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Review

## Genotoxicity of heat-processed foods

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### Abstract

Gene–environment interactions include exposure to genotoxic compounds from our diet and it is no doubt, that humans are regularly exposed to e.g. food toxicants, not least from cooked foods. This paper reviews briefly four classes of cooked food toxicants, e.g. acrylamide, heterocyclic amines, nitrosamines and polyaromatic hydrocarbons. Many of these compounds have been recognised for decades also as environmental pollutants. In addition cigarette smokers and some occupational workers are exposed to them. Their occurrence, formation, metabolic activation, genotoxicity and human cancer risk are briefly presented along with figures on estimated exposure. Several lines of evidence indicate that cooking conditions and dietary habits can contribute to human cancer risk through the ingestion of genotoxic compounds from heat-processed foods. Such compounds cause different types of DNA damage: nucleotide alterations and gross chromosomal aberrations. Most genotoxic compounds begin their action at the DNA level by forming carcinogen–DNA adducts, which result from the covalent binding of a carcinogen or part of a carcinogen to a nucleotide. The genotoxic and carcinogenic potential of these cooked food toxicants have been evaluated regularly by the International Agency for Research on Cancer (IARC), which has come to the conclusion that several of these food-borne toxicants present in cooked foods are possibly (2A) or probably (2B) carcinogenic to humans, based on both high-dose, long-term animal studies and *in vitro* and *in vivo* genotoxicity tests. Yet, there is insufficient scientific evidence that these genotoxic compounds really cause human cancer, and no limits have been set for their presence in cooked foods. However, the competent authorities in most Western countries recommend minimising their occurrence, therefore this aspect is also included in this review.

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**Keywords:** Acrylamide; Heterocyclic amines; Polyaromatic hydrocarbons; Nitrosamines; Formation; Occurrence; Exposure; Cancer risk; Minimising strategies

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## 1. Introduction

The aim of cooking is to produce bacteriologically safe food with optimal sensory properties and the minimum content of possibly harmful substances. Cooking and food processing at high temperatures have been shown to generate various kinds of genotoxic substances or “cooking toxicants”. Today, there is growing concern about the impact of these substances on human health. The exposure varies among individuals due to dietary habits and differences in cooking practice.

During the 1960s and 1970s, much interest was focused on two classes of food toxicants producing tumours in long-term animal studies (i) polycyclic aromatic hydrocarbons (PAHs) and (ii) *N*-nitroso compounds (NOCs). These compounds are found in food as a result from food processing, e.g. curing, drying, smoking, roasting, refining, and fermentation, and also from air pollution. Furthermore, *N*-nitroso compounds may be formed endogenously [1–3].

In the late 1970s, a new, highly mutagenic class of compounds, heterocyclic amines (HCAs), was identified in grilled or broiled meat and fish by Japanese scientists [4,5]. They used the Ames test and detected sev-

eral compounds, which showed extremely high mutagenic potency; 100 to 100 000 times higher than PAHs and NOCs. Once identified and synthesised, HCAs were in long-term animal studies shown to be moderately active in inducing tumours. HCAs are also formed in meat and fish during pan-frying, roasting/baking, barbecuing, deep-fat frying, smoking and grilling.

The most recently detected food toxicant produced by heat processing is acrylamide. Concern over acrylamide in foodstuffs arose in April 2002 when Swedish scientists reported unexpectedly high levels of this potentially carcinogenic compound in carbohydrate-rich foods heated to high temperatures [6]. Since then researchers have devoted great efforts to measure acrylamide levels in a wide variety of foods – such as crisps, French fries, bread and coffee – and begun to search for ways to reduce levels of the compound [7].

The above-mentioned classes of toxicants (PAHs, NOCs, HCAs and acrylamide) have been evaluated by the International Agency for Research on Cancer (IARC), which has come to the conclusion that several of these food-borne toxicants present in cooked foods are possibly or probably carcinogenic to humans. The aim of this paper is to briefly review their presence,

formation, metabolic activation, genotoxicity and human cancer risk along with figures on estimated daily intakes and ways to minimising the occurrence of these heat-induced food toxicants. For more details, see some reviews given in the reference list [7–15].

## 2. Acrylamide

Acrylamide has been manufactured in big scale since the 1950s mainly to produce water-soluble polyacrylamides used as flocculents for clarifying drinking water, for treating municipal and industrial waste waters and as flow control agents in oil-well operations. Other major uses of acrylamide are in soil stabilisation, in grout for repairing sewers and in acrylamide gels used in biotechnology laboratories. Chemically, acrylamide is a water-soluble low-molecular compound (MW 79.01) built up of a reactive ethylenic double bond linked with a carboxamide group (Fig. 1) [16].

The general opinion has been that the main human exposure to acrylamide is of occupational origin in the industrial production of polyacrylamide, while the general public may be exposed by drinking water

that has been treated with polyacrylamide in a refining process [17]. A maximum tolerable level of 0.1 µg acrylamide per liter water has recently been established within the European Union [18]. In addition, tobacco smokers are highly exposed to acrylamide. Bergmark [19] determined the level of acrylamide adducts in blood samples from smokers and non-smokers working with polyacrylamide gels for electrophoresis. The levels of haemoglobin adducts in smokers were twice the level in the non-smoking laboratory personnel. In the same survey, it was concluded that also non-smokers had elevated levels of acrylamide adducts. The high background of acrylamide adducts in the non-smoking control group was unexpected and the authors offered no explanation. A part of the explanation came 3 years later when Tareke et al. [20] found increased haemoglobin adduct levels in rats fed with fried animal standard diet. During the same period, also Peres [21] measured surprisingly high levels of acrylamide in coffee. These early observations in the year 2000 were largely ignored. However, 2 years later, Tareke et al. [6] showed that the high background level of acrylamide in humans was due to compounds in the diet by demonstrating relatively high levels of acrylamide in

