**Carcinogenesis** vol.18 no.10 pp.1931–1935, 1997

# Assessment of the human exposure to heterocyclic amines

**Katarina Augustsson[1,2,4], Kerstin Skog[3], Margaretha Jägerstad[3] and Gunnar Steineck[1,2,4]**

[1]Department of Cancer Epidemiology, Karolinska Insitute, Stockholm, [2]Unit of Oncology and Unit of Cancer Prevention, Radiumhemmet, Karolinska Hospital, Stockholm and [3]Department of Applied Nutrition and Food Chemistry, Center for Chemistry Engineering, University of Lund, Sweden

[4]To whom correspondence should be addressed

**Heterocyclic amines are possible human carcinogens and fried meat is an important source of exposure in the Western diet. To study the effect of heterocyclic amines in humans, accurate assessment of individual food consumption is essential. Parameters influencing the intake include the amount and type of meat ingested, frequency of consumption, cooking method, cooking temperature and the duration of cooking. The aim of the present study was to develop a practical method for assessing individual intakes of specific heterocyclic amines in a large sample of people. This has been done by combining information on food consumption and laboratory findings of heterocyclic amines in food products. Diet was assessed using a semi-quantitative food frequency questionnaire including photos of fried meat and, in all, 22 dishes were cooked and chemically analyzed. The method was employed in an elderly population in Stockholm to estimate the daily mean intake of the five heterocyclic amines 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). The total daily intake ranged from none to 1816 ng, with a mean intake of 160 ng, which is well below estimates reported previously. Highest amounts ingested were of PhIP (mean 72, range 0–865 ng/day) and MeIQx (mean 72, range 0–1388 ng/day), followed by DiMeIQx (mean 16, range 0–171 ng/day), while MeIQ and IQ were ingested only in very small amounts (mean <1 ng/day).**

## Introduction

There is no doubt that heterocyclic amines, formed in the surface layer of meat during frying, are mutagenic in Ames' test and carcinogenic in animal models, but the effect in humans remains to be elucidated. In 1993, IARC (1) judged the heterocyclic amines 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ*), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) to be possible human carcinogens and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) as probably carcinogenic but, in a review of epidemiological studies, we found the available evidence for human carcinogenicity of heterocyclic amines in fried meat to be insufficient for implementing recommendations about food intake (2).

An accurate assessment of the individual consumption of heterocyclic amines is essential for investigation of human carcinogens. Fried meat is an important source of exposure to heterocyclic amines in the Western diet and parameters influencing the intake are the amount and type of meat ingested, frequency of consumption, cooking method, cooking temperature and the duration of cooking. Gravy is also an important source of exposure in countries where gravy and brown sauce are prepared from the pan residue after the meat has been fried. Ideally, measurements of specific heterocyclic amines should be done for each ingested food item, using a double portion method for each individual for a sufficient period of time. However, this is expensive and impossible to carry out in a retrospective case−control study. Any practical method for assessing the intake of heterocyclic amines on a larger scale will therefore have a multitude of potential flaws.

None of the previously reported estimates of the intake of heterocyclic amines in human populations (3–9) are based on empirical data concerning individual patterns of consumption of meat and gravy or the specifics of cooking methods in the appropriate population, combined with chemical analyses of the food eaten in the same population. An accurate assessment of the intake is not only essential to understand whether or not heterocyclic amines are human carcinogens, but is also of great importance to determine the magnitude of a possibly harmless exposure as well as a potential carcinogenic level of exposure.

The aim of the present study was to develop a practical method for assessing individual intakes of specific heterocyclic amines in a large study population. This has been done by combining information on food consumption and laboratory findings of heterocyclic amines in cooked food. Subsequently, the method was employed in an elderly population in Stockholm to estimate the mean daily intake of the five heterocyclic amines IQ, MeIQ, MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), and PhIP.

## Materials and methods

Our method is a linkage of information gained from chemical analyses of heterocyclic amines in popular dishes, including gravy, with food consumption data. The questionnaire used to assess the food intake is based on results from previous studies (Augustsson *et al.*, in preparation) in which we initially identified frequently eaten dishes through open telephone interviews and then determined the intake frequency by means of a standardized food frequency questionnaire sent to another representative sample of subjects 50–75 years of age living in Stockholm. Dishes found to be eaten less than three times per year, on average, were not included in the present study.

