sweden, was used as an internal standard to quantify PhIP. PhIP was obtained from Toronto Research Chemicals, Downsview, Ontario, Canada. Heptafluorobutyric acid anhydride was purchased from Pierce, Rockford, IL, USA, Blue Cotton from Sigma Chem. Co., St Louis, MO, USA, sodium dodecyl sulphate (SDS) from Fluka Chemie AG, Buchs, Switzerland and Soluene-350 from Packard Instrument Co., Meriden, CT, USA. Other chemicals were of HPLC or analytical grade quality.

Protocol

Participants: Fourteen volunteers, eight females and six males, 21–51 years of age, participated in the study.

Questionnaire: Each subject completed a multiple choice questionnaire about frequency of consumption of four meat items: fried or grilled beef /pork, chicken, bacon, and hamburgers, checking one of the following categories for each item: 'never', 'once a week or less', 'two to four times a week' and 'more than four times a week'. The subjects were also asked about smoking habits, as PhIP has been detected in cigarette smoke (Manabe *et al.* 1991).

Sampling of hair: The subjects had their hair washed, dried and cut. The hair was collected in a paper envelope, which was closed and kept at room temperature until the hair was analysed.

Treatment of hair samples

Hair samples were weighed out as cut, no effort was taken to ensure equal hair lengths from all participants. Length of hair samples varied from 0.5 to 20 cm, hair colours from light blond to dark brown. Duplicates are based on separate weighings, and no attempt was made to mix the individual hairs in each sample to ensure homogeneity prior to weighing. However, one sample was weighed out in double amount and divided after solubilization, ensuring duplicates based on identical material prior to extraction. Two of the subjects had brown, slightly greyish hair. From an aliquot of a separate haircut of the two, grey and white hair straws were collected together and analysed.

Extraction procedure

Hair samples weighing 250 mg (in duplicate) were cleaned in 12.5 ml 0.1% sodium dodecyl sulphate by whirlimixing, sonication for 1 min, followed by decanting the liquid. The hair was then rinsed four times in 25 ml of distilled water, each time decanted and the glass placed upside down to drain the water. Care was taken to ensure minimal loss of sample (less than 1% w/w, totally) upon decanting. To the hair sample was then added 500 pg of internal standard ([$^2H_3$]PhIP) dissolved in methanol, followed by 12.5 ml of 1 N NaOH. The suspension was kept at 100 °C for 45 min, followed by successive extractions of HAAs with 10, 5 and 2.5 ml ethyl acetate. The HAAs were transferred from the combined ethyl acetate extracts to the aqueous phase by shaking with 5 ml of 0.1 N HCl, followed by 2.5 ml. The aqueous layers were combined, neutralized to pH 5–6 with a 1 N NaOH solution, and treated with Blue Cotton as described by Reistad *et al.* (1997). The ammonia–methanol eluates from Blue Cotton were dried on a rotary evaporator and derivatized for GC–MS by acylation and methylation (Reistad *et al.* 1997).

Standards

Internal standards of [$^2H_3$]PhIP, 10 and 100 pg μl^{-1}, and various concentrations of PhIP (0–100 pg μl^{-1}) in methanol were prepared, derivatized, stored at -20 °C, and used for standard curves.

Spectrophotometric characterization of melanin

Spectrophotometric characterization of melanin was carried out according to Ozeki *et al.* (1996). Briefly; to hair samples, 5–10 mg, were added 2 ml of Soluene-350, followed by heating at 95 °C for 45 min. This completely solubilized all hair samples, and absorptions were recorded at 500 and 650 nm (A_{500} and A_{650}) in a Perkin Elmer Lambda 6 UV/VIS spectrophotometer. A_{500} reflects the colour intensity of total melanin, and the ratio A_{650}/A_{500} is used as a parameter to estimate the eumelanin/total melanin ratio.

Gas chromatography – mass spectrometry

The mass spectrometers were operated either in the high resolution electron impact (HREI) mode or the low resolution electron capture (LREC) mode, both with selected ion monitoring (SIM). Good

selectivity and sensitivity were obtained by monitoring the main fragment $(M–C_3F_7)^+$ in the HREI mode. An HP 5890 Ser. 2 GC coupled to a VG AutoSpec high-resolution MS instrument was used, with a column of CP-Sil 5 CB, 25 m, i.d. 0.25 mm, film thickness 0.25 μm. Temperature programme: 100 °C, 1 min, raised to 240 °C at 30 °C min^{-1}, raised to 255 °C at 1 °C min^{-1}, raised to 300 °C at 20 °C min^{-1}, held at 300 °C for 3 min. Other MS conditions were: resolution 8000, source temp. 250 °C, and electron energy 41 eV.