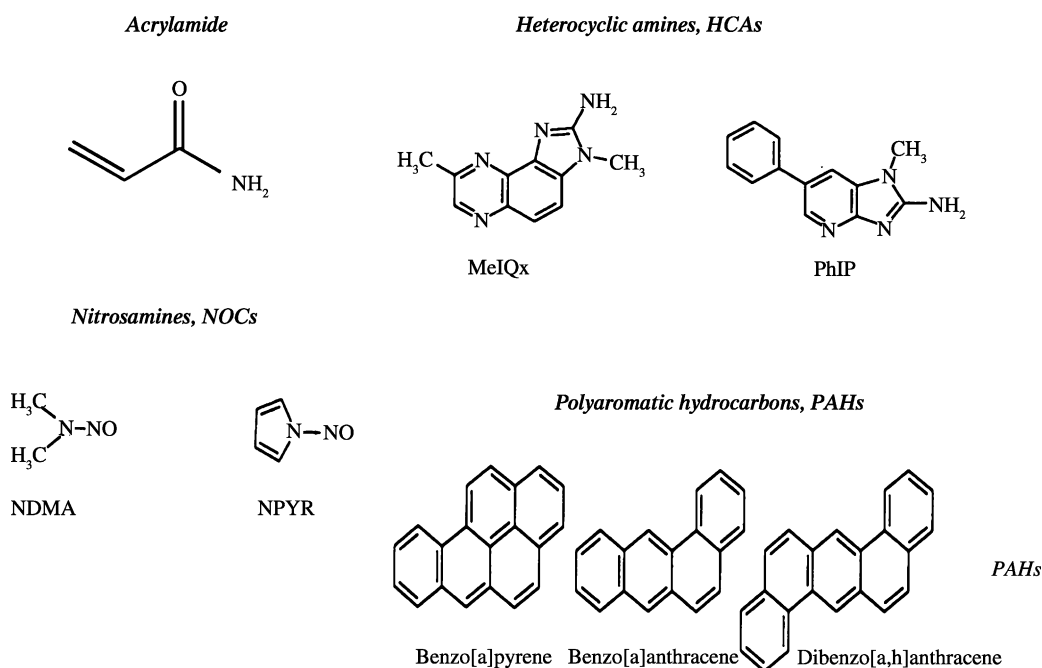


Fig. 1. Carcinogens produced in heat-processed and cooked food.

Table 1  
Acrylamide levels in processed foods listed alphabetically [6,7]

Food	Acrylamide <sup>a</sup> (μg/kg = ppb)
Almonds, roasted	260
Asparagus, roasted	143
Baked products: bagels, breads, cakes, cookies, pretzels	70–430
Beer, malt, and whey drinks	30–70
Biscuits, crackers	30–3200
Cereals, breakfast	30–1346
Chocolate powder	15–90
Coffee powder	170–351
Corn chips, crisps	34–416
Crisp bread	800–1200
Fish products	30–39
Gingerbread	90–1660
Meat and poultry products	30–64
Onion soup and dip mix	1184
Nuts and nut butter	64–457
Peanuts, coated	140
Potato, boiled	48
Potato chips, crisps	170–3700
Potato, French-fried	200–12000
Potato, puffs, deep-fried	1270
Snacks, other than potato	30–1915
Soybeans, roasted	25
Sunflower seeds, roasted	66
Taco shells, cooked	559

<sup>a</sup> Values were selected from several references and websites on acrylamide: (a) CFSN/FDA Exploratory Survey: <http://www.cfsan.fda.gov/~dms/acrydata2.html>; and <http://www.acrylamide-food.org/>; (b) acrylamide Infonet: <http://www.acrylamide-food.org/>; (c) WHO/FAO Acrylamide in Food Workshop: <http://www.jifsan.umd.edu/acrylamide/acrylamideworkshop.html>; (d) JIFSAN/NCFST Acrylamide in Food Workshop: <http://www.jifsan.umd.edu/Acrylamide/acrylamideworkshop.html>.

heat-processed commercial foods and in foods cooked at high temperatures, especially in carbohydrate-rich foods such as crisps and French fries. These widely publicised findings stimulated worldwide studies on determining acrylamide levels in food and on the nature of acrylamide precursors in unprocessed foods.

Acrylamide in food is largely derived from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of reducing sugars as glucose during baking and frying. The acrylamide contents of several food categories are listed in Table 1 [7]. Widely consumed processed foods with high levels of acrylamide include French fries, potato chips, tortilla chips, breads crust, crisp bread and various baked goods and cereal formulations and

coffee. However, the observed wide variations in levels of acrylamide in different food categories as well as in different brands of the same food category (e.g. French fries; potato chips) appear to result not only from the amounts of the precursors present but also from variations in processing conditions (e.g. temperature; time, nature of frying oil; nature of food matrix). No acrylamide has been reported in unheated or boiled food.

## 2.1. Genotoxicity and metabolism

The genotoxicity of acrylamide has been studied extensively. It does not induce mutation in bacteria, but its metabolite, glycidamide, does in the absence of an exogenous metabolic system (IARC). Acrylamide induces sex-linked recessive lethal and somatic mutations in *Drosophila*. It induces gene mutation, structural chromosomal aberrations, sister chromatid exchange and mitotic disturbances in mammalian cells in vitro in the presence or absence of exogenous metabolic systems. It induces structural chromosomal aberrations in vivo in both somatic and germ-line cells. Chromosomal aberrations and micronuclei were induced in mouse bone marrow and in premeiotic and postmeiotic cells in a linear dose–response relationship. Treatment with acrylamide in vivo also caused somatic mutation in the spot test, heritable translocation and specific locus mutations in mice and dominant lethal mutations in both mice and rats in several studies. Acrylamide induces unscheduled DNA synthesis in rat spermatocytes in vivo but apparently not in rat hepatocytes. And glycidamide induced unscheduled DNA synthesis in rat hepatocytes in one study in vitro. Furthermore, acrylamide induces transformation in cultured mammalian cells [16,22].

Acrylamide and glycidamide are equally distributed throughout the tissues and have half-lives of about 5 h in rats; acrylamide itself has also been shown to be uniformly distributed between tissues in several other species. The conversion of acrylamide to glycidamide is saturable, ranging from 50% at very low doses (<5 mg/kg bw) to 13% at 100 mg/kg bw in treated rats. Both agents are detoxified by glutathione conjugation, and glycidamide is also detoxified by hydrolysis. Both agents react directly with haemoglobin in vivo, but DNA adducts result only from the formation of glycidamide (for reference, see [7,16,17,22]).