*Population and administration*

Subjects were randomly selected from a population register covering nearly 100% of the Stockholm population. Altogether, 10 random samples of subjects were drawn from the register, representing a source person−time comprising all individuals born in Sweden who were 50–75 years old and lived in the

***Abbreviations:** MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline.

**Table I.** Description of the participating subjects

| Parameters | Women | Men |
|---|---|---|
| Number of subjects | 267 | 277 |
| Mean age (years) | 68 | 68 |
| BMI (kg/m$^2$) | 24 | 25 |
| Current smokers (%) | 19 | 28 |
| Energy intake (kcal) | 1919 | 2190 |
| Fat intake (energy %) | 35 | 36 |
| Carbohydrate intake (energy %) | 46 | 43 |
| Total protein intake (energy %) | 16 | 16 |
| Alcohol intake (energy %) | 3 | 5 |

Mean values are given for women and men separately. The intake of energy is expressed as the mean daily intake and the macronutrients as percentage of the total energy intake.

county of Stockholm between November 1, 1992 and December 31, 1994. The samples were separated in calendar time and spread over different seasons. The participating subjects are described in Table I and will be used later on as controls in an analytical epidemiological study. A questionnaire was mailed to the subjects after they had received an introductory letter and had been asked after that over the telephone if they were interested in participating. After the questionnaire was returned, missing information was completed by telephone interview.

*Cooking procedures and chemical analyses*

Cooking procedures, chemical analyses and the obtained values of heterocyclic amines used in the present study to calculate the daily intake have been reported in detail elsewhere (10,11). A total of 22 dishes were cooked and analyzed for heterocyclic amines. Three of the dishes were baked/roasted (sausage, meat loaf and chicken) without any addition of fat. The rest of the dishes (15 meat dishes, two fish dishes, eggs and black pudding) were fried in a standardized way, described by Övervik *et al.* (12), at 150, 175, 200 and 225°C. Gravy was made from the pan residue after each cooking session by the addition of distilled water. Six frequently eaten fried meat dishes in the study population (meatballs, pork chops, minute beef, sliced pork belly, ground beef patties and sausage) were photographed to record the degree of browning of the surface of the meat. Heterocyclic amines were extracted from the dish and the corresponding gravy and then quantified using HPLC according to the method described by Gross (13). IQ, MeIQ, MeIQx, DiMeIQx and PhIP were chosen for analysis after reviewing the literature of heterocyclic amines formed in meat and fish products at temperatures used in households and restaurants. The amounts of MeIQx, DiMeIQx and PhIP formed during frying were determined by the frying temperatures (10,11). Also, with the duration of cooking used by us, a visual examination showed a correlation with the degree of surface browning.

*Dietary assessment method*

The diet was assessed by means of a semi-quantitative food frequency questionnaire including a total of 188 food and drink items, 27 of which were fried meat dishes, 16 baked/roasted meat dishes, 11 boiled meat dishes, four grilled meat dishes, four gravies and sauces, seven fish dishes, one fried egg, one omelette and one black pudding. The questions about gravy and sauce included information about how much of the pan residue was used each time to make gravy and intake frequency for different kinds of gravies and sauces based on the pan residue. Altogether, a module of 22 pages, including 12 pages of color photos of fried meat, was devoted in the questionnaire to assessing intake of heterocyclic amines. All questions took account of eating habits 5 years previously and the food frequency module gave information on the average food intake for each subject. The 10 categories of intake frequencies ranged from '2–3 times per day' to 'never', starting with the highest frequency to the left in the table, and had three categories for weekly and monthly intake. Photos were included to show four different standardized portion sizes and six sets of photos showed the meats degree of browning and specific portion sizes.