The base peak $(M\text{-}C_3F_7)^+$ was recorded. Selected ions were: PhIP (*m/z* 265.1089), $[^2H_3]$PhIP (*m/z* 268.1277).

Being very selective and causing no fragmentation, recording the complete molecule plus one electron $(M)^-$, the EC mode is normally the method of choice. However, as we have previously successfully employed the HREI mode, the two were compared in the present experiment. For the LREC analyses, a HP 6890+ GC coupled to a HP MSD 5973 was used, with a column of HP 5 MS, 30 m, i.d. 0.25 mm, film thickness 0.25 μm. Methane was used as reagent gas. Temperature programme: 100 °C, 1 min, raised to 300 °C at 25 °C min^{-1}, held at 300 °C for 5 min. Other MS conditions were: Source temp. 150 °C and electron energy 105 eV.

Selected ions were: PhIP (*m/z* 434), $[^2H_3]$PhIP (*m/z* 437).

RESULTS

PhIP was found in 12 out of 14 hair samples (table 1), in concentrations from about 50 to 5000 pg g^{-1} hair. The detection limit for both MS methods, defined as a signal to noise ratio of 3:1, was about 0.1 pg injected. Recovery through the extraction procedure, assessed by comparison of internal standard peak areas in extracted samples with those of unextracted standards, was at least 50%, resulting in a detection limit for PhIP of about 50 pg g^{-1} hair.

The questionnaires revealed that all subjects ate fried/grilled meat, and frequency of consumed beef/pork, chicken, hamburgers and bacon did not vary greatly between most subjects.

The subjects were classified into three main categories according to the reported intake of fried/grilled meat:

Low (L): Subjects eating each of the four listed meat types once a week or less.

Medium (M): Subjects eating one of the four meat types two to four times a week, the rest of the meat types once a week or less.

High (H): Subjects eating two of the four meat types two to four times a week, the rest of the meat types once a week or less.

Based on the classification above, most of the subjects belonged to categories L and M, only two participants, from the same household, to category H.

The answers can only be used for a very rough estimation of intake, as degree of browning, serving sizes, etc. were not included in the questionnaire. None of the subjects had changed their basic diet during the last year. However, some reported seasonal variations, i.e. a more frequent intake of fried/grilled meat during the summer.

Twelve subjects were non-smokers, one an occasional smoker (< 1 cigarette per day), and one a heavy smoker (> 15 cigarettes per day).

The results show that PhIP, from intake through a regular diet, is deposited in hair in concentrations high enough to be detected and quantified by GC–MS in most samples (table 1). The rather high deviation between duplicates, as observed for some samples, is most likely caused by heterogeneity within a hair sample. Splitting of samples after solubilization, but prior to extraction (table 1, samples 13a** and 13b**), resulted in good agreement (3–5% deviation between duplicates). This suggests that PhIP may be rather heterogeneously distributed in hair, possibly caused by variation in hair quality and/or seasonal variation in intake.

The method of melanin characterization of Ozeki *et al.* (1996) employed on our

Table 1. PhIP in human hair.

Subject	Pg PhIP g^{-1} hair, mean and individual values of duplicates	% Deviation between duplicates	Hair colour	Meat intake based on questionnaire
1	277 (214, 339)	23	B	M
1*	240 (235, 245)	2		
2	222 (219, 224)	1	dB	M
3	134 (113, 155)	15	B(g)	L
4	219 (210, 228)	4	B(g)	M
5	2588 (2273, 2902)	12	B	L
5*	2243 (1897, 2588)	15		
6	138 (131, 144)	5	B	L
7	247 (224, 269)	9	Bl	L
8	n.d.		B	M
9	62 (47, 77)	24	Bl	M
10	n.d.		B	M
11	58 (54, 62)	7	dBl	M
12	129 (113, 144)	12	Bl	M
13	4933 (3338, 6528)	32	B	H
13*	5682 (5558, 5805)	2		
13a**	4181 (4042, 4319)	3		
13b**	7526 (7133, 7919)	5		
14	338 (176, 499)	48	lBl	H

n.d., not detectable.
* Repeated analyses of sample.
** Repeated analyses of sample. Sample split in duplicates after hair solubilization.
dB—dark brown, B—brown, B(g)—brown (greyish), dBl—dark blond, Bl—blond, lBl—light blond, L—low, M—medium, H—high.

