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Exposure to heterocyclic aromatic amines from consumption of cooked red meat and effect on human cancer risk. A review

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7 **Exposure to heterocyclic aromatic amines from consumption of**
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9 **cooked red meat and effect on human cancer risk - a review**
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14 Maite Sanz Alaejos, Venerando González and Ana María Afonso
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Abstract

This review covers the bibliographic data from the last ten years, on the possible carcinogenicity of heterocyclic aromatic amines (HAAs) in humans. Aspects such as red meat intake, cooking methods applied to red meat, and doneness of cooking are discussed from an epidemiological point of view. The role in the HAAs carcinogenicity has been assigned to two main factors, firstly the very high frequency of consumption of red meat, and secondly very darkly browned meats from cooking. However, there are some uncertainties associated with epidemiological results such as the presence of other carcinogens, co-carcinogens and anti-carcinogens in the diet, analytical results on HAAs content in foods, food frequency questionnaires and, mainly, genetic susceptibility to HAAs. It is concluded that there is not sufficient scientific evidence to support the hypothesis that human cancer risk is due specifically to the intake of HAAs in the diet.

Key words: Heterocyclic aromatic amines; intake; human cancer risk

Introduction

To date, more than twenty heterocyclic aromatic amines (HAAs) have been isolated as potent mutagens in the Ames/*Salmonella* test, and have been characterized. Table I shows their structures, chemical and abbreviated names.

Insert Table I about here

These HAAs contain from two to five (generally three) condensed aromatic cycles with one or more nitrogen atoms in their ring system and, usually, one exocyclic amino group. In general, they are formed during heating process of organic products containing nitrogenous compounds, mainly proteins. This formation is mainly temperature-dependent and so HAAs are classified into at least two groups due to the formation process. HAAs formed at temperatures between 100 and

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4 300°C are known as "thermic HAAs", IQ type or aminoimidazoazarenes. The ones formed at higher
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6 temperatures, above 300°C, are known as "pyrolytic HAAs" or non-IQ type. Thermic HAAs are
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8 generated from the reaction of free amino acids, creatin(in)e and hexosas. The precursor
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10 undergoes further dehydration and cyclization to form the observed pyrrole and pyridine derivatives.
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12 The heterocyclic pyridines and pyrazines formed in the Maillard reaction between hexose and
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14 amino acids then undergo further transformation with participation of Strecker aldehydes and
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16 creatin(in)e to produce imidazo-quinoxalines, perhaps through free-radical reactions. However, with
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18 temperatures about 225-250°C, these compounds seem to degrade or react with other compounds.
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20 On the other hand, the formation takes place through pyrolytic reactions between amino acids and
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22 proteins in the case of the non-IQ type. Pyrolysis occurs at temperatures well above 300°C, and
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24 produces many reactive fragments through radical reactions. These fragments are believed to
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26 condense to form new heterocyclic structures, and pyrolytic mutagens might be formed via free-
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28 radical reactions.

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30 HAAs are mutagenic not only for bacteria, but also for some mammalian cell systems and
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32 can produce chromosomal aberrations and sister chromatid exchanges in cultured cells. Some of
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34 them show higher mutagenic activity in bacteria and certain animals than typical
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36 mutagens/carcinogens such as benzo{a}pyrene or aflatoxin B₁. HAAs like harman and norharman
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38 are not mutagenic, but enhance the genotoxicity of mutagenic HAAs. In 1993, the International
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40 Agency for Research on Cancer (IARC, 1993) considered eight of the HAAs tested (MeIQ, MeIQx,
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42 PhIP, A α C, MeA α C, Trp-P-1, Trp-P-2 and Glu-P-1) as possible human carcinogens (class 2B) and
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44 one (IQ) as a probable human carcinogen (class 2A). More recently, in 2002, IQ was included as
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46 reasonably anticipated to be a human carcinogen in the NTP 10th Report on Carcinogens (NTP
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48 2002). Latterly, MeIQ, MeIQx, and PhIP are also listed in the NTP 11th Report on Carcinogens (NTP
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50 2004) as reasonably anticipated to be human carcinogens, together with IQ. These results are
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52 based on the sufficient evidence of carcinogenicity and supporting genotoxicity data obtained in
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54 long-term animal feeding studies. Although epidemiological evidence suggests that consumption of
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56 well-done or grilled meat may be associated with increased cancer risk in humans, the data are
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4 insufficient to support the conclusion that this risk is due specifically to MeIQ, MeIQx, or to PhIP,
5 present in these foods. Case-control studies show very conflicting results. Other case-control
6 studies suggest that any of MeIQ, MeIQx, or PhIP may increase the risk of certain cancers, but they
7 were not associated with other types of cancers. In relation to IQ, published epidemiology studies
8 provide some indication that human cancer risk is related to consumption of broiled or fried foods
9 that may contain IQ and/or other HAAs (NTP 2004). So, a reduction to the exposure of these
10 compounds has been recommended. In any case, it is important to have reliable data on the
11 content of these HAAs in different types of foods prepared in several ways in order to assess the
12 effects associated with their intake.
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22 The present review collects bibliographic data from the last ten years, on the possible
23 relationship between the intakes of certain foods and cancer risk. This paper belongs to a series of
24 reviews on different aspects of heterocyclic amines, such as a) the occurrence and formation of
25 HAAs during the cooking of food, mainly meat and fish products (Sanz Alaejos and Afonso); b)
26 analytical methods applied to HAAs determination in foods (Sanz Alaejos et al. a); and c) HAAs
27 intake, genetic aspects and human cancer risk (Sanz Alaejos et al. b).
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36 **Possible relationships between HAAs and human cancer**

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40 Epidemiological evidence appears to imply that an excessive consumption of very well-done red
41 meat (HAAs-containing meat products), may induce certain types of cancer (Navarro et al. 2004;
42 Nowell et al. 2002). "Red meat" usually refers to beef, veal, pork, mutton, and lamb. The so-called
43 "processed meat" groups ham, cured or smoked meats, cold cuts, charcuterie, and meat-products.
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48 The introduction of the Ames test in 1975 provided a rapid method to isolate potential
49 carcinogens from food on the basis of their mutagenic activity. If comparing the mutagenic activity in
50 meat samples to the one related to the known HAAs, most samples present mutagenic activity that
51 cannot be exclusively associated to the aromatic amines currently identified. This suggests that
52 additional compounds other than HAAs are present in these foods and need to be investigated,
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4 particularly those grilled over open flames (Anderson et al. 2005; Balbi et al. 2001; Jägerstad and
5
6 Skog 2005).

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8 Several epidemiological studies have been performed to test the hypothesis of an
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10 association between meat intake and human cancers (Zimmerli et al. 2001). It becomes really
11
12 difficult to link meat consumption to the HAAs intake in practically all these studies. Many other
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14 substances and factors which possibly contribute to the etiologies of these cancers cannot be
15
16 excluded, e.g. nitrosamines, polycyclic aromatic hydrocarbons (PAHs), acrylamide, high fat or salt
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18 intake, physical activity and others (Anderson et al. 2005; Augustsson et al. 1999; Balbi et al. 2001;
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20 De Stefani et al. 1997, 1998a, 2001; Gunter et al. 2005; Jägerstad and Skog 2005; Ji et al. 1995;
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22 LeMarchand et al. 1997, 2002; Murtaugh et al. 2004; Navarro et al. 2004; Nowell et al. 2002;
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24 Oreglia et al. 2001; Probsttensch et al. 1997; Singh and Fraser 1998; Sinha et al. 1998, 1999,
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26 2001, 2005; Zheng et al. 1998).

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28 Cancer development attributed to HAAs intake is shown in Table II together with some
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30 other dietary factors for comparison purposes.