*Intake of heterocyclic amines from meat and fish*

Parameters required to assess the intake of heterocyclic amines are the amount and type of meat ingested, frequency of consumption, cooking method, temperature and concentration of heterocyclic amines in various dishes. Twenty four color photos showed six dishes, each fried at four different temperatures and with varying degrees of surface browning. Each photo corresponded to a specific frying temperature and amount of heterocyclic

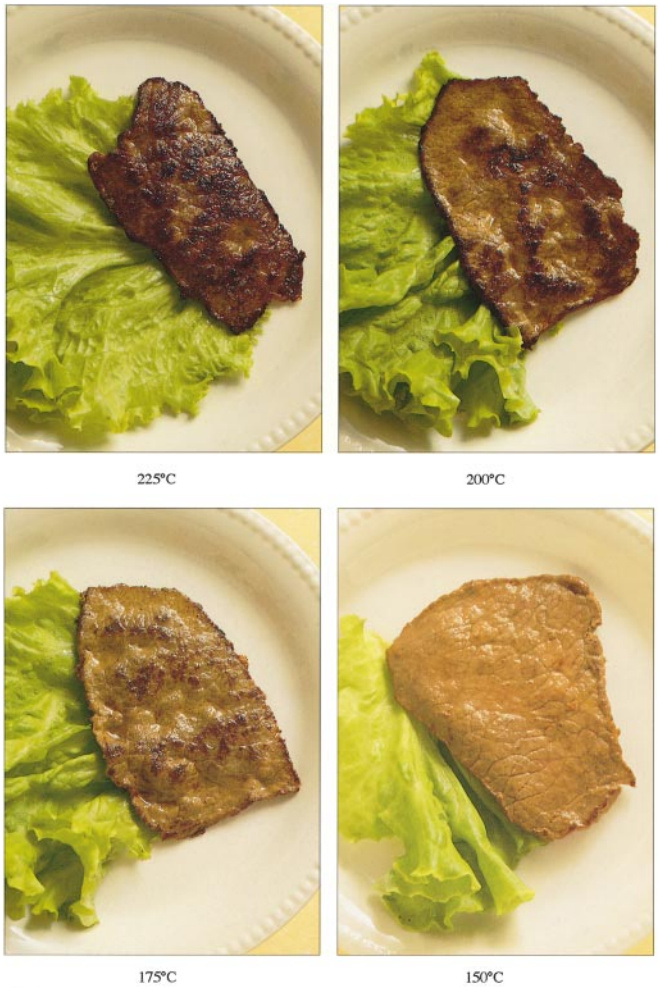


**Fig. 1.** Minute beef fried at four different temperatures.

amines (Figure 1 shows minute beef). By looking at the photos, participants indicated their preference for degree of browning and the amount eaten for each dish and meal. The weight in grams of each fried item was taken from our own cooking sessions (each fried meatball weighed 20 g, each pork chop 101 g, including bone, a slice of minute beef 27 g, a slice of pork belly 9 g, a ground beef patty had a weight of 48 g and a piece of sausage 20 g).

For all other dishes, a general standard portion was chosen from photos showing four portion sizes with 60, 90, 120 and 150 g of meat or fish each, except fried black pudding (150 g), of which only one portion size was available. Information about the degree of browning of the meat was taken from a dish judged to be the most similar of the six dishes in the photos. The classification of the dishes is shown in Figure 2. For example, the degree of browning of pork chops was also used to classify the degree of browning of other kinds of pork dishes listed in the questionnaire, such as loin of pork, fillet of pork and smoked-cured loin of pork. The degree of browning was not considered in the case of baked/roasted dishes. Values for baked Falu sausage (a Swedish specialty) at 200°C were also used for all other baked sausages. For the four grilled/barbecued dishes in the questionnaire (sausage, pork, steak and ground beef) the value for the corresponding fried dish was used. Fried eggs were excluded when calculating the daily intake, since no heterocyclic amines were found in the chemical analysis.

