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Carcinogens formed when Meat is Cooked

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1. Introduction

Diet has been associated with varying cancer rates in human populations for many years, yet the causes of the observed variation in cancer patterns have not been adequately explained (Wynder et al. 1977). Along with the effect of diet on human cancer incidence is the strong evidence that mutations are the initiating events in the cancer process (Vogelstein et al. 1992). Foods, when heated, are a good source of genotoxic carcinogens that very likely are a cause for some of these events(Doll et al. 1981). These carcinogens fall into two chemical classes: heterocyclic aromatic amines (HAA) and polycyclic aromatic hydrocarbons (PAH). There is ample evidence that many of these compounds are complete carcinogens in rodents(El-Bayoumy et al. 1995; Ohgaki et al. 1991). Heterocyclic aromatic amines are among the most potent mutagenic substances ever tested in the Ames/*Salmonella* mutagenicity test (Wakabayashi et al. 1992). Both classes of carcinogen cause tumors in rodents at multiple sites, (El-Bayoumy et al. 1995; Ohgaki et al. 1991) many of which are common tumor sites in people on a Western diet. An HAA, PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), and a PAH, B[a]P (benzo[*a*]pyrene), of comparable carcinogenic potency caused mammary gland tumors in a feeding study in female rats (El-Bayoumy et al. 1995). In addition, PhIP has recently been shown to cause carcinomas in the prostate of the male rat (Shirai et al. 1997). Complementing the rodent cancer studies are numerous human case-control and prospective studies suggesting a relationship between overheated beef, chicken, and lamb, and cancer of the colon, breast, prostate, and stomach (Sinha et al. 1999; Ward et al. 1997; Zheng et al. 1998).

Thus, it is important to estimate human exposure to the HAA and PAH food carcinogens by accurate dietary intake data to determine the amounts and types of carcinogens to which humans are exposed.

2. Formation

The cooking process is responsible for the formation of HAA and PAH from natural constituents in foods, with cooking time and temperature being important determinants in both the qualitative and the quantitative formation of these compounds (Knize et al. 1985; Skog et al. 1995). Higher temperatures and longer cooking times favor the formation of HAA. A number of studies have shown the precursors for the formation of the HAA to be amino acids, such as phenylalanine, threonine, and alanine; creatine or creatinine; and sugars (Skog et al. 1993). HAA are frequently formed in muscle meats during frying, broiling, and grilling. But methods using lower temperatures such as stewing, boiling, and baking usually do not form HAA.

PAH are products of combustion and pyrolysis of protein, carbohydrate or lipids by condensation of smaller units at high temperatures to form stable polynuclear aromatic compounds (Lijinsky 1991). PAH levels in foods are strongly dependent on the method of cooking, including the distance of food from the heat source, design of cooking device and fat content of the foodstuff (Lijinsky 1991). Smoke deposited on the surface of charcoal-grilled meats appears to be the major source of PAH carcinogens in food.

The HAA and PAH carcinogens formed during cooking have stable multi-ring aromatic structures. The heterocyclic amines have an exocyclic amino group and several nitrogen heteroatoms. Structures of those compounds commonly detected in foods are shown in Figure 1. Additional heterocyclic aromatic amines have been found in foods and the whole

set of HAA compounds has been reviewed (Robbana-Baranat et al. 1996). Over 25 PAH have been identified in curing smoke and approximately 40 others have been identified but not characterized in this type of smoke. An extensive database of PAH in foods was recently published (Kazerouni et al. 2001). The variables influencing the formation of PAH and HAA create a wide range of concentrations in food, requiring the analysis of a large number of food samples cooked under various conditions to determine the sources and amounts of carcinogens in the human diet.