## 2.2. Cancer

IARC [16] makes an overall evaluation that acrylamide is probably carcinogenic to humans (Group 2A) based on the following overall evaluation: “There is inadequate evidence in humans for the carcinogenicity of acrylamide. There is sufficient evidence in experimental animals for the carcinogenicity of acrylamide. In making the overall evaluation, the Working Group took into consideration the following supporting evidences: (i) acrylamide and its metabolite glycidamide form covalent adducts with DNA in mice and rats; (ii) acrylamide and glycidamide form covalent adducts with haemoglobin in exposed humans and rats; (iii) acrylamide induces gene mutations and chromosomal aberrations in germ cells of mice and chromosomal aberrations in germ cells of rats and forms covalent adducts with protamines in germ cells of mice *in vivo*; (iv) acrylamide induces chromosomal aberrations in somatic cells of rodents *in vivo*; (v) acrylamide induces gene mutations and chromosomal aberrations in cultured cells *in vitro*; (vi) acrylamide induces cell transformation in mouse cell lines”.

When calculating cancer risk for humans, reliable data from epidemiological studies are of course better than an extrapolation from animal studies using high doses of acrylamide. Due to the fact that acrylamide is produced in many different types of foodstuffs, it may be difficult to find sufficiently large differences in exposure between the studied groups, which is a prerequisite in epidemiological studies. So far a few epidemiological studies on cancer risk with dietary acrylamide intake have been published. Mucci et al. [23] found a lack of an excess risk of cancer of the large bowel, bladder or kidney in Swedish consumers of foods containing moderate (30–299  $\mu\text{g}$ ) or high (300–1200  $\mu\text{g}/\text{kg}$ ) levels of acrylamide. Pelucchi et al. [24] and Dybing and Sanner [25] reported similar results. Although the absence of an association in a population-based study seems reassuring, there is a need to extend the epidemiological evaluation to other cancer sites (e.g. lung, pancreas, testis) in view of the fact that smoking significantly increases the body burden of acrylamide. Moreover, epidemiological studies have so far not been designed to detect any estimated stochastic (random) small increase in the incidence of cancer related to acrylamide.

## 2.3. Exposure

Based on all analyses of acrylamide in foods that already have been published, WHO [26] estimated an intake of acrylamide to be in the range of 0.3–0.8  $\mu\text{g}/\text{kg}$  bodyweight per day for an adult corresponding to 21–56  $\mu\text{g}/\text{day}$  for a person weighing 70 kg. It is important to take into account that there may also be certain groups of people with much higher intake of acrylamide. A higher estimated daily exposure, about 100  $\mu\text{g}$  per person including acrylamide originating from cosmetics and tobacco based on average haemoglobin adducts level in the Swedish population, was also reported [6].

## 3. Heterocyclic amines (HCAs)

Research leading to the discovery of a series of mutagenic and carcinogenic heterocyclic amines (HCAs) was inspired by the idea that smoke produced during cooking of food, especially meat or fish, might be carcinogenic [4,5]. More than 20 derivatives of HCAs, actually produced by cooking or heating of meat or fish, have now been isolated and their structures determined, most being previously unregistered compounds [14,15]. Generally, they all consist of two or three rings with an exocyclic amino group attached to one of the rings. Depending on their chemical structures they can be divided into the following sub-groups: amino-carbolines (e.g. AAC), imidazo-quinolines (e.g. IQ) and imidazoquinoxalines (e.g. MeIQx) and imidazopyridines (e.g. PhIP). The chemical structure and trivial names of some HCAs are shown in Fig. 1. For full names, see the list of abbreviations.

The imidazo-quinolines, imidazoquinoxalines and imidazopyridines may be formed from creatine or creatinine, certain free amino acids and sugars via the Maillard reaction [27]. These three groups of precursors are all present in uncooked meat and fish muscle, where free amino acids and sugars are supplied from muscle protein and glycogen, respectively, whereas creatine is an energy metabolite only present in significant levels in muscle cells. Following cooking at high temperatures, HCAs are produced in ng/g. In general pan-frying and grilling produce high yield of HCAs at cooking temperatures from 200 °C and above, boiling yields little or no HCAs, and deep-fat frying, roasting, and

Table 2  
HCA levels in processed foods [28]

Food	MeIQx (ng/g)	PhIP (ng/g)
Beef burger, fried	0–7	0–32
Meat balls, fried	0–0.8	0–0.6
Chicken, fried	0–3	0–70
Salmon, fried	0–5	0–23
Beef burger, pan residue	0–6	0–13
Meat extract	0–80	0–4
Beef flavour	0–20	0–4
Beef stock cube	0–0.6	0–0.3

baking procedure give variable yields. Extremely high yield of HCAs have been reported in pan residues (up to 82.4 ng/g from minute beef [28]) from frying, roasting or baking, while most commercial bouillon cubes contain modest amounts. Some typical amounts of HCAs formed in cooked foods are displayed in Table 2. For more details, see also [29,30].

### 3.1. Genotoxicity and metabolism

Sugimura and his colleagues [4,5] demonstrated already in 1977 that the charred surface of fish and beef, broiled over a direct flame or charcoal were highly mutagenic in the Ames bacterial system (*Salmonella typhimurium*); the mutagenic activities were far more potent than expected from the amount of benzo(a)pyrene contained in these materials. The Japanese group reported that the mutagenicity in *Salmonella* varied more than 160 000 times between the strongest and the weakest HCAs, affected by number and positions of exocyclic substituents, especially the 2-amino-group of the imidazo part of the molecular structure present in most HCAs. Genotoxic activity of the HCAs has now been studied in various organisms including bacteria, yeast, *Drosophila*, mammalian cells in vitro and experimental rodents in vivo [14].

The proposed bioactivation pathway of HCAs is initiated by oxidation to hydroxyamine derivatives by cytochrome P450s, e.g. *N*-hydroxylation by CYP1A2 [31], and subsequent acetylation [32,33] by *N*-acetyltransferase type 2 (NAT2). The nitrenium ion (derived from the exocyclic amino group of the imidazo-moiety present in most HCAs) is the likely ultimate carcinogen binding to the DNA bases [34] producing DNA adducts through the formation of N–C bonds at guanine bases. Metabolic activation by

CYP1A2 was documented in people after extensive characterisation in vitro and in animal models as reviewed elsewhere [35,36]. The activation by CYP1A2 can be induced in humans fed a diet rich in HCAs [37] and is affected by polymorphisms of phase II activating enzymes [33,38–41].