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Insert Table II about here

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36 Table III shows the enhancing and protective effects exerted by dietary components on
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38 cancer risk development when associated with consumption of foods potentially containing HAAs.
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40 Usually, odds ratios (OR) are used. Sometimes relative risks (RR) are used, but in some studies no
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42 indication is made.

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Insert Table III about here

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48 Animal fat intake has been frequently, but inconsistently, associated to colorectal cancer
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50 risk. Intakes of total fat, saturated fat (S), and polyunsaturated fat (P) are not related with the risk of
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52 this disease. However, an inverse association is found for the P/S ratio. So, this ratio may be an
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54 indicator of colorectal cancer (LeMarchand et al. 1997). The fat content of red meat diminishes with
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56 the intensity of cooking. Therefore, potential higher risk associated with well-done beef may be
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4 counterbalanced with the lower fat content of meat. Red and total meat intakes are associated with
5 strong increases in risk of squamous cell laryngeal cancer. This effect disappears after controlling
6 the total fat intake. On the other hand, total fat intake displays a strong association with risk of
7 laryngeal cancer when red meat is also included. Total fat intake combines its effect multiplicatively
8 with tobacco smoking (Oreglia et al. 2001). However, MeIQx consumption was inversely associated
9 with smoking habits in relation to lung cancer. Neither DiMeIQx nor PhIP show an association with
10 smoking categories on lung cancer histology, and probably, are not associated with lung cancer risk
11 (Sinha et al. 2000).

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20 Vitamins C and E, vegetables and fruits are associated with reduced risks of prostate and
21 other cancers in population-based studies (Chan et al. 2005; Chiu et al. 2003; Deneo Pellegrini et
22 al. 1999; De Stefani et al. 1998a; Hu et al. 1999; La Vecchia et al. 1996; Nomura et al. 2003; Sinha
23 et al. 1999). The possible explanation is that vitamins C and E inhibit the nitrosation when
24 nitrosamines or other N-nitroso compounds are present in tissues. Also, fiber and carotene seem to
25 exert a decline in cancer risk (Chiu et al. 2003; Deneo Pellegrini et al. 1999; Hu et al. 1999;
26 Kampman et al. 1995; La Vecchia et al. 1996; Shannon et al. 1996; Singh and Fraser 1998; Slattery
27 et al. 1997). However, in relation to pancreatic cancer, high intakes of grilled/barbecued red meat
28 increase odds ratios, but no significant changes are further detected by adjustment the fruit,
29 vegetables, calories, total fat, or alcohol consumption (Anderson et al. 2002). Although the high
30 meat-low vegetable diet is considered the reference high-risk diet of colorectal cancer, other risk
31 dietary patterns are emerging, such as high intakes of processed meat and refined carbohydrates.
32 The strong association with refined cereal products is consistent with the hypothesis of a role of
33 hyperinsulinism in colorectal carcinogenesis (Boutron-Ruault et al. 1999). Total dietary diversity is
34 not associated with colon cancer (Slattery et al. 1997). However, eating a diet with a great diversity
35 of meats, poultry, fish and eggs is associated with a 50% increase in risk among all the men.
36 Women who eat a diet with a more diversified pattern of vegetables presented, approximately, a
37 20% lower risk than women who ate a non-diversified diet in vegetables (Slattery et al. 1997).
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4 There are few epidemiological studies in which an exclusive association between HAAs and
5 human cancer can be assumed.
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9 1. **Red meat intake:** Experts from the World Cancer Research Fund and the American Institute for
10 Cancer Research (WCRF/AICR, 1997) reviewed data on red meat intake and cancer risk. Those
11 experts concluded that a high intake of red meat *probably* increases the risk of developing
12 colorectal cancer, and *possibly* increases the risk of pancreas, breast, prostate and kidney cancers.
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16 Some reviews on meat consumption and cancer of the large bowel have been published
17 (Norat and Riboli 2001; Truswell 2002). One of these reviews concludes that the relationship
18 between meats and colorectal cancer looks generally weaker than the “probable” status stated by
19 the WCRF in 1997. Nonetheless, it is possible that certain meats or sausages cooked at very high
20 temperature can imply some risk (Truswell 2002). The hypothesis that consumption of red and
21 processed meat increases colorectal cancer risk is reassessed in a meta-analysis of articles
22 published during 1973-1999. Total meat consumption is not significantly associated with colorectal
23 cancer risk (Kato et al. 1997; LeMarchand et al. 2002; Navarro et al. 2003; Norat and Riboli 2001;
24 Sinha *et al.* 1999, 2005), but high intake of red meat, and particularly of processed meat, is
25 associated with a moderate increase on colorectal cancer risk in all sexes (LeMarchand et al. 2002;
26 Norat and Riboli 2001; Sinha et al. 1999), or only in men (LeMarchand et al. 1997). Sinha et al.
27 (1999) found a higher risk of colorectal adenoma mostly confined to intake of well-done/very well-
28 done red meat and meats cooked at high temperature, such as grilled and, possibly, fried red meat.
29 However, other authors (Navarro et al. 2003) do not find any relation between high consumption of
30 total-, red- and fatty beef meats with colorectal cancer risk. A slight increase in risk is detected for
31 relatively high intake of cold cuts, sausages and bovine viscera, whereas lean beef is associated
32 with a decreased risk (Navarro et al. 2003).
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50 Other investigations show that red meat intake is unrelated to colon cancer (LeMarchand et
51 al. 2002; Sinha et al. 2005) although individuals with low intake present lower risk for rectal cancer
52 (LeMarchand et al. 2002). Three wide studies were performed in Norway (Gaard et al. 1996) and
53 Sweden (Augustsson et al. 1999; Larsson et al. 1996). Although in these countries the HAA
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4 concentrations in the diet are usually lower than in other western European countries, differences
5 are found when high consumption (Augustsson et al. 1999; Larsson et al. 1996) or very high
6 frequency of consuming (Gaard et al. 1996) are studied. So, high frequency of sausages
7 consumption shows a relative risk of colon cancer of 3.5 (95% CI: 1.02-11.9) only for women. The
8 RR for total meat consumption is 1.87. However, both parameters are not statistically significant for
9 men (Gaard et al. 1996). In the prospective study for 61433 Swedish women, with ages between 40
10 and 75 years, a significant positive association between red meat consumption and risk of distal
11 colon cancer is observed, but not of cancers of proximal colon nor rectum (Larsson et al. 1996). In a
12 case-control study, risks for colon and rectal cancers are lower for the highest estimated intake
13 category than for the lowest intake category of HAAs. Relative risks for each one of the HAAs are
14 similar. Intake of meat and fish together, without considering the cooking method, is associated with
15 increased risk of colon and bladder cancer (Augustsson et al. 1999).
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28 Consumption of white meats is generally associated with lower cancer rates, even when
29 these meats have HAAs contents greater than red meats (Sanz Alaejos and Afonso). Furthermore,
30 chicken eaten without skin seems to decrease colorectal cancer risk (LeMarchand et al. 1997). Red
31 meat is no more implicated than any other meat type (Kampman et al. 1999; LeMarchand et al.
32 2002; Sinha et al. 2000, 2005).
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38 **2. Cooking methods:** The influence of cooking methods and cancer risk was also evaluated by the
39 WCRF/AICR (1997). The report established that there is not convincing evidence that any cooking
40 method modifies the risk of any cancer; neither there is evidence of any probably causal
41 relationship. However, high intakes of grilled/barbecued red meat possibly increase the risk of
42 stomach and colorectal cancer (WCRF/AICR, 1997). Higher risks are also observed for methods
43 that require higher temperatures and close contact of the food to the heating source, such as
44 barbecuing or iron-pan cooking and especially for red meats. Iron-pan means a kind of ground cast
45 iron skillet used in Argentina to cook meat without adding oil or fat. No associations are found for
46 boiled meats (Navarro et al. 2004).
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However, the results of studies on cooking methods are not consistent. Cooking methods hypothetically considered “hazardous”, such as frying and broiling, were found to increase colorectal cancer risk in some studies but not in others (Jägerstad and Skog 2005; LeMarchand et al. 2002; Navarro et al. 2004; Norat and Riboli 2001). The association between colorectal cancer and total HAAs intake is limited to men, and it is more clearly observed with MeIQx. This relation with MeIQx seems to result partially from an association with pan-fried meat and fish (LeMarchand et al. 2002). Furthermore, MeIQx intake is associated with slightly increased risk of lung cancer. However, DiMeIQx and PhIP intakes show no association (Sinha et al. 2000). When various factors are controlled, such as total-, red-, barbecued- or smoked meat, a statistically significant lung cancer risk associated to MeIQx remains. This MeIQx effect is still present, but no longer statistically significant, when the model includes well-done- or fried red meat. The opposite is also true; the previously significant effects of well-done and fried red meat are no longer significant after adjusting for MeIQx (Terry et al. 2003). Grilling/barbecuing, oven broiling or intakes of well-done meat and fish do not appear to affect cancer risk (LeMarchand et al. 2002; Sinha et al. 2000). The exception appears when a darkly browned surface is preferred but excluding red roasted meat. Roasted chicken and all barbecued meats were associated with a risk for colorectal cancer (Navarro et al. 2004). Pancreatic cancer cases reported high levels of grilled/barbecued meat intake and low levels of broiled meat intake relative to controls. Other meat variables do not show statistically significant associations with risk, neither alter the findings for barbecued meat intake. These meat variables include processed-, total-, total broiled-, total fried-, total red-, or total meat cooked by means other than grilling (Anderson et al. 2002). In a population-based case-control study (Anderson et al. 2005), dietary intake of HAAs and benzo(a)pyrene formed in grilled/barbecued red meat and fried red meat, was studied in relation to pancreatic cancer. Cases, when compared with controls, had higher mean levels of all the carcinogens studied (PhIP, MeIQx, DiMeIQx, and benzo(a)pyrene), as well as total mutagenic activity. The highest ORs were seen in the highest quintiles of intake and, with the exception of MeIQx, there were all statistically significant. Grilled/barbecued red meat intake was a statistically significant predictor of pancreatic