3. Analysis

There are several factors that make the analysis of carcinogens from foods a difficult problem. PAH and HAA are present in foods at low nanogram per gram levels. The low levels require that chromatographic efficiency and detector sensitivity be optimized, and these are typically analyzed by gas or liquid chromatography with detection by light absorbance, fluorescence or mass spectrometry. Several of the compounds are formed under the same reaction conditions, so the number of compounds to be quantified requires that the extraction, chromatographic separation, and detection be general enough to detect several of the carcinogens in each separation. A single solid-phase extraction scheme was used to isolate the PAH and HAA classes of genotoxic compounds from charcoal-grilled meat (Rivera et al. 1996), and was developed from an innovative solid-phase extraction method for HAA (Gross et al. 1992).

4. Results

Comparison of the formation of PAH and HAA shows that open flames are required to make PAH, but high temperature by a variety of heat sources can form HAA (Table 1). The mass amounts of PAH and HAA are within the same order of magnitude for high

temperature propane-grilling of ground beef with 30% fat by weight. Further work is needed to analyze other food types for both classes of carcinogen.

5. Reducing the formation of food carcinogens

Many studies have shown the effect of cooking time and temperature on the formation of mutagenic activity (Commoner et al. 1978; Sugimura et al. 1977) and specific HAA in various meats (Gross et al. 1992; Knize et al. 1985; Skog et al. 1995). Food doneness is difficult to quantify. In our experience, measuring temperatures with thermocouples is too dependent on probe placement. Surface appearance, too, is not a good doneness indicator because color can be affected by other variables such as pH differences in meat. Reducing the cooking temperature seems to be the most practical way to reduce HAA content, but avoiding the conditions where the temperatures are below those needed to kill harmful bacteria is also important. The formation of PAH can be reduced by not exposing the food directly to the heat source and resulting smoke when grilling foods (Larsson et al. 1983).

Figure 2 shows two methods that reduce the formation of HAA during cooking of beef patties to an internal temperature of 70°C. The pan temperature was varied and the sum of the detectable HAA measured. When meat patties were turned over just once at 5 min (open bars), and cooked until the internal temperature of 70°C was reached, there was a great effect of pan temperature on the formation of the HAA. If the meat patties were turned every minute during cooking, much less of the HAA was detected. So cooking at a lower surface temperature (160-180°C) and turning the meat over every minute greatly reduces the formation of HAA when frying ground beef.(Salmon et al. 2000). In the same study it was shown that the time needed to produce a beef hamburger that is safely heated

to 70°C is only slightly decreased by increasing the pan temperature from 160 to 250°C (Figure 3). Figure 3 also shows that if meat is turned over every minute, cooking times are slightly reduced compared to flipping the meat over just once during the entire cooking time. Thus, a high pan-temperature accelerates the cooking process only a little, but turning every minute both accelerates the cooking and reduces the HAA formation. Another means of decreasing HAA formation is to remove the precursors from the meat before cooking (Felton et al. 1994). Figure 4, upper, illustrates that ground beef patties contain small molecule precursors of HAA: amino acids, sugars, and creatinine. When fried at high temperatures, these precursors form the HAA. Alternatively, a microwave oven pretreatment of 1.5 to 2 min reduces the precursors, which can be discarded as meat drippings, so meat then cooked at high temperatures results in lower HAA exposure to the consumer.

6. Risk assessment of meat carcinogens

The analysis of foods for HAA and PAH is important because there is widespread human exposure to these compounds, there is suggestive epidemiology for cause and effect, and these chemicals are potent mutagens and animal carcinogens as stated in the introduction. Exposures differ among individuals, since dietary preferences and methods of food preparation can vary greatly. This area of research provides a unique opportunity in cancer etiology, the chance to evaluate carcinogens in human populations. The variability in the formation of these compounds also provides the opportunity for intervention, to reduce exposure if it is warranted from risk evaluation of humans and rodents exposed to these compounds in their diet.

Which class of carcinogenic compounds and which specific compounds within each class are the most important in human health are difficult questions to answer at this time. All of the HAA and PAH tested are rodent carcinogens, so it could be argued that the total mass of carcinogen is the most important risk factor.

The compounds do differ in tumor-site specificity in rats, with most HAA causing tumors at sites commonly seen for humans. The metabolism of each class of chemical and the enzymes involved in their metabolism are known to be polymorphic, suggesting a distribution in risk across the population, varying from individual to individual.