The formation of covalent adducts with DNA is assumed to be a prerequisite for the initiation of the carcinogenic process. The parent HCAs, their metabolites and biologically effective doses determined by DNA and protein adducts have been measured in human studies using accelerator mass spectrometry [42,43–45] and a variety of other very sensitive analytical methods [42,46,47]. The results using accelerator mass spectrometry showed a linear relation between adduct levels and dose, except at high chronic doses, where a plateau was reached, and that humans form more DNA adducts per dose than rats. This indicates that linear extrapolation from high-dose animal studies may underestimate human DNA damage at low doses [48].

Alterations in genes that might provide clues to the induction mechanism include APC, b-catenin, Ha-ras and p53 tumour-suppressor genes and p53 tumour-suppressor genes [14].

### 3.2. Cancer

HCAs have been found to be potent carcinogens, which induce a variety of histologic types of tumours in multiple organs following long-term oral administration [11,49,50]. Tumours are induced in liver, lung, haematopoietic system, forestomach, and blood vessels in *mice*, and colon, small intestine, prostate, mammary gland, hematopoietic system, liver, Zymbal gland, skin, clitoral gland, oral cavity, and urinary bladder in *rats* (usually, doses between 0.01 and 0.06% in diet for 48–112 weeks). It is notable that some HCAs induced tumours of the colon (PhIP, IQ, MeIQ, Glu-P-1, Glu-P-2), mammary gland (PhIP, MeIQ, Trp-P-2) and prostate (PhIP), which are common cancers in Western countries and have been associated with Western life style, i.e. high fat/meat consumption [51]. It has also been shown that IQ induces liver tumours in cynomolgus monkeys (non-human primates), after chronic dosage of 10 or 20 mg/kg for 5 days per week. A high-fat diet has been shown to increase the carcinogenicity of low levels of IQ in several target organs in rats, es-

pecially the mammary gland [52]. Neonatal mice are highly sensitive to test chemicals, and a two-generation study showed PhIP to increase the risk of mammary carcinoma development in the second generation [53]. Moreover, HCA at a total dose of 5–10 000-fold less than the standard chronic bioassays, have caused tumours on neonatal mice.

Based on animal experiments, one of the HCAs, IQ, has been classified by IARC as a possible human carcinogen (Group 2A) and eight other HCAs (MeIQ, MeIQx, PhIP, AaC, MeAaC, Trp-P-1, Trp-P-2 and Glu-P-2) as probable human carcinogens (group 2B) [49]. In the past few years, several epidemiological studies have focused on meat consumption and cancer. Conflicting data exist regarding the relative risk associated with the intake of (fried) meat, and the results of many studies have wide confidence intervals, and therefore no reliable conclusions can be drawn. However, no investigation has directly assessed the intake of HCAs in relation to cancer development.

There is also good epidemiological evidence correlating a high intake of HCAs with colon cancer [54–56] although this correlation is not consistent [57]. The mutations in the APC suggest a connection to the exposure to HCAs [58], but further research is needed.

Some people may be more susceptible to HCAs than others. Supportive data have also been obtained from studies on polymorphic enzymes involved in the metabolism of HCAs, cytochrome P4501A2 (CYP1A2) and *N*-acetyltransferase type 2 (NAT2). Individuals possessing rapid CYP1A2 and rapid NAT2 phenotypes are considered to be more susceptible to colorectal cancer because they rapidly activate HCAs to reactive forms [59,60]. In two [61,62] of three reported studies [61–63], rapid acetylators determined by phenotype were more frequent in colorectal cancer patients than in control subjects. Minchin et al. [59] reported that rapid acetylators accounted for 47% (147/313) of colorectal cancer patients and 33% (94/286) of controls ( $p = 0.001$ ). A case–control study of 75 cases and 205 controls demonstrated that presence of both the rapid CYP1A2 and rapid NAT2 phenotypes was associated with a 2.8-fold ( $p = 0.002$ ) increase in the risk of colorectal cancer and polyps combined [64]. However, a case–control study of colon adenomas found no measurable difference in the genetically determined NAT2 status between 447 cases and 487 controls [65].

Table 3

Assessment of daily intake of some HCAs

MeIQx	DiMeIQx	PhIP	Total HCAs	Reference
			1820	[67]
			976 <sup>a</sup>	[68]
72	16	72	160	[69]
33–45	4	285–458	322–507	[70]
20–33	1.5–2.2	78–110	99–145	[71] <sup>b</sup>
9.8–11.2	0.7–6.3	39–47	58–74 <sup>d</sup>	[72] <sup>c</sup>
93–135	6.5–10.7	160–218	260–364	[73] <sup>b</sup>
34	2	63	103	[74]

The data are mean values and are expressed in nanograms per person per day.

<sup>a</sup> The intake of IQ, MeIQ, MeIQx, DiMeIQx, PhIP, AaC, MeAaC, Trp-P-1 and Trp-P-2.

<sup>b</sup> Mean values for control subjects and colon cancer cases.

<sup>c</sup> Based on 70 kg body weight.

<sup>d</sup> Total HCAs also include Trp-P-1 and MeIQ.

### 3.3. Exposure

Human exposure to HCAs has been estimated to range from a few ng/day to some µg/day, depending on dietary habits and cooking practices (Table 3) [66–74]. HCAs have been detected in urine from volunteers consuming a normal diet, but not from patients receiving parenteral alimentation, suggesting that humans are normally exposed to HCAs [66]. Other studies have shown that MeIQx and PhIP are absorbed and rapidly metabolised by humans [13,44]. Although the consumption of these compounds is very low, several of the HCAs are consumed at the same time and the combined effect has not been sufficiently investigated.

The content of HCAs in dishes consumed in ordinary life is low and perhaps not sufficient in itself to explain human cancer. However, the coexistence of many other mutagens/carcinogens of either autotoxic or xenotoxic type and the possibility that HCAs induce genomic instability and heightened sensitivity to tumour promoters suggest that minimising the exposure to HCAs or reduction of HCAs' biological effects as far as possible are highly recommended [14]. Numerous environmental chemicals found in food or the atmosphere can impact the exposure, metabolism, and cell proliferation response of HCAs [15].