*Intake of heterocyclic amines from gravy and sauce*

The intake of heterocyclic amines from gravy and sauce was calculated using a model giving the concentration in one standard serving of gravy or sauce. In the model we used information on the concentrations of heterocyclic amines in gravy made from the pan residues after the meat and fish were fried, as well as gravy from the baked/roasted dishes. The contribution of heterocyclic amines was given by the concentration in gravy (which was expressed as the amount of heterocyclic amines per 100 g of corresponding cooked meat or fish product and the proportion of pan residue used for the sauce and gravy, 100, 50 or 25%) and information about the corresponding dish (the reported degree of browning, portion size and intake frequency). The sum of heterocyclic

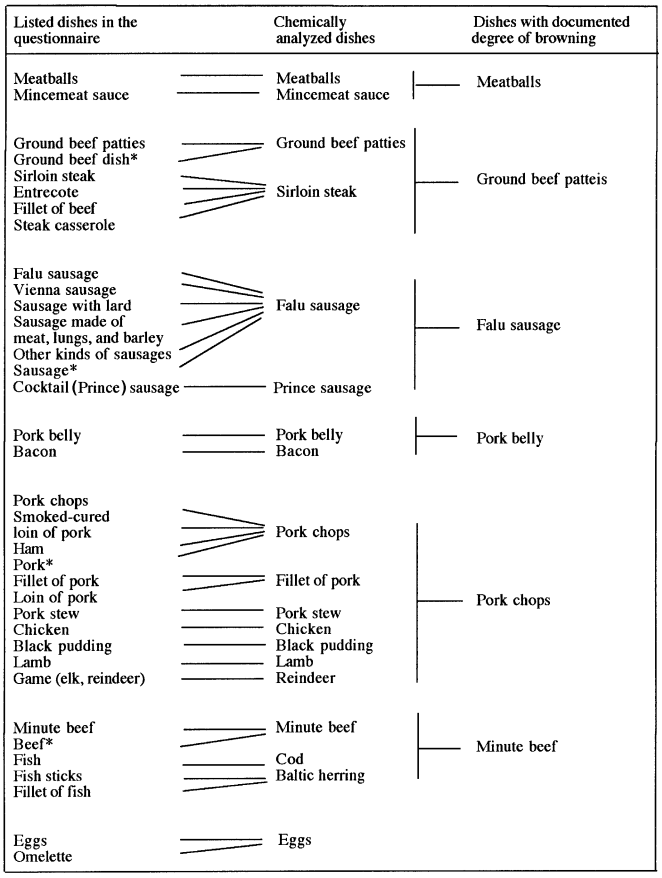


**Fig. 2.** Classification of the degree of browning and the amount of heterocyclic amines for calculating the daily intake of heterocyclic amines from the frequency questionnaire. The Falu sausage and the Prince sausage are Swedish specialties. Eggs and omelette were not considered in the calculation, since no heterocyclic amines were found in the chemical analysis. *Indicates grilled dishes.

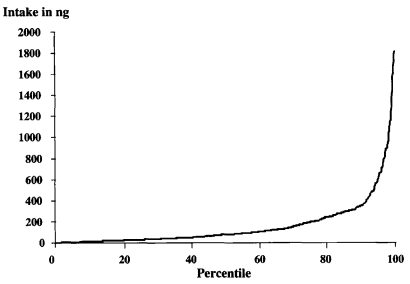


**Fig. 3.** The distribution of the intake of heterocyclic amines.

amines was thereafter divided by the total number of consumption events for all dishes, to obtain the content in one standard serving of gravy or sauce. This value was thereafter multiplied by the intake frequency for sauce and gravy.

*Databases and computer programs*

A computer program linked information from the questionnaire on cooking method, intake frequency, portion size and preference for the degree of browning to a database with information on the amount of heterocyclic amines (in ng) in 100 g of cooked meat, fish, black pudding and gravy. A median value based on all subjects that answered was used when information about intake frequency was missing. Another computer program linked consumption data to the National Food Administration's database on energy and nutrients in various food products in order to calculate the daily intake.

## Results

Out of the 692 selected subjects, 548 (79%) participated in the present study, but due to missing information analyses are based on 544 individuals. The average intake of meat and fish, taken together, was among women 131 g per day, and men 168 g per day. When classifying the degree of surface browning of the six fried dishes, the photos corresponding to 175 and 200°C were chosen by 84% of the subjects. The daily exposure to heterocyclic amines from meat and gravy, reported separately for women and men, is shown in Table II. In general, the mean contribution from gravy and sauce was ~30% of the total intake and the women's exposure was generally 40% less than that of men.