samples is shown in figure 1. A_{500} is plotted against the ratio A_{650}/A_{500}, giving an impression of location of samples with high/low total melanin concentration, as well as locations with predominantly eumelanic/pheomelanic samples. Furthermore, according to Ozeki *et al.* (1996) A_{650}/A_{500} expresses the ratio of eumelanin to total melanin, black hair giving the highest (0.33), red hair the lowest ratio (0.12). Calculated for our samples, the ratios ranged from 0.12 to 0.26, covering colours from light blond to dark brown, in good agreement with Ozeki *et al.* (1996).

Each point in figure 1 is marked with the subject number as given in table 1. Blond hair is found mainly in the lower, pheomelanic part of the figure, brown in the upper, eumelanic part. However, total melanin concentration does not generally seem to be higher in brown than in blond hair. It is also noted that the two samples with the highest PhIP concentrations (samples 5 and 13) are rich in eumelanin (figure 1). Otherwise, PhIP was found in samples of all hair colours (table 1).

Except for one subject (no. 5), all reporting a low/medium intake of fried meat (table 1) had PhIP concentrations from below detection limit up to about 300 pg g^{-1} hair (table 1, samples 1–4, 6–12, 14). Only two subjects (from the same household) reported a high intake of fried meat. One of them (table 1, no. 13) had the highest hair concentration of PhIP, and brown hair, rich in melanin (figure 1). The other (table 1, no. 14), had light blond hair with a more than ten-fold lower concentration of PhIP. Total concentration of melanin was low, and predominantly of the pheomelanic type (figure 1). This points to melanin, and possibly eumelanin, as a major factor in the binding of PhIP. Interestingly, subject 14 was also a heavy smoker, which did not seem to add significantly to PhIP accumulated in this hair.

Also, subjects 5 and 6 belonged to the same household. Both reported a low

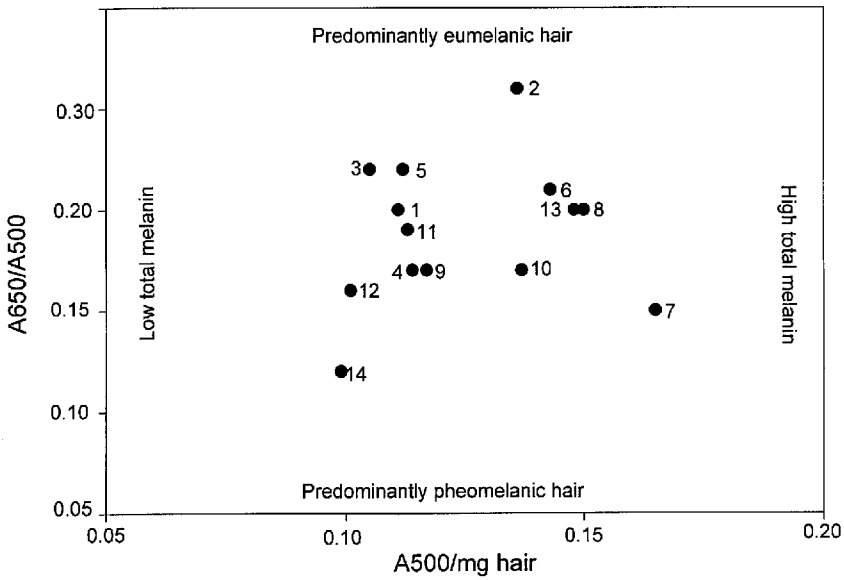

Figure 1. Distribution of hair samples according to melanin types and concentrations. Absorbancies per mg hair in 1 ml of solvent.

consumption of fried meat, but the samples exhibited widely different PhIP hair concentrations (table 1), despite roughly the same hair colour (figure 1). These discrepancies may be caused by differences in serving sizes of meat or by unknown PhIP sources.