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4 cancer risk. Fried red meat intake also increased risk, but was not statistically significant, because
5 fried meats in general do not contain appreciable concentrations of PAHs (Anderson et al. 2005).
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9 3. **Doneness:** Meat doneness was weakly and inconsistently associated with prostate cancer risk
10 for individual types of meat, but an increased risk was observed for very well-done beefsteak
11 (Norrish et al. 1999). The incidence rates of prostate, pancreas and colon cancers seem to be
12 positively related to exposure to HAAs among men of different races in Los Angeles County (USA).
13 This result can partially explain the higher incidence among blacks with respect to white men in the
14 studied human group, because black men consume higher amounts of well-done meats, bacon,
15 pork and cold cuts (Bogen and Keating 2001). Moreover, an increased risk of breast cancer is
16 observed in healthy women who consume very well-done meats. PhIP is detected in their milks at
17 levels of 59 pg/mL, but not in milk from a vegetarian donor. Preference for well-done red meat is not
18 significantly associated with colon cancer, but seems to increase risk somewhat for rectal cancer
19 (LeMarchand et al. 2002; Sinha et al. 1999). On the other hand, MeIQx, PhIP, and benzo{a}pyrene
20 were associated with a slight increased risk of adenoma of colon, non-advanced adenoma, and
21 single adenomas in a recent and wide population-based case-control study, related to a high intake
22 of well-done red meat, bacon and sausage. However, in a fully adjusted model, total mutagenicity,
23 DiMeIQx, and MeIQx intakes are not associated with colorectal adenoma risk when all adenomas
24 were combined. Rectal cancer is not associated with any of the meat variables, such as meat types
25 or doneness/cooking methods (Sinha et al. 2005). Pancreatic cancer cases reported slightly higher
26 mean levels of intake for total-, total red-, and processed meat, and lower mean levels of intake for
27 white meat, when comparing with controls. The relative amounts of meat intake by doneness levels
28 are not different between cases and controls (Anderson et al. 2002). However, other studies have
29 shown no such association among well-done red meat consumption and cancer (Augustsson et al.
30 1999; Gunter et al. 2005; Kampman et al. 1999; Norrish et al. 1999; Sinha et al. 2000, 2001).
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51 As conclusion, in general, the three high-temperature cooking methods (pan-frying, oven-
52 broiling and grilling/barbecuing) seem to produce the highest HAAs concentrations. Preparation
53 methods like boiling, oven roasting, deep-frying, and charcoal grilling are generally “milder”, and
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4 much lower amounts of HAAs are formed. The degree of doneness, which is often closely related to
5 surface browning and total cooking time, is a key issue for HAAs production in cooked meat. The
6 formation of the crust is the result of steady transportation of water and dissolved compounds such
7 as amino acids and creatinine to the surface by capillary flow to the evaporation zone. Thus, the
8 precursors of HAAs are concentrated on or near the surface of the meat where the temperature is
9 highest.

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16 However, there is insufficient scientific evidence to prove that these genotoxic compounds
17 really cause human cancer, and so, no limits have been set for their presence in cooked foods.
18 Nevertheless, the competent authorities in most Western countries recommend minimizing their
19 occurrence. The reasons are the co-existence of other mutagens/carcinogens in foods, and the
20 possibility that the HAAs induce mutations, therefore increasing the sensitivity to tumor promoters.
21 So, some recommendations could be followed: Choose lean cuts. Apply lower temperatures and
22 shorter cooking times. Avoid browning of foods or, at least, remove the crust and charred parts of
23 fried or grilled meat, poultry and fish. Do not allow drippings from meat to become dry before
24 making gravy. To lessen the levels of HAAs produced it may be useful cooking meat and fish
25 together with foodstuffs containing phenolic antioxidants or with tomatoes, carrots or other
26 vegetables rich of carotenoids and antioxidant vitamins.
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40 **Uncertainties associated to epidemiological results**