Table III summarizes the mean daily intake of heterocyclic amines for the whole group (both women and men) and the total intake for all five heterocyclic amines ranged from 0 to 1816 ng, with a mean intake of 160 ng. Of the five heterocyclic amines analysed, PhIP and MeIQx were ingested in the largest amounts, 72 ng/day each, followed by DiMeIQx. Small amounts of IQ and MeIQ were consumed, primarily from the gravy. The intake of IQ ranged from 0 to 10 ng and the highest noted intake of MeIQ was 2 ng. Dividing the intake into quintiles gave the following cut-off points between the quintiles: IQ (0.02, 0.09, 0.20, 0.55 ng/day), MeIQ (0, 0, 0, 0.01 ng/day), MeIQx (12.12, 25.37, 46.35, 98.89 ng/day), DiMeIQx (3.27, 6.84, 12.63, 23.54 ng/day), PhIP (8.31, 19.66, 43.36, 105.09 ng/day) and total heterocyclic amines (24.77, 52.85, 104.15, 241.28 ng/day).

## Discussion

Altogether, we found the mean daily intake of IQ, MeIQ, MeIQx, DiMeIQx and PhIP to amount to 160 ng, with PhIP and MeIQx accounting for the highest amounts, followed by DiMeIQx. These relative levels of intake are in line with previous reports on the amounts of heterocyclic amines occurring in cooked food (5). Our calculated intake of heterocyclic amines is well below estimates reported by others, regardless of study design (see below). The total intake of meat and fish per day in the present study is in line with a recently published national study on food intake in Sweden among people in the same age group, in which women were found to consume 118 g of meat and fish per day and men 153 g, regardless of the cooking method (14). Also, we found the mean daily intake of several nutrients, including protein, to be in agreement with other studies on food and nutrient intake in elderly Swedish populations (14,15).

Previous estimates of the intake of heterocyclic amines (3–9), shown in Table III, are based on either (i) excretion in the urine, (ii) general levels in cooked food or (iii) a combination of concentrations in different food products and dietary intake assessments. Also, there are many laboratory reports on concentrations in different food products (16–19), but they are not accompanied by information on the actual consumption (portion size, intake frequency, cooking specifics, etc.), making it impossible to estimate the intake at an individual level. A plenitude of factors influence the formation and intake of heterocyclic amines and differences in study design give rise to divergent estimates. In contrast to our study, none of the previous studies have estimated, on a large scale, the daily exposure based on individual preferences for the degree of browning of the meat, intake frequencies, portion sizes and concentrations of heterocyclic amines in dishes eaten by the same population.

Studies on the excretion of heterocyclic amines in the urine reflect the individual's present exposure and cannot be used

**Table II.** The intake of heterocyclic amines from meat, fish and gravy, in ng/day/person, separately for women ($n$ = 267) and men ($n$ = 277), expressed as mean values and range

| | Meat and fish (ng) | | Gravy (ng) | | Meat, fish and gravy (ng) | |
|---|---|---|---|---|---|---|
| Heterocyclic amines | Women | Men | Women | Men | Women | Men |
| IQ | 0 (0–0) | 0 (0–0) | <1 (0–9) | <1 (0–10) | <1 (0–9) | <1 (0–10) |
| MeIQ | 0 (0–0) | 0 (0–0) | <1 (0–2) | <1 (0–2) | <1 (0–2) | <1 (0–2) |
| MeIQx | 39 (0–644) | 66 (0–1379) | 14 (0–391) | 24 (0–244) | 53 (0–778) | 90 (0–1388) |
| DiMeIQx | 9 (0–123) | 15 (0–131) | 3 (0–68) | 5 (0–55) | 12 (0–171) | 20 (0–140) |
| PhIP | 30 (0–555) | 51 (0–767) | 21 (0–479) | 40 (0–420) | 52 (0–732) | 91 (0–865) |
| Total[a] | 78 (0–1128) | 132 (0–1733) | 38 (0–944) | 69 (0–722) | 117 (0–1607) | 201 (0–1816) |

[a]The totals do not add up due to rounding off.