Two of the subjects (nos 3 and 4) had some grey/white hair straws naturally mixed with the pigmented ones. In an aliquot of a separate haircut, the grey and white hair straws of the two subjects were collected and analysed by the two MS-modes HREI and LREC, together with samples of the natural hair mixture of each subject. The two MS-modes proved interchangeable. Both of the grey/white samples showed a substantial decrease (about 40 and 55%, respectively) in PhIP concentration (figure 2) as compared with the natural hair, consisting of pigmented, grey and white hair straws.

DISCUSSION

The results of the present study clearly answer the primary question whether it is possible to determine HAAs in hair samples from humans consuming a regular diet containing cooked meat. It is possible to quantify at least PhIP in a reliable way, as the analytical method shows good reproducibility. The interchangeability of the two MS modes, HREI and LREC, using the same derivatives, makes the analytical work quite flexible. This is promising with regard to the possible use of HAAs, i.e. PhIP, in hair as a biomarker for the exposure to this compound through the diet. Previous studies in this and other laboratories show that HAAs in urine only give an indication of the very recent intake. Hence, hair seems to be an ideal type of sample material. It grows about 1 cm per month, is easily collected, tells the intake story of selected compounds for as many months as the hair length in cm, and is stable at room temperature.

It is, however, difficult to answer whether the concentration of PhIP in hair

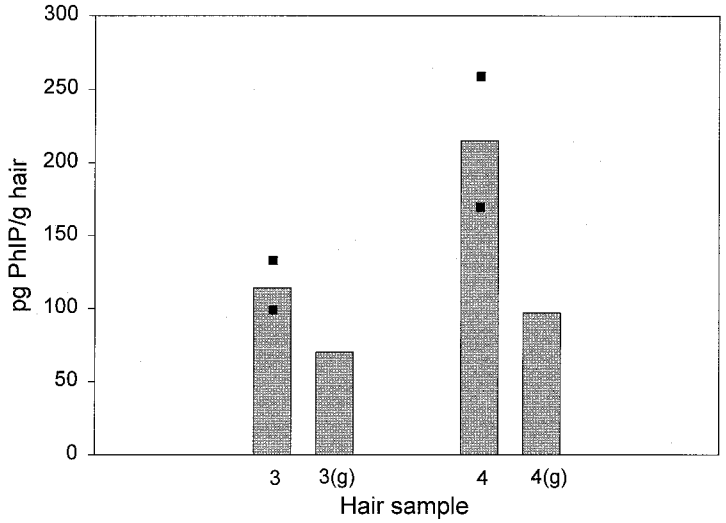

Figure 2. PhIP in the natural hair mixture and in grey/white (g) hair straws from subjects 3 and 4, respectively. The PhIP concentration in the natural hair mixture is the mean of duplicates (black squares), while analysis of grey/white hair straws is based on one sample only, due to shortage of material.

truly reflects the exposure, whether there is a dose–response relationship, and whether equal exposures give rise to the same concentration in hair among different individuals.

Reported dietary intakes are weak parameters of exposure, as formation of these substances is highly dependent upon cooking conditions (Knize *et al.* 1994, Sinha *et al.* 1995, 1998a, b, Skog *et al.* 1995, Tikkanen *et al.* 1996). Questionnaires, even accompanied by pictures of serving sizes and degrees of browning of various dishes to estimate HAA intake, combined with chemical analyses of foods, have flaws (Augustsson *et al.* 1997). Exposure as reported through the questionnaire in the present study is probably very inaccurate. Thus, it is difficult to validate the concentration of PhIP in hair as a biomarker for exposure.

Regarding intake of drugs, determinations of concentration in hair is an accepted method, although difficulties and uncertainties regarding interpretation of results are recognized (Pötsch *et al.* 1997). The binding in hair is probably influenced by several factors. Melanin has affinity for many different types of drugs, is considered a major binding substance in hair, and varies greatly between individuals with different hair colour. Therefore, a reservation concerning the method seems to be related to the binding compounds of HAAs in hair.

The colour intensity of melanin and ratio of eumelanin to total melanin were therefore measured in the hair samples. The differences in PhIP concentrations of the hair samples of the two subjects from the same household with very different hair colours and content of melanin (figure 1, subjects 13 and 14), indicate that melanin, and possibly eumelanin, is important for the uptake of PhIP in hair.