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43 The lack of consistency of the results for all types of meats together with the lack of information
44 from cohort studies do not always permit a judgment about the possible differences in the effect of
45 meat on e.g., colon and rectal cancer risks, separately (Norat and Riboli 2001). In any case, the
46 present data are not enough to evaluate the dose response of the possible relationship between
47 HAAs and human cancer, in its widest sense. Besides, a simplistic approach to evaluate only HAAs
48 with respect to cancer risk may not be too appropriate, taking into account that diet is a complex
49 mixture that contains carcinogens, co-carcinogens and anti-carcinogens (Jägerstad and Skog 2005;
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4 Matos and Brandani 2002; Sinha and Rothman 1999; Sugimura et al. 1996). Furthermore, it must
5
6 be noticed that the development of cancer takes a long time, and so there is a delay of several
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8 years between exposure and disease outcome.
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10 Metabolic aspects of HAAs have been reviewed (Gooderham et al. 2001; Sanz Alaejos et
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12 al. b). The major pathway for the metabolic activation of HAAs starts with the N-hydroxylation of the
13
14 exocyclic amino group, catalyzed mainly by cytochrome P450 1A2 (CYP1A2). Although these
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16 metabolites may directly react with DNA, this N-hydroxylation step is usually followed by sulphation
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18 or acetylation by means of sulfotransferase 1A1 (SULT1A1), or N-acetyltransferases (NAT),
19
20 respectively (Sanz Alaejos et al. b).
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22 Urinary excretion of MeIQx and its metabolites has been also studied (Ji et al. 1994; Lynch
23
24 et al. 1992; Sinha et al. 1995; Stillwell et al. 1994, 1999a, 1999b; Turesky et al. 1998). MeIQx
25
26 metabolism and disposition in humans are more strongly influenced by CYP1A2 activity than those
27
28 of PhIP. Biotransformation of MeIQx via CYP1A2 oxidation to form 8-CH₂OH-MeIQx and the
29
30 hydroxylamine NHOH-MeIQx, followed by N-2-glucuronidation is a general pathway for the MeIQx
31
32 metabolism in humans. The formation of both 8-CH₂OH-MeIQx and NHOH-MeIQx is catalyzed by
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34 CYP1A2 and may be useful biomarkers of CYP1A2 activity in humans (Turesky et al. 1998). Low
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36 levels of total unconjugated MeIQx are associated with high CYP1A2 activity when adjusted for
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38 amount of ingested meat, whereas NAT2 activity shows no relationship with the latter (Sinha et al.
39
40 1995). However, significant interracial differences are observed for black, white and Asian men.
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42 This is because urinary levels of MeIQx are positively associated with intake frequencies of pork,
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44 bacon and cold cuts (Ji et al. 1994).
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46 The theoretical cancer risk by HAAs in the Swiss diet is calculated by two different
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48 procedures and results in $0.7 \cdot 10^{-5}$ or, roughly, 10^{-5} (Zimmerli et al. 2001). If it is assumed that
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50 humans may be 10 to 100 times more susceptible to HAAs than male rats, the hypothetical excess
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52 cancer risk would be in the order of 10^{-4} to 10^{-3} . Cancer potency estimates for several of the HAAs
53
54 were as high as one in a 100,000 per ppb in the total diet. Based on HAAs exposures ranges of
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56 0.02 to 83 ppb in the human total diet, and rodent data, Gaylor and Kadlubar (1991) estimated that
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4 the upper limit on cancer risk due to HAAs formed during cooking is on the order of one per 10,000.
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6 Although rodents, monkeys and humans have the capacity to activate HAAs, studies using hepatic
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8 microsomes demonstrated that humans have a greater capacity to activate HAAs than rodents or
9
10 cynomolgus monkeys. Human risk, based on linear extrapolation of TD₅₀ calculations from mouse,
11
12 rat, or primate tumor data, and on mean estimated mutagen exposures for the U.S. population,
13
14 suggests potential risks of 10⁻⁵ to 10⁻³ (Bogen 1994; Felton et al. 1992; Gaylor and Kadlubar 1991;
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16 Layton et al. 1995). Adamson (1996) suggests potential risks of 10⁻⁴ to 10⁻³. On the other hand,
17
18 interspecies differences in CYP1A2 enzyme expression and catalytic activities may be significant
19
20 and must be carefully considered when assessing human health risk (Kim and Guengerich 2005).
21
22 So, important differences between human and rat CYP1A2 were found in the C8- and N-oxidation
23
24 of MeIQx (Turesky et al. 2001), and in the levels of expression and catalytic activities for HAAs N-
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26 hydroxylation (Turesky et al. 1999), although rat and human CYP1A2 have identical 75% amino
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28 acid sequence (Guengerich 1997). The value 10⁻³ is considered as a maximum credible risk (Felton
29
30 et al. 1992). Similar theoretical cancer risks result from human exposure to other compounds
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32 unavoidably present in the diet, such as naturally occurring radionuclides (potassium 40) or excess
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34 calories (Zimmerli et al. 2001).
35

36 There are several major uncertainties in the present estimation of cancer risk, including
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38 human susceptibility, exposure, carcinogenic potencies of individual HAA, as well as possible
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40 interactions between different HAAs and other substances normally present in a diversified diet.
41
42 The most likely target organs for most HAAs are the liver and the gastrointestinal tract.
43
44 Nevertheless, in Switzerland the cancer mortality trends for these organs (gastric and colon
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46 cancers) show a permanent slight decrease, although meat, fish and alcohol consumption have
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48 greatly increased in Switzerland over the last 50 years, and milk intake (including yogurt) has
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50 decreased (Zimmerli et al. 2001).
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52 A major trouble of the epidemiological studies is the obtaining of a correct estimation for the
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54 exposure to HAAs (Alexander et al. 2002; Keating and Bogen 2001; Keating et al. 1999; Sinha and
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56 Rothman 1997; Skog 2002). Dietary assessment in combination with analytical data on HAAs levels
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4 in various foods must be used. In this sense, food frequency questionnaires including not only the
5 type and amount of foods consumed but also the cooking methods and the level of doneness are
6 required. There are several flaws including bias, inconsistent reporting, difficulty in quantifying
7 cooking doneness by such methods, and the day-to-day variation in the diet. Moreover, HAAs
8 belong to a class of numerous compounds and the quantification of each of them in a large number
9 and variety of food samples is not an easy task. Consequently, it is not surprising that bibliographic
10 data are very incomplete (Sanz Alaejos and Afonso). Table IV shows some estimates for HAAs
11 mean intakes in various countries. The dispersion of the results is evident, and there are scarce
12 data referred to individual HAAs.
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Insert Table IV about here