## 4. *N*-nitroso compounds (NOCs)

Humans are exposed to *N*-nitroso compounds (NOCs) in diet from a variety of cured meats and fish

products [75,76]. Moreover, NOCs can be formed in vivo during simultaneous ingestion of nitrite or nitrogen oxides and a nitrosable substrate such as a secondary amine [77]. *N*-nitroso compounds are the general term covering all substances with *N*-nitroso groups. Currently, several hundred such compounds are known (for references see [3,10,78]).

Structures of two nitrosamines, commonly reported in cooked foods, NDMA and NPYR, are shown in Fig. 1. A large group of *N*-nitroso compounds occurring in food are the volatile carcinogenic *N*-nitrosoamines: NDMA (*N*-nitrosodimethylamine), NDEA (*N*-nitrosodiethylamine), NPYR (*N*-nitrosopyrrolidine) and NPIP (*N*-nitrosopiperidine). However, the main forms of *N*-nitroso compounds in food are non-volatile, including a large number of compounds that could be potentially formed, e.g. proteins containing *N*-nitrosated peptide linkages, such as NPRO (*N*-nitrosoproline). Non-volatile *N*-nitroso compounds have not been reported as mutagenic or carcinogenic, but they might act as precursors to volatile carcinogenic nitrosamines. Another group of *N*-nitroso compounds, the nitrosamides, contains substances such as *N*-nitrosoureas, *N*-nitrosocarbamates and *N*-nitrosoguanidines.

Nitrite is added to certain foods, especially meat products, to inhibit the growth of *Clostridium botulinum*, a bacteria which can produce one of the most toxic substances known; a very small amount of the toxin can cause life-threatening neurological symptoms. When bacon or smoked belly of pork is fried, NPRO is produced through nitrosation of the amino acid proline. NPRO is decarboxylated to NPYR, which is carcinogenic. High temperature and long frying time increase the amounts of NPYR formed. In addition, the formation of other volatile nitrosamines increases during frying of cured meat products. While uncooked cured meats may contain between not detectable to 25 ppb NPYR, fried bacon might contain up to 200 ppb of NPYR [8]. Equal parts of volatile *N*-nitroso compounds are found in the bacon and in the dripping after frying (Table 4). Moreover, according to some reports, as much as 90% of the volatile nitrosamines produced during cooking is vaporised [10,78–81].

#### 4.1. Genotoxicity and metabolism

Nitrosamines require metabolic activation to be mutagenic/carcinogenic, whereas nitrosamides are active

Table 4  
Volatile *N*-nitrosamine in fried bacon, smoked pork and corresponding cooked-out fat (from [80])

Sample	No. of samples	NDMA	NPIP	NPYR	Total
Unfried bacon	29	0.5	tr	tr	0.5
Fried Bacon	15	0.9	nd	10.9	11.8
Cooked-out fat	15	1.2	nd	7.0	8.2
Unfried smoked pork	18	0.9	tr	tr	1.0
Fried Smoked pork	5	0.9	tr	2.8	3.7
Cooked-out fat	5	1.7	0.1	2.7	4.5

Pan-frying at  $175 \pm 5$  °C, frying time 3 min/side. Mean levels of nitrosamines ( $\mu\text{g}/\text{kg}$ ) [80]. NDMA = *N*-nitrosodimethylamine; NPIP = *N*-nitrosodipropylamine; NPYR = *N*-nitrosopyrrolidine.

without metabolism. The hydroxylation is catalysed mainly by CYP2E1 [81,82], but other cytochrome P450 isoforms including CYP2A6 have been implicated [83,84]. The liver is the main site of metabolic activation of nitrosamines, but other human tissues can also metabolise nitrosamines, at least the simple symmetrical dialkyl nitrosamines. Interestingly, large quantitative differences in metabolic rate (up to 150-fold) have been found to occur between individuals (for a review see [85]). *N*-nitrosodimethylamine undergoes enzymatic hydroxylation and subsequent hydrolysis to an aldehyde and a monoalkyl nitrosamine that rearranges and releases a carbocation that is reactive toward DNA bases [86,87]. The *O*<sup>6</sup>-methylguanine is mostly responsible for the mutagenicity and carcinogenicity of alkylating agents [88,89]. The *O*<sup>6</sup>-methylguanine leads to GC–AT transitions in cell culture [90] and in animal models if not repaired by the *O*<sup>6</sup>-methylguanine methyltransferase [91,92]. Although the *O*<sup>6</sup>-methylguanine is a promutagenic lesion, it is technically easier to measure the 7-methylguanine, which is not promutagenic, as surrogate marker for exposure and genetic susceptibility, because the 7-methylguanine occurs at levels ca. 10-fold higher. Human studies of *N*-nitrosamine adducts in different tissues and the use of susceptibility markers should help elucidate the risk of *N*-nitrosamine exposures. Of the volatile nitrosamines most commonly found in food, NDEA appears to be the most potent carcinogen, whereas NDMA has somewhat lower potency, and the heterocyclic NPYR and NPIP even lower.

## 4.2. Cancer

There is overwhelming evidence that some *N*-nitroso compounds are carcinogenic in most animals [8,80,93,94]. Experimental animal models strongly support the carcinogenic properties of dietary *N*-nitrosamines [76]. In fact, there is a large concordance between animal species and strains albeit with different organ specificity, type of compound and dose [95]. Cancer of the lung, liver, kidney, mammary gland, stomach, pancreas, bladder or esophagus has been observed [96].

These sites also are considered to be the target organs in humans. Dietary *N*-nitrosamines have been linked to esophageal and other gastrointestinal cancers [76,97]; for example, *N*-nitrosamines are considered an important carcinogen in parts of China and Japan. In Chinese studies, several sources of evidence suggest a correlation between either dietary nitrosamines or endogenous nitrosation of dietary amines, and increased incidence of esophageal cancer. In addition, several studies have shown an association between intake of salted/preserved fish and the induction of several cancer forms: gastric cancer in Japan and Norway, cancer of the nasal cavity and oesophagus in China, and colorectal cancer in Finland. Biomarker studies show *N*-nitrosamine adducts are higher in the popu-

lations in these parts of the world compared to low-*N*-nitrosamine areas [98,99]. Although tobacco smoke and tobacco-specific nitrosamines cause lung cancer [93,100], dietary *N*-nitrosamines might also contribute to lung cancer [101–103].