For PAH carcinogens, occupational studies have been used to establish risk of human exposure, with only suggestions of excessive risk at some sites (Nadon et al. 1995). The health risk to the human population consuming HAA has been recently discussed (Layton et al. 1995; Zimmerli et al. 2001), and supports the linkage between HAA consumption and higher cancer risk.

Acknowledgments

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References

Figure legends

Figure 1. Chemical structures and abbreviated names of HAA and PAH carcinogens. B[*a*]A= benzo[*a*]anthracene, B[*a*]P=benzo[*a*]pyrene, B[*b*]F=benzo[*b*]fluoranthene, B[*k*]F=benzo[*k*]fluoranthene, DBA=dibenzo[*a,h*]anthracene, DiMeIQx=2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline, Indenopyrene= indeno[1,2,3-*c,d*]pyrene, MeIQx=2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, PhIP=2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine.

Figure 2. Sum of HAA formed in beef patties fried to an internal temperature of 70°C, either turned over once at 5 min (open bars) or turned over every minute (filled bars) until done. Four different pan temperatures were used. Error bars are the standard deviation of 5 replicate samples.

Figure 3. Cooking time to reach an internal temperature of 70°C for beef patties fried at a pan temperature of 160, 180, 200 or 250°C, and either turned over once during frying (open bars) or turned over every minute (filled bars). Frying time varied little despite the greatly different pan temperature. Turning every minute decreased the cooking time needed.

Figure 4. Schematic depiction of formation of HAA carcinogens after frying (right side) or reduction of HAA carcinogens after microwave pretreatment of ground beef patty before frying (left side). Microwave pretreatment reduces precursors resulting in lower exposure to consumers.

Table 1. Carcinogenic HAA and PAH in hamburgers, ng/g

Sample	MeIQx	PhIP	B[*a*]P	B[*b*]F	B[*k*]F	B[*a*]A	DBA	Indenopyrene
Propane grilled	2.2	15	6.2	18	2.0	5.2	0.5	5.1
Charcoal grilled	nd	nd	0.6	4.1	nd*	3.1	nd	nd
Pan fried	3.8	16	nd	nd	nd	nd	nd	nd

nd=Not detected.

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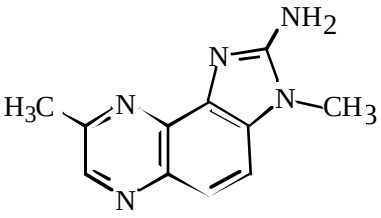
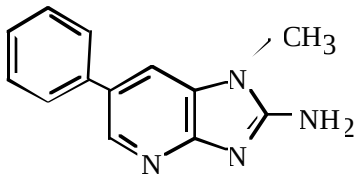
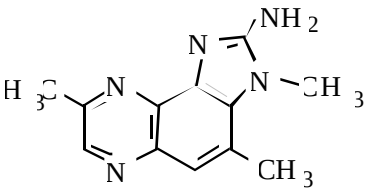