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26 Some attempts have been made to develop biomarkers in order to overcome some of the
27 problems associated with the use of questionnaires in the assessment of HAAs exposure.
28 Biomarkers of diet promise to provide a more accurate measure of dietary intake. They are more
29 objective because they do not rely on the subject's memory when being interviewed, or on the
30 accuracy of recording the food diary. The use of biomarkers does not solve the problem of
31 exposure estimation back in time for years, since HAAs do not accumulate in body tissues. Hence,
32 HAAs in body fluids or tissues would only give a measure of recent exposure (Frandsen et al. 2002;
33 Lynch et al. 1992; Stillwell et al. 1997; Strickland et al. 2001; Wild et al. 2001). However, these
34 short-term markers are useful tools to study mechanisms of action of HAAs, and to validate
35 questionnaires (Sinha 2002). So, Peters et al. (2003) when combining the measurements of urinary
36 mutagenicity and the estimated intake of meat-derived mutagenicity, found a stronger positive
37 association with risk for colorectal adenomas than did either variable separately, when comparing
38 the highest with the lowest quintile of combined mutagenicity (OR=5.6; 95% CI=2.3-13.9). In
39 consequence, urinary mutagenicity may be considered a useful adjunct to questionnaire data and
40 may have value as a direct biomarker of exposure and/or risk for colorectal adenoma (Peters et al.
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4 2003). Also, the formation of both 8-CH₂OH-MeIQx and NHOH-MeIQx may be useful biomarkers of
5
6 CYP1A2 activity in humans (Turesky et al. 1998).
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8 On the other hand, protein adducts in blood and HAAs in hair are biomarkers reflecting
9
10 longer-term exposure. The use of PhIP (and other HAAs) in hair is attractive. It would integrate the
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12 dose over several months depending on the length of the hair, which grows approximately 1 cm per
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14 month. Hair is easy to sample and store for the purpose of later analysis in prospective studies on
15
16 the relationship between HAAs exposure and cancer (Alexander et al. 2002; Kobayashi et al. 2005).
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18 A main problem when using literature data related to HAAs contained in cooked dishes is
19
20 that many experiments have been performed under unspecified cooking conditions, or using high
21
22 cooking temperatures during a long time to maximize the HAAs production. This last case can lead
23
24 to a non-representative form of the usual way of cooking meats by the general population in a
25
26 certain country. The relation between the degree of doneness and the surface browning may differ
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28 because some people fry their meats at a high temperature for a short time to get a brown surface
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30 but the interior is not so cooked through, while others fry their meats at low temperatures but during
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32 a longer time. This can lead to the same degree of surface browning but very different HAAs
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34 amounts. The colored photos are sometimes used to assess the preference of the consumer on the
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36 degree of doneness and, indirectly, the amount of HAAs (Alexander et al. 2002; Keating and Bogen
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38 2001; Keating et al. 1999; Sinha and Rothman 1997; Skog 2002). The results seem to lead to an
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40 underestimation of exposure (Skog 2002). However, HAAs levels in home-cooked meat samples
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42 are significantly different when samples are visually classified for doneness, but not when self-
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44 reported doneness preference is used to classify doneness or to categorize samples (Keating et al.
45
46 2000). HAAs levels in meats obtained from homes vary considerably from the laboratory data. In a
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48 study (Knize et al. 2002), samples were taken from volunteers responding in survey that they
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50 preferred their meat well-done or very well-done, leading to expect high HAAs exposures in these
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52 households. But, surprisingly, in that collection of meats samples, approximately 25% of the
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54 samples had undetectable amounts of MeIQx or PhIP. Besides, although well-done chicken breast
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56 prepared in the laboratory may contain large amounts of PhIP, a survey of flame-grilled meat
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4 samples cooked in private homes showed PhIP levels in beefsteak and chicken breast that were
5 not significantly different. The extremely high PhIP levels reported in some studies of grilled chicken
6 are not observed in home-cooked samples (Knize et al. 2002). Considerable differences between
7 equivalent products from different manufacturers are found in many cases. In addition, the
8 terminology used for different cooking methods varies around the world. In Argentina and Uruguay
9 the term “roasting/broiling” does not expose foods directly to flame, whereas “grilling” may use a hot
10 surface (i.e., a pre-heated heavy iron plate), or a grill directly above burning coals (Matos and
11 Brandani 2002; Navarro et al. 2004). Cooking practices and eating habits differ between different
12 populations, and conclusions drawn from one population may not be applicable to another
13 (Alexander et al. 2002; Ferguson 2002; Keating and Bogen 2001, 2004; Keating et al. 1999; Matos
14 and Brandani 2002; Sinha and Rothman 1997; Skog 2002). In some countries pan residues are
15 used for preparing gravies, and pan residues contain substantial amounts of HAAs compared with
16 the corresponding food item. Besides, meat extracts and bouillon (cubes) contribute to the HAAs
17 intake. Therefore, it is important to establish databases on HAAs content in cooked foods that are
18 representative for the eating habits of the population being studied, and taking into account each
19 ingredient of the recipe, spices, condiments, etc. The dishes should be prepared in a way that
20 reflects regular household and restaurant cooking conditions.
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38 Really, recent advances in the analytical instrumentation, concretely in LC-MS and GC-MS,
39 have greatly facilitated the ability to measure HAAs in foods, as well as HAAs and their metabolites
40 in urine of meat eaters. Nevertheless, the accurate determination of HAAs is a difficult analytical
41 task since traces of these compounds have to be determined in highly complex food matrices. This
42 problem can only be solved by combining both elaborate sample preparation steps with selective
43 separation steps, and then followed by sensitive detection methods to quantify low levels of HAAs.
44 Tedious clean-up procedures that include extraction, purification, and pre-concentration steps,
45 followed by a separation technique, such as liquid or gas chromatography and capillary
46 electrophoresis are usually used. The main detection systems used are UV, fluorescence,
47 electrochemical and MS (Sanz Alaejos et al. a). In addition, the different analytical methods applied
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4 might not be comparable. Some problems in determining appropriate estimates of extraction
5 recovery rates must also be taken into account. Some HAAs or HAAs derivatives can bind with
6 other food components. All of these formed compounds cannot be extracted from food by the usual
7 extraction methods and, therefore, the dietary assessment of genotoxic compounds may be
8 underestimated. Therefore, different extraction procedures have to be applied to cooked and
9 uncooked meat, before and after enzymatic proteolysis. Consequently, the use of absolute or
10 relative values of HAAs content in food can lead to erroneous conclusions when possible cancer
11 risks are established for the intake of these foods.
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20 Divergent results can be due to several causes, one of them is the meat type and the
21 ingredients added. Also, analytical results on HAAs content in foods obtained under unspecified
22 cooking conditions or at high cooking temperatures may be not representative. It should very
23 convenient: a) to establish databases on HAAs content in cooked foods that are representative for
24 the eating habits of the population being studied, and taking into account each ingredient of the
25 recipe; b) to record the inside and outside food appearance in the food frequency questionnaires,
26 instead of simply recording the "doneness level"; c) to consider the possible role of HAAs in the
27 cancer development in conjunction with PAHs and other toxic compounds; and d) to use
28 biomarkers in order to determine the HAAs exposure.
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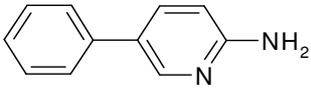
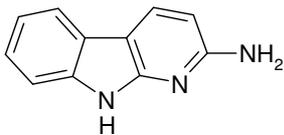
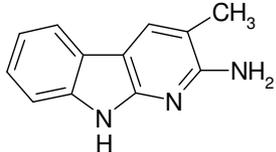
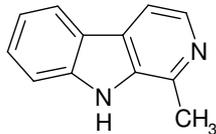
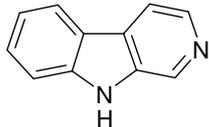
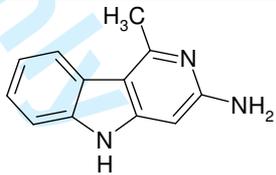
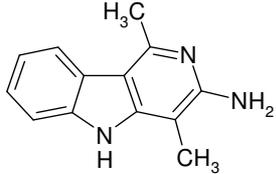
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Table I. Classification of HAAs

Chemical name	Abbreviated name	Structure
ISOLATED THERMIC HAAs: AMINOIMIDAZOAZARENES		
Imidazopyridine derivatives		
2-amino-1,6-dimethylimidazo(4,5-b)-pyridine	DMIP	
2-amino-1,5,6-trimethylimidazo(4,5-b)-pyridine	1,5,6-TMIP	
2-amino-3,5,6-trimethylimidazo(4,5-b)-pyridine	3,5,6-TMIP	
2-amino-1-methyl-6-phenylimidazo(4,5-b)-pyridine	PhIP	
2-amino-1-methyl-6-(4'-hydroxyphenyl)-imidazo(4,5-b)-pyridine	4'-OH-PhIP	
2-amino-1,6-dimethyl-furo(3,2-e)imidazo(4,5-b)-pyridine	IFP	
Imidazoquinoline derivatives		
2-amino-3-methyl-imidazo(4,5-f)-quinoline	IQ	
2-amino-3,4-dimethyl-imidazo(4,5-f)-quinoline	MeIQ	

Imidazoquinoxaline derivatives		
2-amino-3-methyl-imidazo(4,5-f)-quinoxaline	IQx	
2-amino-3,4-dimethyl-imidazo(4,5-f)-quinoxaline	4-MelQx	
2-amino-3,8-dimethyl-imidazo(4,5-f)-quinoxaline	8-MelQx	
2-amino-3,7,8-trimethyl-imidazo(4,5-f)-quinoxaline	7,8-DiMeIQx	
2-amino-3,4,8-trimethyl-imidazo(4,5-f)-quinoxaline	4,8-DiMeIQx	
2-amino-4-hydroxymethyl-3,8-dimethyl-imidazo(4,5-f)-quinoxaline	4-CH2OH-8-MelQx	
2-amino-3,4,7,8-tetramethyl-imidazo(4,5-f)-quinoxaline	TriMeIQx	
2-amino-1,7,9-trimethyl-imidazo(4,5-g)-quinoxaline	7,9-DiMeIQx	

ISOLATED PYROLYTIC HAAs: CARBOLINES		
Phenylpyridine derivatives		
2-amino-5-phenylpyridine	Phe-P-1	
Pyridoindole derivatives:		
α – carbolines		
2-amino-9H-pyrido(2,3-b)indole	A α C	
2-amino-3-methyl-9H-pyrido(2,3-b)indole	MeA α C	
β – carbolines		
1-methyl-9H-pyrido(3,4-b)indole	Harman	
9H-pyrido(3,4-b)indole	Nor-harman	
γ - carbolines		
3-amino-1-methyl-5H-pyrido(4,3-b)indole	Trp-P-2	
3-amino-1,4-dimethyl-5H-pyrido(4,3-b)-indole	Trp-P-1	