In an attempt to further address the question of melanin binding, PhIP was determined in grey/white hair straws from the two subjects with brown, greyish hair (figure 2). The reduced content of PhIP found in grey/white hair straws suggests that the melanin concentration is an important determinant for binding of

PhIP in hair. However, as a substantial amount of PhIP was still present, apparently not only melanin functions as a binding substance. Keratin, the main hair polymer, may also contribute to HAA binding, especially in grey/white hair straws where concentration of melanin is low. Pötsch *et al.* (1997), discussing the conservation of drug molecules during keratinization, suggest that drug molecules may be localized in proteins in keratin intermediate filaments.

Validation of PhIP in hair as a biomarker for exposure has to be developed along several lines. A larger human study, with more subjects in better-defined groups, like vegetarians and persons with high intakes, is in progress and will give additional information. Secondly, experiments in progress exposing animals with different hair colours to HAAs will probably give information of dose–response relationships and the role of different melanin types in the binding of these compounds in hair.

Acknowledgements

We are indebted to Espen Jensen, Marit Hindrum and Tone Rasmussen for skilful technical assistance. S.H. Nyholm is a research fellow of The Norwegian Research Council.

References

AUGUSTSSON, K., SKOG, K., JÄGERSTAD, M. and STEINECK, G. 1997, Assessment of the human exposure to heterocyclic amines. *Carcinogenesis*, **18**, 1931–1935.

BRITTEBO, E.B., SKOG, K.I. and JÄGERSTAD, M. 1992, Binding of the food mutagen PhIP in tissues of mice. *Carcinogenesis*, **13**, 2263–2269.

BERGMAN, K. 1985, Autoradiographic distribution of 14C-labelled 3H-imidazo(4,5-*f*)quinoline-2-amines in mice. *Cancer Research*, **45**, 1351–1356.

BRANDT, I., GUSTAFSSON, J.-Å. and RAFTER, J. 1983, Distribution of the carcinogenic tryptophan pyrolysis product Trp-P-1 in control, 9-hydroxyellipticine and beta-naphthoflavone pretreated mice. *Carcinogenesis*, **4**, 1291–1296.

BRANDT, I., KOWALSKI, B., GUSTAFSSON, J.-Å. and RAFTER, J. 1989, Tissue localisation of the carcinogenic glutamic acid pyrolysis product Glu-P-1 in control and beta-naphthoflavone-treated mice and rats. *Carcinogenesis*, **10**, 1529–1533.

DUPONT, R.L. and BAUMGARTNER, W.A. 1995, Drug testing by urine and hair analysis: complementary features and scientific issues. *Forensic Science International*, **70**, 63–76.

GERSTENBERG, B., SCHEPERS, G., VONCKEN, P. and VÖLKEL, H. 1995, Nicotine and cotinine accumulation in pigmented and unpigmented rat hair. *Drug Metabolism and Disposition*, **23**, 143–148.

GREEN, S.J. and WILSON, J.F. 1996, The effect of hair color on the incorporation of methadone into hair in the rat. *Journal of Analytical Toxicology*, **20**, 121–123.

JI, H., YU, M., STILLWELL, W.G., SKIPPER, P.L., ROSS, R.K., HENDERSON, B.E., and TANNENBAUM, S.R. 1994, Urinary excretion of 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline in white, black and Asian men in Los Angeles county. *Cancer Epidemiology, Biomarkers and Prevention,* **3**, 407–411.

JOSEPH, R.E. JR, SU, T.-P. and CONE, E.J. 1996, In vitro binding studies of drug to hair: Influence of melanin and lipids on cocaine binding to caucasoid and africoid hair. *Journal of Analytical Toxicology*, **20**, 338–344.

KNIZE, M.G., DOLBEARE, F.A., CARROLL, K.L., MOORE, D.H. and FELTON, J.S. 1994, Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. *Food and Chemical Toxicology*, **32**, 595–603.

LYNCH, A.M., KNIZE, M.G., BOOBIS, A.R., GOODERHAM, N.J., DAVIES, D.S. and MURRAY, S. 1992, Intra- and interindividual exposure in humans to 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, carcinogens present in cooked beef. *Cancer Research*, **52**, 6216–6223.

MANABE, S., TOHYAMA, K., WADA, O. and ARAMAKI, T. 1991, Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in cigarette smoke condensate. *Carcinogenesis*, **12**, 1945–1947.