**Table III.** Estimated daily exposure of heterocyclic amines expressed in ng/day/person in different studies

| Reference | Method | MeIQx | DiMeIQx | PhIP | Total heterocyclic amines | Remarks |
|---|---|---|---|---|---|---|
| 3 | Based on levels in urine | 200–2600 | | | | |
| 4 | Based on levels in cooked meat and fish | | | 100–13 800 | | |
| 5 | Based on levels in cooked meat and fish | 100–1300 | | | | |
| 6 | Based on levels in cooked meat and fish | | | | 800–8400 | Heterocyclic amines not specified |
| 7 | Based on levels in fried meat, fish and gravy | | | | 40–7000 | The sum of IQ, MeIQ, MeIQx, DiMeIQx and PhIP |
| 8 | Based on levels in fried meat and gravy | | | | <500–4000 | The sum of MeIQx, DiMeIQx and PhIP |
| 9 | Based on consumption data and chemical analyses | | | | 1820 | The sum of IQ, MeIQx, DiMeIQx, PhIP and AαC. The mean intake for a person weighing 70 kg |
| Present study | Based on consumption data and chemical analyses | 72 | 16 | 72 | 160 | Mean intake |

retrospectively. Findings have been reported from a small number of healthy subjects and the method demands that the participants collect their urine accurately. Estimations based on average levels in cooked food do not take into account individual variation, such as intake frequency, and apply only at the population level. The latter is true of course only if the portion size is representative of the population and concentrations of heterocyclic amines are determined in dishes eaten by the same population and cooked according to normal household or restaurant procedures. In 1995 Layton *et al.* (9) combined published data on concentrations of heterocyclic amines with consumption data covering 3 days for 3563 people. Detailed information about frying patterns was not available and the variability in the content of heterocyclic amines as a consequence of differences in frying methods could not be taken into account. They found the mean daily intake of the major heterocyclic amines from meat to be 26 ng/kg body wt. Thus, the intake for an average person weighing 70 kg is 1820 ng, which is a 10 times higher intake than we estimated. They found the intake of heterocyclic amines to be, in descending order, PhIP, MeIQx, DiMeIQx and, finally, IQ.

Even though the method for estimating the individual level of exposure to heterocyclic amines is based on two previous studies in Stockholm, it is possible that the method has a number of potential flaws. In order to minimize misclassification the cooking was done according to standardized procedures. Also, it is common in Sweden to consume gravy together with meat and, in contrast to estimates done by others, our method takes into account the contribution from both meat/fish and gravy. The contribution from gravy is based on a model, which for example assumes that the serving size of gravy or sauce is in proportion to the portion size of the meat, which probably makes the estimate less precise than that for meat. For practical reasons the number of photo sets was limited to six and the classification of the degree of browning for other dishes therefore introduces some degree of misclassification, as well as the fact that only 22 of the 53 fried/baked/roasted dishes in the questionnaire were chemically analyzed. In the two articles giving the underlying data for the present calculation of intake (10,11) it was clearly shown that formation of MeIQx, DiMeIQx and PhIP are temperature dependent. For example, formation of PhIP rose from 0.2 ng/g fried bacon at 175°C to 4.5 ng/g fried bacon at 225°C; such a 20-fold increase was also typically seen for the other dishes. However, it is possible that by adjusting the duration of frying, one could achieve a similar degree of browning at other temperatures, at least above 175°C. We have not explored such possible situations and know of no other investigation of these relations. The cooking times used in the frying sessions were taken from widely used cookbooks or from the food packaging.

The questions in the questionnaire referred to eating habits 5 years previously in order to avoid differential misclassification. The questionnaire will later on be used in a case–control study on cancer and, therefore, we want to assess diet before

diagnosis, in case the disease has had some impact on present food habits. Also, we wanted to include subjects at an age relevant to the incidence of cancer.

An accurate assessment of consumption at the individual level is essential for investigations of human carcinogens and, in the present study, we have developed a practical method for assessing the intake of heterocyclic amines. The total mean daily intake of IQ, MeIQ, MeIQx, DiMeIQx and PhIP was found to be <200 ng in this elderly population in Stockholm.

## Acknowledgement

We wish to express our thanks to the Swedish Cancer Society for their financial support.