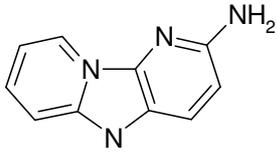
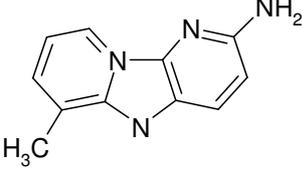
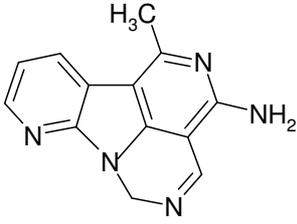
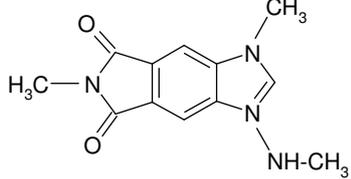
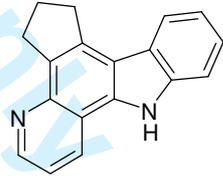
Pyridoimidazole derivatives		
δ – carbolines		
2-aminodipyrdo(1,2- α :3',2'-d)imidazole	Glu-P-2	
2-amino-6-methyldipyrdo(1,2- α :3',2'-d)imidazole	Glu-P-1	
Tetraazafluoranthene derivatives		
4-amino-6-methyl-1H-2,5,10,10b-tetraazafluoranthene	Orn-P-1	
Benzimidazole derivatives		
4-amino-1,6-dimethyl-2-methylamino-1H,6H-pyrrolo(3,4-f)benzimidazole-5,7-dione	Cre-P-1	
Carbazole derivatives		
3,4-cyclopenteno-pyrdo(3,2-a)carbazole	Lys-P-1	

Table II. Influence of HAAs consumption on cancer risk development

Cancer sites	Cases	Controls	Odds ratios	95% confidence interval	Intakes (observations)	Geographical location	Ref.
Aero-digestive	140	286	Non significant		Fried meat, white meat	Uruguay	De Stefani et al. 1998
			2.0	1.0-4.3	Broiled meat		
			2.2	1.1-4.2	Total HAAs		
			2.8	1.4-6.0	Red meat		
Bladder	273	553	0.8	0.5-1.5	Total meat and fish	Sweden	Augustsson et al. 1999
			1.2	0.7-2.1	HAAs or PhIP		
			5.0	3.3-7.6	(Current smoking)		
	144	576	Non significant		Well-done fried meat	Uruguay	Balbi et al. 2001
			0.38	0.23-0.64	Fruits, cooked vegetables, potatoes, cheese		
			4.04	2.24-7.27	Barbecued meat, salted meat, fried eggs, N-nitroso compounds		
Breast	352	382	3.34	1.85-6.02	For IQ, in red meat, total meat, beef, fried meat (OR: 2.6 for PhIP)	Uruguay	De Stefani et al. 1997
			5.3	2.8-10.2	Fried red meat		

	273	657	1.54	0.96-2.47	Very well-done hamburger	USA (Iowa)	Zheng et al. 1998
			1.64	0.92-2.93	Very well-done bacon		
			2.21	1.30-3.77	Very well-done beef steaks		
			4.64	1.36-15.70	Very well-done meats		
Colon	352	553	0.6	0.4-1.0	HAAs	Sweden	Augustsson et al. 1999
			0.9	0.5-1.4	Total meat and fish		
			1.0	0.7-1.4	(current smoking)		
	1542	1860	0.9		Red and white meat	USA	Kampman et al. 1999
			1.2	0.9-1.5	Well-done red meat		
			1.4	1.0-1.9	Processed meat (men)		
	2474	34817	1.11	0.97-1.27	DiMeIQx; red meat (processed, roasted)	USA	Sinha et al. 2005
			1.17	1.01-1.35	PhIP; red meat (processed, roasted)		
			1.18	1.01-1.38	MeIQx; red meat (processed, roasted)		
			1.18	1.02-1.35	Benzo{a}pyrene; red meat (processed, roasted)		

	513	513	0.8	0.5-1.2	Grilled/barbecued meat and fish	USA (Hawaii)	LeMarchand et al. 2002
			0.9	0.6-1.3	Well-done meat and fish		
			1.0	0.7-1.5	Total red meat; red meat, well- or very well-done		
			1.3	0.8-1.9	Pan-fried/oven broiled meat and fish		
	289	289	1.0	0.6-1.1	MeIQx (men)		
			1.0	0.6-1.6	PhIP (men)		
			1.1	0.7-1.7	4,8-DiMeIQx (men)		
	620	1038	1.7	1.2-2.5	Well-, very well-done red meat	USA (North Caroline)	Butler et al. 2003
			1.8-2.0		4,8-DiMeIQx		
			2.0	1.3-3.2	Red meat, pan-fried red meat		
Colorectal	88	200	0.74	0.52-1.05	Boiling/stewing white meat, >21.6 g/day	Argentina (Córdoba)	Navarro et al. 2004
	87	200	0.93	0.65-1.34	Boiling/stewing red meat, >31.2 g/day		
	110	199	1.08	0.76-1.54	Roasted red meat, darkly browned		
	148		2.19	1.53-3.12	Roasted white meat, darkly browned		
	128		1.42	1.00-2.02	Fried white meat, darkly browned		

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	145	200	1.74	1.23-2.45	Fried red meat, darkly browned		
	64	198	1.51	0.98-2.32	All meats and methods, lightly browned		
	189	202	4.57	3.10-6.73	All meats and methods, darkly browned		
	133	199	1.91	1.33-2.74	Barbecued white meat, darkly browned		
	176	200	2.85	1.97-4.10	Barbecued red meat, darkly browned		
	167	200	2.44	1.71-3.47	Iron-pan cooked red meat, darkly browned		
	102	537	0.5	0.2-1.07	Poultry and fish (women)	Germany	Tiemersma et al.
			2.7	1.1-6.7	Red meat (men)		2002
	431	433	1.2	0.8-1.9	Meat	Germany	Tiemersma et al.
							2004
	500	500	1.51	1.03-2.23	Total meat	United Kingdom	Barrett et al.
			1.97	1.30-2.98	Red meat		2003
	3696	34817	0.92	0.78-1.09	Broiled red meat	USA	Sinha et al. 2005
			0.95	0.74-1.22	Pan-fried red meat		
			0.99	0.80-1.23	Grilled meat		
			1.03	0.91-1.16	White meat		

		1.04	0.90-1.19	Processed red meat with N-nitroso compounds		
		1.05	0.92-1.22	Total meat		
		1.07	0.92-1.24	Red meat		
		1.14	1.00-1.30	High intake of bacon and sausages		
		1.21	1.06-1.37	Well-done red meat, as hamburgers, hot-dogs, sausages, bacon (HAAs+PAHs)		
	32	100	1.75	0.78-4.05	MelQx low exposure	USA (Arkansas and Tennessee) Nowell et al. 2002
	53	79	4.09	1.94-9.08	MelQx high exposure	
	54	80	4.36	2.08-9.60	Well-, very well-done red meat	
			0.8	0.4-1.4	Total HAAs (women)	USA (Hawaii) LeMarchand et al. 2002
			1.8	1.1-3.1	Total HAAs (men)	
	146	228	1.04	0.98-1.09	Total meat	USA (Maryland) Sinha et al. 1999
			0.19	0.01-2.86	Microwaved red meat	
			0.94	0.74-1.20	Broiled red meat	
			1.01	0.79-1.28	Baked red meat	
			1.10	0.96-1.26	Rare-, medium-done red meat	