## 4.3. Exposure

For most Western countries, the average exposure to carcinogenic volatile *N*-nitroso compounds from the diet is generally of the order of 0.3–1.0 µg per person per day, with cured meats (cooked and uncooked) and beer as major sources. Table 5 displays daily intakes of NDMA according to dietary surveys published 1978–1991 [12]. Corresponding figures for non-volatile *N*-nitrosoamines are estimated to be 10–100 µg per person per day. In Asia, the dietary exposure to volatile nitrosamines is much higher due to the intake of fish products derived from dried and nitrite-salted fish. Cigarette smoke is another significant source of *N*-nitrosated compounds. Estimates show that an average smoker may be exposed to more than 15 µg volatile *N*-nitroso compounds per day [104]. Furthermore, the formation of *N*-nitroso compounds inside the human body is another source of great concern. Formation of *N*-nitroso compound in the body is based on reac-

Table 5  
Daily intake of nitrosodimethylamine (NDMA) in different countries (from [12])

Country	NDMA intake (µg/day)	Major NDMA source
UK <sup>a</sup>	0.53	Cured meats (81%)
UK	0.6	Beer, cured meats
The Netherlands	0.38	Beer (71%)
The Netherlands <sup>b</sup>	0.10	Not evaluated
FRG (1979/1980)	1.10 (men)	Beer (65%)
FRG (1979/1980)	0.57 (women)	Cured meats (10%)
FRG (1981)	0.53 (men)	Beer (40%)
FRG (1981)	0.35 (women)	Cured meats (18%)
FRG (1989/1990)	0.28 (men)	Beer (31%)
FRG (1989/1990)	0.17 (women)	Cured meats (36%)
Japan	1.8	Dried fish (91%)
Japan	0.5	Fish products (68%), beer (30%)
Sweden	0.12	Meat products (61%), beer (32%)
Finland <sup>c</sup>	0.08	Smoked fish (75%)
China	No data	Marine foods
Italy	No data	Cured meats
USSR	No data	Meat and fish products

<sup>a</sup> Beer not included in the survey.

<sup>b</sup> Determined by 24 h duplicate diet analysis.

<sup>c</sup> Based on limited data.

tion between for example, nitrite and amines, amides or alkyl ureas. Since *N*-nitrosation is acid-catalysed, generally with a pH-optimum between 2 and 4 depending on the substrate, this means that conditions favouring nitrosation reactions exist in the human stomach. On the other hand, conversion of nitrate to nitrite is rather limited at low pH. In the normal acidic stomach, nitrite of dietary and salivary origin is utilised in the nitrosation reactions. Saliva is the major site of nitrite production in humans [76,85]. One study reported urinary excretion of several micrograms of nitrosoproline (non-carcinogenic) per day following the ingestion of extra proline together with the ordinary diet. In addition, some individuals may be exposed occupationally, for example, those working in leather tanneries and rubber and tyre industries.

## 5. Polycyclic aromatic hydrocarbons (PAHs)

Grilling (broiling) meat, fish or other foods with intense heat over a direct flame result in fat dripping on the hot fire and yielding flames containing a number of polycyclic hydrocarbons (PAHs). These chemicals adhere to the surface of the food. The more intense the heat, the more PAHs are present [105]. Fig. 1 shows structures of the most commonly detected PAHs in cooked foods, of which benzo[a]pyrene (B[a]P) is regarded as the most carcinogenic [2,9]. As seen, PAHs are composed mainly of compounds consisting of three or more fused benzene rings without any acyclic groups.

PAHs are produced from organic compounds by condensation of smaller units at high temperatures forming stable polynuclear aromatic compounds. The mechanism of formation of PAHs is not fully understood, but two principal pathways are considered to be involved, pyrolysis and pyrosynthesis. At high temperatures, organic compounds are easily fragmented into smaller compounds, mostly free radicals, which may then recombine to form a number of relatively stable PAHs. At temperatures below 400 °C, only small amounts of PAHs are formed. However, the amounts of PAHs increase linearly in the range 400–1000 °C [2,9].

Cooking methods involving grilling can produce marked differences in the levels of carcinogens. For example, fat dripping on hot surfaces can form PAHs, while oven-grilling prevents reflux of pyrolysed drip-

Table 6  
Levels of B[a]P in frankfurters grilled by different technique (from [111])

Grilling method	Number of samples	B[a]P (µg/kg), average	Range
Ungrilled	2	0.2	0.1–0.3
Frying pan	5	0.1	nd–0.2
Electric oven	2	0.2	0.1–0.3
Charcoal fire	13	0.3	nd–1.0
Cone fire	7	18	2–31
Log fire	17	54	6–212
Log fire embers	9	8	<1–25

pings and results in much lower levels of PAHs in the cooked food. Precoating with sauces can often result in burned meat surface.

PAHs are present in grilled meat or fish in very variable amounts (0–130 ng/g). The content of the B(a)P in these foods ranges from 0.2 to 50 ng/g [2]. Grilled meat in general is estimated to contain around 10.5 ng/g B(a)P [106]. Table 6 shows PAHs content in foods.

### 5.1. Genotoxicity and metabolism

B(a)P is the best-characterised PAH compound in the diet. Metabolic activation by CYP1A and CYP1B result in formation of epoxides are required for adduct formation. The bay-region diol epoxide binds covalently to DNA mostly as the N<sup>2</sup>- deoxyguanose adduct [107]. B(a)P adducts can be quantified by several sensitive methods. Other methods exist for detecting PAH-metabolites, e.g. urinary B(a)P-tetrol and 3-hydroxy-B(a)P. Data on B(a)P adducts reflect the biologically effective dose and suggest a link to cancer risk in the lung. The adducts are also associated with site-specific hotspot mutations in the p53 tumour-suppressor gene and mutations observed in lung cancer of smokers. Similar evidence for dietary PAH-associated cancer should be sought, for example, in gastrointestinal cancers (for reference, see [108]).