MURRAY, S., GOODERHAM, N.J., BOOBIS, A.R. and DAVIES, D.S. 1989, Detection and measurement of MeIQx in human urine after ingestion of a cooked meat meal. *Carcinogenesis*, **10**, 763–765.

NAKAHARA, Y., TAKAHASHI, K. and KIKURA, R. 1995, Hair analysis for drugs of abuse X. Effect of physicochemical properties of drugs on the incorporation rates into hair. *Biological and Pharmaceutical Bulletin,* **18**, 1223–1227.

ORTONNE, J.-P. and PROTA, G. 1993, Hair melanins and hair color. *Journal of Investigative Dermatology,* **101** (Suppl. 1), 82S-89S.

OZEKI, H., ITO, S., WAKAMATSU, K. and THODY, A.J. 1996, Spectrophotometric characterization of eumelanin and pheomelanin in hair. *Pigment Cell Research*, **9**, 265–270.

POIRIER, M.C. 1997, DNA adducts as exposure biomarkers and indicators of cancer risk *Environmental Health Perspectives*, **105** (Suppl. 4), 907–912.

PÖTSCH, L., SKOPP, G. and MOELLER, M.R. 1997, Biochemical approach on the conservation of drug molecules during hair fiber formation. *Forensic Science International*, **84**, 25–35.

REISTAD. R., ROSSLAND, O.J., LATVA-KALA, K.J., RASMUSSEN, T., VIKSE, R., BECHER, G. and ALEXANDER, J. 1997, Heterocyclic aromatic amines in human urine following a fried meat meal. *Food and Chemical Toxicology*, **35**, 945–955.

SINHA, R., ROTHMAN, N., BROWN, E.D., SALMON, C.P., KNIZE, M.G., SWANSON, C.A., ROSSI, S., MARK, S., LEVANDER. O.A. and FELTON, J.S. 1995, High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) occur in chicken but are dependent on the cooking method. *Cancer Research*, **55**, 4516–4519.

SINHA, R., ROTHMAN, N., SALMON, C.P., KNIZE, M.G., BROWN, E.D., SWANSON, C.A., RHODES, D., ROSSI, S., FELTON, J.S. and LEVANDER, O.A. 1998a, Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. *Food and Chemical Toxicology*, **36**, 279–287.

SINHA, R., KNIZE, M.G., SALMON, M.G., BROWN, E.D., SWANSON, C.A., RHODES, D., FELTON, J.S., LEVANDER, O.A. and ROTHMAN, N. 1998b, Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. *Food and Chemical Toxicology*, **36**, 289–297.

SKOG, K., STEINECK, G., AUGUSTSSON, K. and JÄGERSTAD, M. 1995, Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. *Carcinogenesis*, **16**, 861–867.

TIKKANEN, L.M., LATVA-KALA, K.J. and HEINIÖ, R.-L. 1996, Effect of commercial marinades on the mutagenic activity, sensory quality and amount of heterocyclic amines in chicken grilled under different conditions. *Food and Chemical Toxicology*, **34**, 725–730.

TJÖTTA, K., INGEBRIGTSEN, K. and AUNE, T. 1992, Tissue distribution of 2-amino-3, 8-dimethylimidazo(4,5-*f*)quinoxaline (MeIQx) in two strains of male rats. *Carcinogenesis*, **13**, 779–782.

TURESKY, R.J., GROSS, G.A., STILLWELL, W.G., SKIPPER, P.L. and TANNENBAUM, S.R. 1994, Species differences in metabolism of heterocyclic aromatic amines, human exposure, and biomonitoring. *Environmental Health Perspectives*, **102** (Suppl. 6), 47–51.

UEMATSU, T., MIYAZAWA, N., OKAZAKI, O. and NAKASHIMA, M. 1992, Possible effect of pigment on the pharmacokinetics of ofloxacin and its excretion into hair. *Journal of Pharmaceutical Sciences*, **81**, 45–48.

USHIYAMA, H., WAKABAYASHI, K., HIROSE, M., ITOH, H., SUGIMURA, T. and NAGAO, M. 1991, Presence of carcinogenic heterocyclic amines in urine of healthy volunteers eating normal diet, but not of inpatients receiving parenteral alimentation. *Carcinogenesis*, **12**, 1417–1422.