			2.11	0.90-4.92	Very well-done red meat		
			1.15	0.97-1.36	Pan-fried red meat		
			1.22	0.92-1.61	Well-, very well-done pan-fried red meat		
			1.26	1.06-1.50	Grilled/barbecued red meat		
			1.85	1.24-2.75	Well-, very well-done grilled/barbecued red meat		
	146	228	1.11	1.03-1.19	Red meat	USA (Maryland, Missouri)	Sinha et al. 1999, 2001
			1.29	1.08-1.54	Well-, very well-done red meat		
	146	228	2.1	1.0-4.3	MeIQx	USA (Missouri)	Sinha et al. 2001
			2.2	1.2-4.1	4,8-DiMeIQx		
			2.5	1.1-5.5	PhIP		
			3.1	1.4-6.8	HAAs		
Colorectal adenomas	488	488	2.2	1.1-4.3	Fried red meat, darkly browned (high frequent intake)	USA (California)	Probsttensch et al. 1997
	565	565	Non significant		Well-done red meat (HAAs)	USA (California, southern)	Gunter et al. 2005

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Esophagus (squamous cell)	150	745	2.4	1.2-4.8	Fried meats, pan juices	Sweden	Terry et al. 2003
Esophagus (adenocarcinoma)	157		0.7	0.4-1.2	High intake of MeIQx, 4,8-DiMeIQx, and PhIP		
Gastric cardia (adenocarcinoma)	233		1.3	0.8-2.1			
Kidney	138	553	1.0	0.5-1.9	HAA's or total meat and fish	Sweden	Augustsson et al. 1999
			1.3	0.8-2.1	(Current smoking)		
	121	243	0.78	0.45-1.39	Processed meat	Uruguay	De Stefani et al. 1998
			2.07	1.03-4.19	High intake of barbecued meat		
			2.18	1.14-4.19	PhIP (>15.6 ng/d)		
3.42	1.76-6.65	High intake of red meat					
Lung	303	765	1.0	0.5-2.4	Stir-fried meat less often than daily (smokers, women)	China	Seow et al. 2000
			2.7	1.3-5.5	Stir-fried meat daily (smokers, women)		
			3.7	1.8-7.5	Stir-fried meat daily (kitchen fumes with oily fumes during cooking, women)		
			4.3	2.7-6.8	Fumes from meat cooking (ex-smokers, women)		
			5.0	3.4-7.3	Fumes from meat cooking (current smokers, women)		

	256	284	Significant		Red meat, fried meat	Uruguay	Deneo Pellegrini et al. 1996
	593	623	1.5	1.1-2.1	Fried or well-done red meat (women)	USA (Missouri)	Sinha et al. 1998
			1.6	1.1-2.4	Total meat (women)		
			1.8	1.2-2.7	Red meat (women)		
	593	623	1.3	0.9-1.9	Fried or well-done red meat (women)	USA (Missouri)	Sinha et al. 2000
			1.4	1.0-1.9	Total meat or red meat (women)		
			1.5	1.1-2.0	Barbecued red meat or smoked meat (women)		
			0.9	0.8-1.1	PhIP (women)		
			1.2	0.9-1.6	DiMeIQx (women)		
			1.5	1.1-2.0	MeIQx (women)		
	343	200	1.0	0.7-1.5	MeIQx (women, heavy smokers)		
	203	340	2.1	1.3-3.3	MeIQx (women, light/moderate smokers)		
	47	83	3.6	1.3-10.3	MeIQx (women, non smokers)		
Lung, squamous cell carcinoma			1.9	1.2-3.1	MeIQx (women)		
Lung, other histological types			1.6	1.1-2.5	MeIQx (women)		

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Pancreas (exocrine)	193	674	1.5	0.9-2.7	MeIQx; well-done barbecued meat and pan-fried meat	USA	Anderson et al. 2005
			1.8	1.0-3.1	PhIP; well-done barbecued meat and pan-fried meat		
			2.0	1.2-3.5	DiMeIQx; well-done barbecued meat and pan-fried meat		
			2.2	1.2-4.0	Benzo{a}pyrene; well-done barbecued meat and pan-fried meat		
			2.4	1.3-4.3	Mutagenic activity; well-done barbecued meat and pan-fried meat		
Pancreas	193	674	0.7	0.4-1.2	Broiled red meat	USA (upper Midwest area)	Anderson et al. 2002
			1.4	0.7-2.6	Fried red meat		
			2.2	1.4-3.4	Grilled/barbecued red meat		
Prostate	317	480	Non significant		Meat doneness and major HAAs	New Zealand	Norrish et al. 1999
			Weakly significant		IFP		
			1.68	1.02-2.77	Very well-done beef steaks		
	464	459	1.68	1.20-2.36	Well-done meat	USA	Nowell et al. 2004
Rectum	249	553	0.7	0.4-1.1	HAAs	Sweden	Augustsson et al. 1999
			1.0	0.6-1.6	Total meat and fish		
			0.9	0.6-1.4	(Current smoking)		

	688	34817	Non significant		PhIP, MeIQx, DiMeIQx, benzo{a}pyrene	USA	Sinha et al. 2005
	952	1205	1.33	0.98-1.79	Well-done red meat (men)	USA (California and Utah)	Murtaugh et al. 2004
			1.37	0.98-1.92	Mutagen index (men)		
	210	210	0.8	0.4-1.6	Oven broiled, grilled/barbecued meat and fish	USA (Hawaii)	LeMarchand et al. 2002
			1.2	0.7-2.0	Well-done meat and fish		
			1.5	0.9-2.4	Well-, very well-done red meat		
			1.6	1.0-2.5	Pan-fried meat and fish		
			1.7	1.0-3.0	Total red meat		
	137	137	1.7	0.3-3.8	PhIP (men)		
			2.7	1.1-6.3	4,8-DiMeIQx (men)		
			3.1	1.3-7.7	MeIQx (men)		
Stomach	123	282	1.7	0.7-4.0	Total meat and proteins, total energy and total fat	Uruguay	De Stefani et al. 2001
			1.9	1.1-3.5	Energy adjusted for high intake of processed meat		
			4.6	2.3-9.0	Total meat		
			Non significant		Fried, barbecued, salted meat and PhIP		

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Table III. Influence of foods/nutrients intake on cancer risk development

Cancer sites	Higher risk (odds ratio)	Protective effect (odds ratio)	No association (odds ratio)	Geographical location	Ref.
Bladder	Red meat (1.6)			Italy (Northern)	Tavani et al. 2000
Brain	Salted vegetables, salted fish	Vegetables, fruit, fresh fish, poultry, Ca, vitamins E, C, β -carotene		China (Northeast)	Hu et al. 1999
Breast	Well-done deep-fried red meat (1.92), freshwater fish	Non-hydrogenated soybean oil (0.48)		China (Shanghai)	Dai et al. 2002
	Red meat (1.2)			Italy (Northern)	Tavani et al. 2000
Colon	High intake of preserved animal or vegetal foods (men: 2.0; women: 2.7), eggs (1.3), fish (men: 1.7), red meat (1.5)	Fresh fruit (0.6), vitamins E, C, β -carotene, fiber	Fish (women: 1.2)	China (Shanghai)	Chiu et al. 2003
	Fresh red meat (women: 2.35), high	Vegetables (0.4)	Fresh red meat (men: 0.89)	Germany	Kampman et al.