### 5.2. Cancer

Oral administration of PAHs in an oily base has been shown to induce squamous carcinoma of the stomach in mice and to a lesser extent in rats, cancer of the mammary gland in sensitive strains of rats, and lymphomas or leukaemias in certain strains of mice and rats. Also

lung cancer can be induced. Eleven PAH compounds have been classified as carcinogenic to laboratory animals [2,3]. However, The Committee on Diet, Nutrition and Cancer, appointed by the National Research Council of the USA, stated in their report that of the PAHs occurring in the average American diet only three, namely B[a]P, benz[a]anthracene, and dibenz[a,h]anthracene, have been found to be carcinogenic in animals following oral administration.

These three food PAHs have been considered *probably* (Group 2A) carcinogenic to humans on the basis of data obtained from animal experiments and in vitro test systems. In addition, experimental data indicate that other such compounds may *possibly* (Group 2B) be carcinogenic to humans [1,2]. The effects of human PAH exposure are measured mostly in relationship to tobacco smoking and the work place, and data indicate that the target organs for PAH compounds are the lung, oropharynx, breast and genitourinary and gastrointestinal tracts. However, dietary PAH exposure and cancer risk has received little attention. A fairly consistent association between grilled (broiled), but not fried, fish and meat and stomach cancer suggests that dietary exposure to PAHs may be involved in human gastric carcinogenesis. In humans there is also some evidence for association of dietary PAH exposure with colon cancer.

There is however no conclusive evidence concerning a possible relationship between ingestion of PAH-contaminated food and human cancer. A few studies (for reference, see [109]) have suggested a link between an increased incidence of stomach cancer and frequent consumption of smoked food. However, as smoked food may contain other potentially carcinogenic substances, such as nitrite and nitrosamines, the observed effects cannot definitively be related to PAHs.

Animal and human studies suggest that dietary PAH is distributed to other organs besides the locally exposed tissues, so it is plausible to consider that dietary PAH might contribute to lung or breast cancer risk, for example. Although the dietary contribution of PAH to the total body burden may be sizeable, the ubiquitous presence of PAH in the environment and the presence of other carcinogens in the same foods makes the interpretation of epidemiological studies of cancer risk due to dietary PAH difficult. It may be possible to distinguish PAH exposure in diet from smoking by measuring biomarkers specific to each, e.g. simultaneous measurement of 1-hydroxypyrene to evaluate total PAH intake and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol to evaluate the contribution due to smoking. In addition, dietary PAH is invariably a complex mixture of compounds with hard-to-predict metabolic and carcinogenic consequences. Corroborated

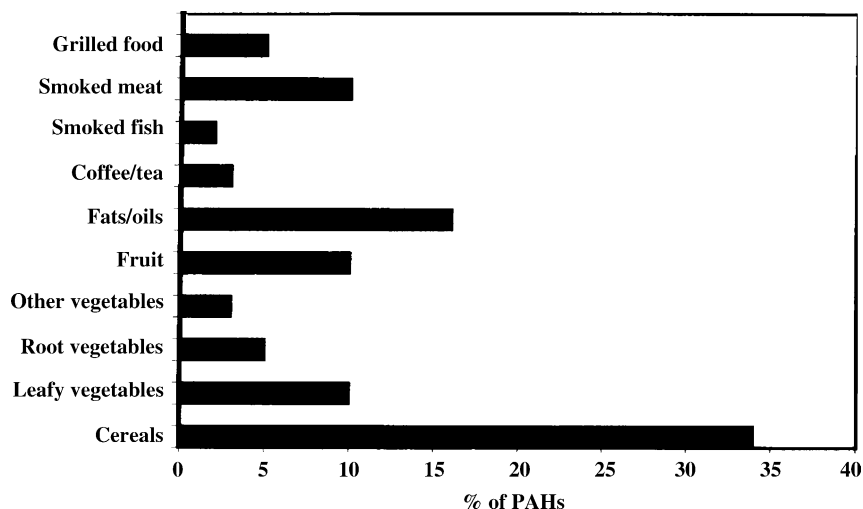


Fig. 2. Estimated contribution (in %) of various food groups in an average Swedish diet to the total dietary intake of nine PAHs (fluoranthene, pyrene, benz[a]anthracene, chrysene/triphenylene, benzo[b]fluoranthene, benzo[j]fluoranthene/benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene) (Larsson [105]), permission to be applied for).

tive data that indicate that PAH exposure from diet is important are the findings that the intake of charcoal-broiled meat is more correlated to blood PAH DNA adducts than smoking [108].

### 5.3. Exposure

The dietary intake of PAHs in Europe, USA and Japan has been estimated to be around 1 mg per person per year. Most data are for B[a]P, with values in the range 0.01–0.61 mg per person per year. Thus, a daily average intake of 2–3 µg PAHs per person seems reasonable, of which B[a]P constitutes 0.5–5%. The average daily intake of PAHs compares to an exposure of 2–5 µg PAHs per pack of cigarettes in a regular smoker. Fig. 2 illustrates the estimated contribution of various food groups in an average Swedish diet to the intake of nine commonly reported PAHs in food. The major part of the PAHs intake is due to contamination through air pollution and only about 25–30% of the PAHs originate from cooking, mainly grilled or smoked foods (for reference, see [110]).

## 6. Minimising strategies

Following a number of evaluations, the IARC has come to the conclusion that several of these food-borne toxicants present in cooked foods are possibly (2A) or probably (2B) carcinogenic to humans, based on both high-dose, long-term animal studies and in vitro and in vivo genotoxicity tests. Generally, animal experiments concerning genotoxic effects are conducted in high doses on rodents, which are exposed during their whole life span, 1–3 years. The test-doses used in animal experiments are based on synthetic compounds of authentic single food processing toxicants amounting to 0.1–2% of the feed. These amounts exceed the daily human intakes by a factor of 1000–10 000. Usually humans are exposed to food processing toxicants in µg-levels on daily basis during a lifespan of around 70–80 years. Based on studies of biomarkers or surrogate markers, e.g. adducts, specific mutations and urinary excretion, a growing number of human studies indicate that food processing toxicants are bioavailable in a dose-dependent way [40–48]. Yet, there is insufficient scientific evidence that these toxicants really cause human cancer, and no limits have been set for

their presence in cooked foods. However, the competent authorities in most Western countries recommend minimising their occurrence for safety reasons.