## References

1. IARC (1993) *Monographs of the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Natural Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation*, IARC Scientific Publication no. 56. IARC, Lyon, France, pp. 165–195.
2. Steineck,G., Gerhardsson de Verdier,M. and Övervik,E. (1993) The epidemiological evidence concerning intake of mutagenic activity from the fried surface and the risk of cancer cannot justify preventive measures. *Eur. J. Cancer Prevent.*, **2**, 293–300.
3. Ushiyama,H., Wakabayashi,K., Hirose,M., Itoh,H., Sugimura,T. and Nagao,M. (1991) Presence of carcinogenic amines in urine of healthy volunteers eating normal diet, but not of inpatients receiving parenteral alimentation. *Carcinogenesis*, **12**, 1417–1422.
4. Wakabayashi,K., Nagao,M., Esumi,H. and Sugimura,T. (1992) Food-derived mutagens and carcinogens. *Cancer Res.*, **52** (suppl.), 2092s–2098s.
5. Wakabayashi,K., Ushiyama,H., Takahashi,M., Nukaya,H., Kim,S.B., Hirose,M., Ochiai,M., Sugimura,T. and Nagao,M. (1993) Exposure to heterocyclic amines. *Environ. Hlth Perspect.*, **99**, 129–134.
6. Eisenbrand,G. and Tang,W. (1993) Food-borne heterocyclic amines. Chemistry, formation, occurrence and biological activity. A literature review. *Toxicology*, **84**, 1–82.
7. Johansson,M. and Jägerstad,M. (1994) Occurrence of mutagenic/carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. *Carcinogenesis*, **15**, 1511–1518.
8. Johansson,M., Fredholm,L., Bjerne,I. and Jägerstad,M. (1995) Influence of frying fat on the formation of heterocyclic amines in fried beef burgers and pan residues. *Fd Chem. Toxicol.*, **12**, 993–1004.
9. Layton,W.D., Bogen,T.K., Knize,G.M., Hatch,T.F., Jonson,M.V. and Felton,S.J. (1995) Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. *Carcinogenesis*, **1**, 39–52.
10. 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.
11. Skog,K., Augustsson,K., Steineck,G. and Jägerstad,M. (1997) Polar and non-polar heterocyclic amines in fish and meat products and their corresponding pan residues. *Fd Chem. Toxicol.*, **35**, 555–565.
12. Övervik,E., Nilsson,L., Fredholm,L., Levin,Ö., Nord,C.E. and Gustavsson,J.Å. (1984) High mutagenic activity formed in pan-broiled pork. *Mutat. Res.*, **135**, 149–157.
13. Gross,G.A. (1990) Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. *Carcinogenesis*, **9**, 1597–1603.
14. Becker,W. (1994) *Food Habits and Nutrient Intake in Sweden 1989*. National Food Administration, Uppsala, Sweden.
15. Becker,W., Enghart,H. and Robertson,A.-K. (1994) *Dietary Assessment Surveys in Sweden 1950–1990*. National Food Administration, Uppsala, Sweden.
16. Gross,G.A. and Grünter,A. (1992) Quantification of mutagenic/carcinogenic heterocyclic aromatic amines in food products. *J. Chromatogr.*, **592**, 271–278.
17. Murray,S., Lynch,A.M., Knize,M.G. and Gooderham,N.J. (1993) Quantification of the carcinogens 2-amino-3,8-dimethyl- and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in food using a combined assay based on gas chromatography–negative ion mass spectrometry. *J. Chromotogr.*, **616**, 211–219.
18. Felton,J.S., Fultz,E., Dolbeare,F.A. and Knize,M.G. (1994) Effect of microwave pretreatment on heterocyclic aromatic amine mutagens/carcinogens in fried beef patties. *Fd Chem. Toxicol.*, **32**, 897–903.
19. Knize,M.G., Dolbeare,F.A., Carroll,K.L., Moore,D.H. and Felton,J.S. (1994) Effect of cooking times and temperature on the heterocyclic amine content of fried beef patties. *Fd Chem. Toxicol.*, **32**, 595–601.

*Received on November 13, 1996; revised on June 19, 1997; accepted on June 19, 1997*