	consumption of red meat and low consumption of vegetables and fruits (women: 3.1)				1995
	Red meat (1.9)			Italy (Northern)	Tavani et al. 2000
	Sausages (women 3.5)		Meat, meat stews, roasted meat, fish, milk, fat, energy, fiber, Ca	Norway	Gaard et al. 1996
	For distal colon: High intake (≥ 94 g) of red meat, (women, prospective study: 2.22)	Poultry	Broiled red meat (1.03), fish. For rectum or proximal colon: red meat	Sweden	Larsson et al. 2005
	For distal colon: Long-term consumption of red meat (1.53), or processed meat (1.50)	Poultry, fish		USA	Chao et al. 2005
	Diverse diet with greater proportion of meat, fish, poultry and eggs (men: 1.5); or with greater proportion of refined grain products (men: 1.7)	Diverse diet with greater proportion of fruits, vegetables or whole grains (women: 0.7)	Diverse diet	USA (California, Minnesota, Utah)	Slattery et al. 1997

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	For distal colon: total meat (men: 2.20)	Vegetables and fruits (women: 0.48), cereals (women: 0.47; men: 0.43), dairy products (women: 0.40), water (women: 0.55; men: 0.68)	Total meat (women: 0.94; men: 1.17), red meat (women: 0.72; men: 1.48)	USA (Seattle)	Shannon et al. 1996
Colorectal	Bovine viscera (1.73), cold cuts/sausages (1.47)	Lean beef (0.67)	Red meat (0.63), total meats (0.70), white meat (0.73),	Argentina (Córdoba)	Navarro et al. 2003
			Red meat; fried or processed meats; fruits, vegetables	Finland	Pietinen et al. 1999
	Processed meat (2.4); offal (2.1); refined cereal products (rice, pasta, pastry), delicatessen, butter, pates (1.7)		Fish (1.5); fresh meat (1.2); eggs (1.1); dairy products (1.0)	France (Burgundy)	Boutron-Ruault et al. 1999
	Bread/cereal (1.7); refined sugar (1.4); potatoes (1.2); cakes/desserts (1.1)	Vegetables, fruit, fish	Meat (white, red or processed), eggs	Italy	Franceschi et al. 1997
	Red meat (1.6)	Vitamin C, β -carotene	Processed meat	Italy (Northern)	La Vecchia et al. 1996
	Red meat (1.03-2.22)	Poultry	Fish	Sweden	Larsson et al. 2005

	Red meat (1.54); refined grain (1.32); pork or processed meat (1.27)	Vegetables, fruit, garlic, whole grain, coffee		Switzerland (Canton of Vaud)	Levi et al. 1999
	White meat (3.29), red meat (1.90), total meat (1.85)	Legume (0.53)		USA (California) (Seventh Day Adventist)	Singh and Fraser 1998
		Protein	Total calories, total fat, subclasses of fat, carbohydrates, dietary fiber	USA (Florida, New York)	Kato et al. 1997
	Eggs (men: 2.7; women: 2.3), processed meat (men: 2.3), red meat (1.6)	Chicken without skin	Fat-trimmed red meat, fish; total fat, saturated fat, polyunsaturated fat	USA (Hawaii)	LeMarchand et al. 1997
	Well-/very well-done red meat (fried or grilled/barbecued)	Vegetables (0.7), fruit/fruit juice (0.8), microwaved red meat (0.19)	Broiled red meat (0.94), total meat, red meat, grilled/barbecued red meat (rare/medium done), pan-fried red meat (rare/medium done), fat, alcoholic beverages	USA (Maryland)	Zheng et al. 1998
Endometrium	Red meat (1.5)			Italy (Northern)	Tavani et al. 2000
Esophagus			Red meat		

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	Soups, butter	Vegetables, fruit, olive oil, pasta, poultry		Italy (Northern)	Bosetti et al. 2000
			Fried foods (1.16)	Italy, Switzerland	Galeone et al. 2005
	Pan-fried red or processed meat; pan juices	Barbecued meat, vitamin C, fiber	Total meat, red meat, white meat, fish	Sweden	Terry et al. 2003
	Red meat, barbecued/grilled		Doneness level; beef, chicken, or pork (broiled or fried)	USA (Nebraska)	Ward et al. 1997
Gall bladder			Red meat	Italy (Northern)	Tavani et al. 2000
Gastric	Red meat (1.6)				
	Processed meat, bacon	Vegetables (vitamins E, C, β -carotene, folate)	Vegetables with processed meat, or with bacon	USA (Hawaii)	Nomura et al. 2003
	Barbecued/grilling well-done meat (3.2), red meat		Beef, chicken or pork (broiled or fried)	USA (Nebraska)	Ward et al. 1997
Kidney			Red meat	Italy (Northern)	Tavani et al. 2000
	Red meat (3.4), protein (2.2),	Vegetables (0.5)	Salted meat, processed meat, milk	Uruguay	De Stefani et al.

	barbecued meat (2.1), fruits (1.7)				1998a
Larynx			Red meat	Italy (Northern)	Tavani et al. 2000
	Red meat or fried fish (3.1), fried eggs or fried potatoes (1.9), processed meat (1.7), fried meat (1.6)	Vegetables, fruit, olive oil		Italy (Northern), Switzerland (Canton of Vaud)	Bosetti et al. 2002a, b
	Total fat from red meat (7.05), red and total meat (3.32)			Uruguay	Oreglia et al. 2001
Liver			Red meat	Italy (Northern)	Tavani et al. 2000
Lymphoma (Hodgkin's and non Hodgkin's disease)			Red meat	Italy (Northern)	Tavani et al. 2000
Lymphoma (non Hodgkin's)	Trans-unsaturated fat (2.4); beef, pork, or lamb (2.2)		Broiled or barbecued red meat	USA (Massachusetts)	Zhang et al. 1999
Myeloma			Red meat	Italy (Northern)	Tavani et al.

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					2000
Oral cavity/pharynx			Red meat	Italy (Northern)	Tavani et al. 2000
			Fried foods (1.11)	Italy, Switzerland	Galeone et al. 2005
	Pork or processed meat (3.2), eggs (2.3), red meat (2.1)	Vegetables, fruit, milk, fish		Switzerland (Canton of Vaud)	Levi et al .1998
Ovary	Red meat (1.53)	Vegetables, fish		Italy	Bosetti et al. 2001
	Red meat (1.3)			Italy (Northern)	Tavani et al. 2000
Pancreas	Deep-fried, grilled, cured, or smoked foods		Preserved animal foods, fresh red meat, organ meat, poultry	China (Shanghai)	Ji et al. 1995
	Red meat (1.6)			Italy (Northern)	Tavani et al. 2000
Prostate			Red meat	Italy (Northern)	Tavani et al. 2000

	Red meat (2.0), total energy (1.9), total fat and desserts (1.8)	Vegetables and fruit (0.5), vitamin E (0.6), vitamin C (0.4)		Uruguay	Deneo Pellegrini et al. 1999
Rectum	Red meat (1.7)			Italy (Northern)	Tavani et al. 2000
	High intake (≥ 94 g) of red meat (women, prospective study: 1.28)	Poultry	Fish	Sweden	Larsson et al. 2005
	Red meat (1.71)			USA	Chao et al. 2005
Thyroid			Red meat	Italy (Northern)	Tavani et al. 2000

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Table IV. Estimated mean intakes of HAAs.

Geographic location	ng/person/day	ng/kg body mass/day	Ref.
Japan	5-300 (PhIP) 300-3900 (MeIQx)		Wakabayashi et al. 1997
Sweden	160 (PhIP: 72) (MeIQx: 72) (DiMeIQx: 16)	2.3	Augustsson et al. 1997
	77		Augustsson et al. 1999
	8.53		Olsson et al. 2005
Switzerland	330	5	Zimmerli et al. 2001
USA (2001)		1.4-2.1 (PhIP: 1.1-1.6) (MeIQx: 0.29-0.47) (DiMeIQx: 0.021-0.031)	Sinha et al. 2001
	455	9	Bogen and Keating 2001
USA (Missouri) (1998)	70 g red meat/day		Sinha et al. 1998
	(PhIP: 158.3) (MeIQx: 52.1) (DiMeIQx: 3.5)		Sinha et al. 2000