Friedman [7] suggests several actions to reduce acrylamide content of food in his comprehensive review, e.g. better understanding of kinetics and cooking/processing conditions that reduce the acrylamide formation. Moreover different ways to reduce the presence of the acrylamide precursor asparagin and sugars in food by careful selecting of raw materials and studies of breeding and/or suppressing genes that encode enzymes which govern the biosynthesis of asparagine in plant foods. Use of chemical and biochemical tools, e.g. changes in pH, use of hydrolysing enzymes from yeast or lactic acid producing bacteria during bread making, addition of inhibitors and modifiers, and changes in recipe of foods are other options.

Recently, Persson et al. investigated ways of modifying cooking practise to reduce the formation of HCAs [112,113]. Various factors that influence the formation of HCAs during cooking of chicken breasts and beef burgers were investigated. The factors studied included cooking method, time, temperature, heat transfer, weight loss during cooking, the chemical composition of beef burgers and antioxidants. The conditions for the cooking experiments were similar to those used in normal domestic restaurants and industrial cooking. The analysis of HCAs was focused mainly on MeIQx and PhIP and a few more. The HCA concentrations ranged from undetectable amounts to 30 ng/g cooked meat. Cooking temperature was shown to be an important factor, especially during frying, since large concentration of HCAs can be formed at high temperatures. Avoiding over-cooking of meat seems thus to be an important way to decrease the content of HCAs. Reducing the weight loss during cooking may be means of decreasing the formation of HCAs, and this correlation is probably due to the transport of water-soluble precursors to the meat surface. This transport can be lowered by the addition of water-holding ingredients, such as common salt or potato starch. Addition of carbohydrates may also affect the formation of HCAs chemically. Frying in oil containing antioxidants may also provide a way of reducing the amount of HCAs formed, although the amounts of antioxidants and the effect of storage must be taken into consideration. A kinetic model was used to describe the formation of HCAs and this can be further developed to predict the formation of

HCAs [27]. The results from this research can be used to design cooking equipment and processes and to form a basis for guidelines to consumers, restaurants and industry, on how to obtain a product with good sensoric properties and minimised contents of HCAs.

The natural variation of the concentrations of HCA precursors in pig meat has been investigated [114]. While production system, sex and feeding regime showed little effect on precursor concentrations in pig meats, a common genotype (RN<sup>-</sup>-allele carriers) was characterised by resulting in high glycogen concentrations. The high glycogen content in carriers of the RN allele led to a browner crust and considerably lower levels of HCAs in fried meat compared to that of normal genotype [115].

Except minimising the intake of HCAs there are also an emerging interest among many scientists to investigate the numerous environmental chemicals found in food or the atmosphere which can impact the exposure, metabolism and cell proliferation response of heterocyclic amines, e.g. it may be wise to include food items rich in compounds protecting bioactivation of HCAs, e.g. soy-isoflavones [15].

During recent years, the levels of *N*-nitroso compounds in the human diet have decreased significantly. This is due to reductions in the levels of nitrates and nitrites added to foods, and the simultaneous addition of ascorbic acid, which inhibits the formation of *N*-nitroso compounds in cured meat products. Recommendations have been made to fry bacon with caution, not to use lids and not to consume the frying fat rendered out of the bacon, i.e. the pan residue. Soaking bacon in water before frying, as well as microwave cooking instead of using a griddle or a frying pan, significantly lowers the NPYR levels [79].

For any cooking involving wood fires, the type of wood used can also be important. For example, hardwood such as oak and hickory, burn cleanly, whereas some woods such as mesquite, but also cones generate copious quantities of PAHs. Ways of minimising exposure to PAHs from cooked foods include preventing the smoke from the combustion of wood or fossil fuels to come into direct contact with the food, when barbecuing or smoking foods, unless the smoke is purified with regard to PAHs. Cooking over a log fire in direct contact with the flames should be avoided. It is important to wait until the log fire has turned into embers. In addition, the use of special grills, designed to prevent

melted fat from dripping onto the heat source, reduces the PAHs contamination significantly. In some countries, legislative limits have been set for PAHs in certain foods, with the aim of minimising the intake of PAHs. In Germany, Poland and Austria a limit of 1 µg/kg is currently imposed for B[a]P in smoked meat products.

## 7. Conclusions

Several lines of evidence indicate that cooking conditions and dietary habits can contribute to human cancer risk through the ingestion of food mutagens. This is of concern for the general public and also for the food industry and national authorities. Food mutagens cause different types of DNA damage: nucleotide alterations and gross chromosomal aberrations. Most mutagens begin their action at the DNA level by forming carcinogen–DNA adducts, which result from the covalent binding of a carcinogen or part of a carcinogen to a nucleotide. However, the effect of food mutagens in carcinogenesis can be modified by heritable traits, namely, low-penetrant genes that affect mutagen exposure of DNA through metabolic activation and detoxification or cellular responses to DNA damage through DNA repair mechanisms or cell death [108].

This article has focused on the most commonly studied food toxicants present in cooked foods. The fact is however, that new process-induced genotoxic compounds are continuously reported. One large group of cooked food mutagens not reviewed in the present paper includes oxidised sterols arising from oxidation of cholesterol and phytosterols during processing and storage of foods. For cholesterol and phytosterols more than 100 different oxidation products are reported among which different epoxides are frequently seen. While cholesterol oxides have been found to be mutagenic, cytotoxic or atherogenic, the oxidation products from phytosterols have been studied to a very little extent. Yet, preliminary reports show many different foods, in particular French fries, to contain as high concentrations of oxidised sterols as acrylamides, for reference, see [116].

Traditionally, scientists are specialised on only one aspect of one group of food mutagens at a time. There is now an urgent need of a new approach to investigate the impact of known food mutagens using more integrated and sophisticated animal and human models based on

more realistic exposure levels. The rapid development of more specific biomarkers or surrogate biomarkers is encouraging and promising for future risk assessment studies and should enable researchers from different disciplines to work together.

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