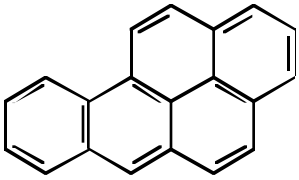
MeIQx



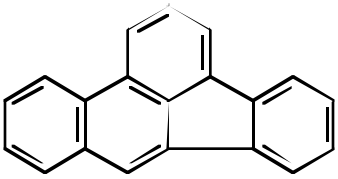
PhIP



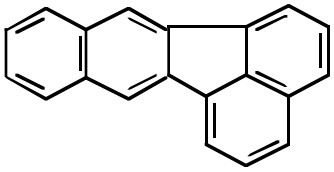
DiMeIQx



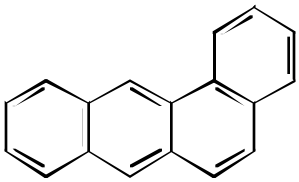
B[*a*]P



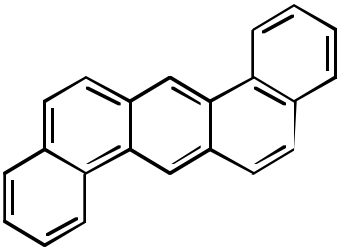
B[*b*]F



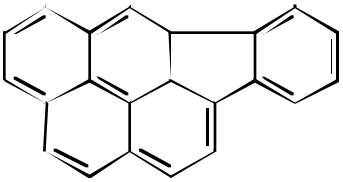
B[*k*]F



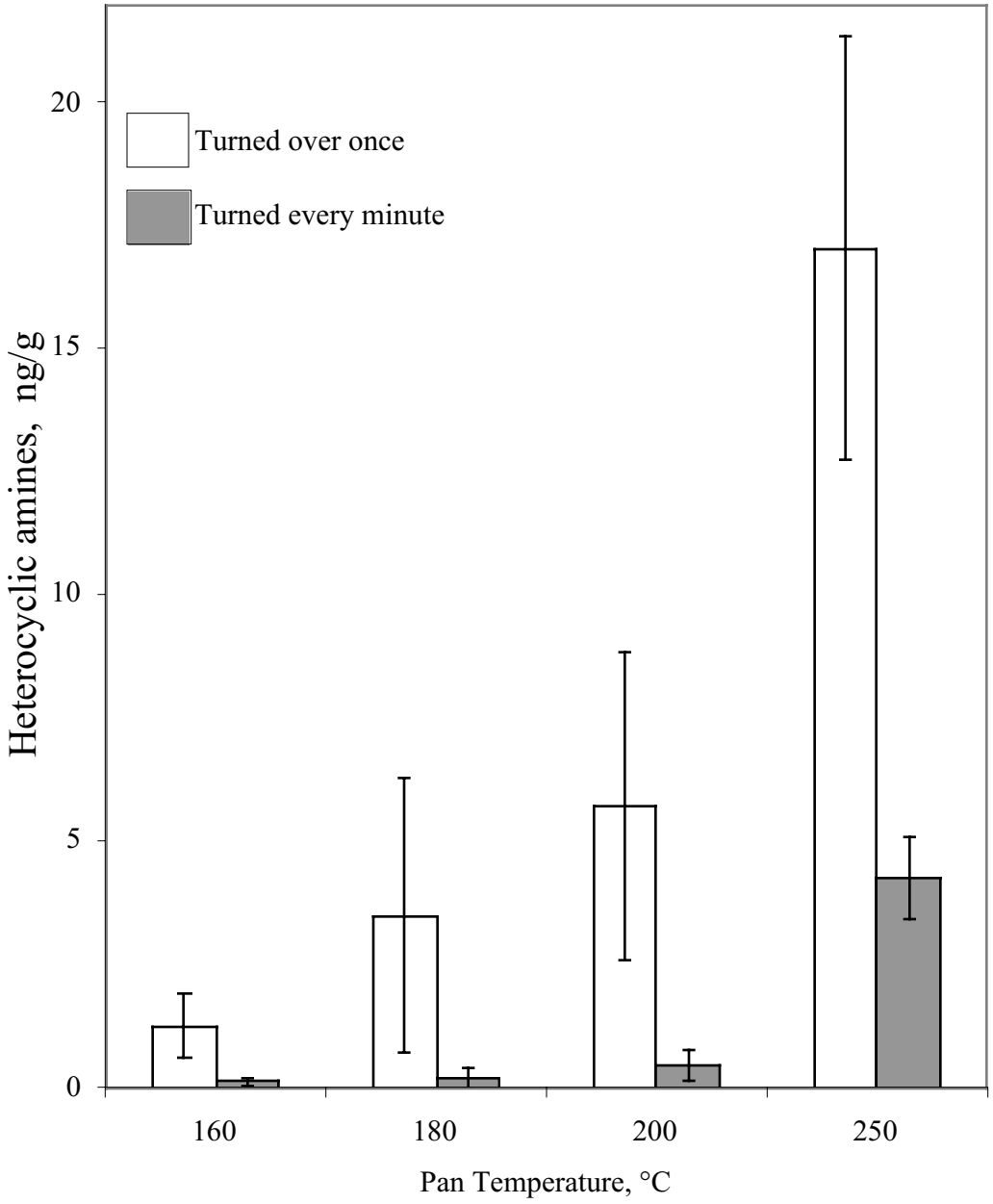
B[*a*]A

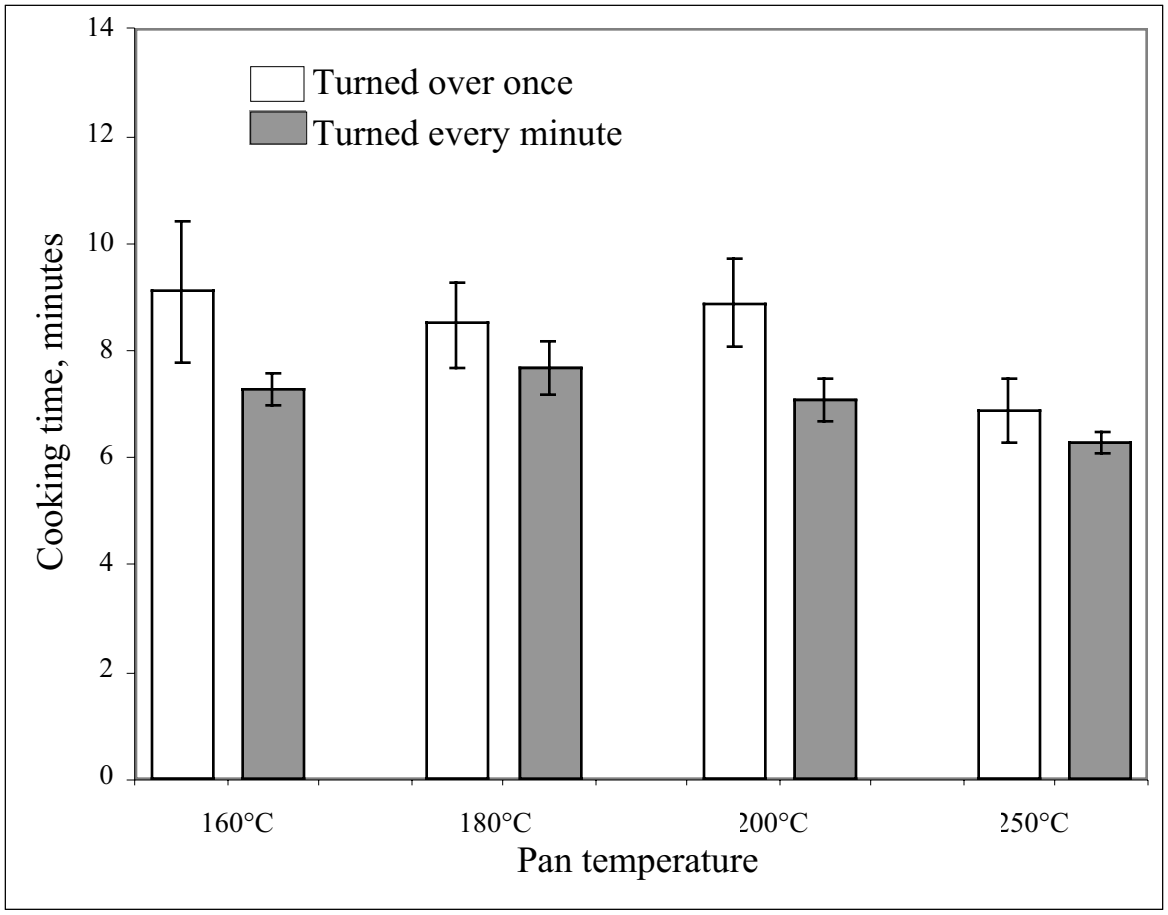


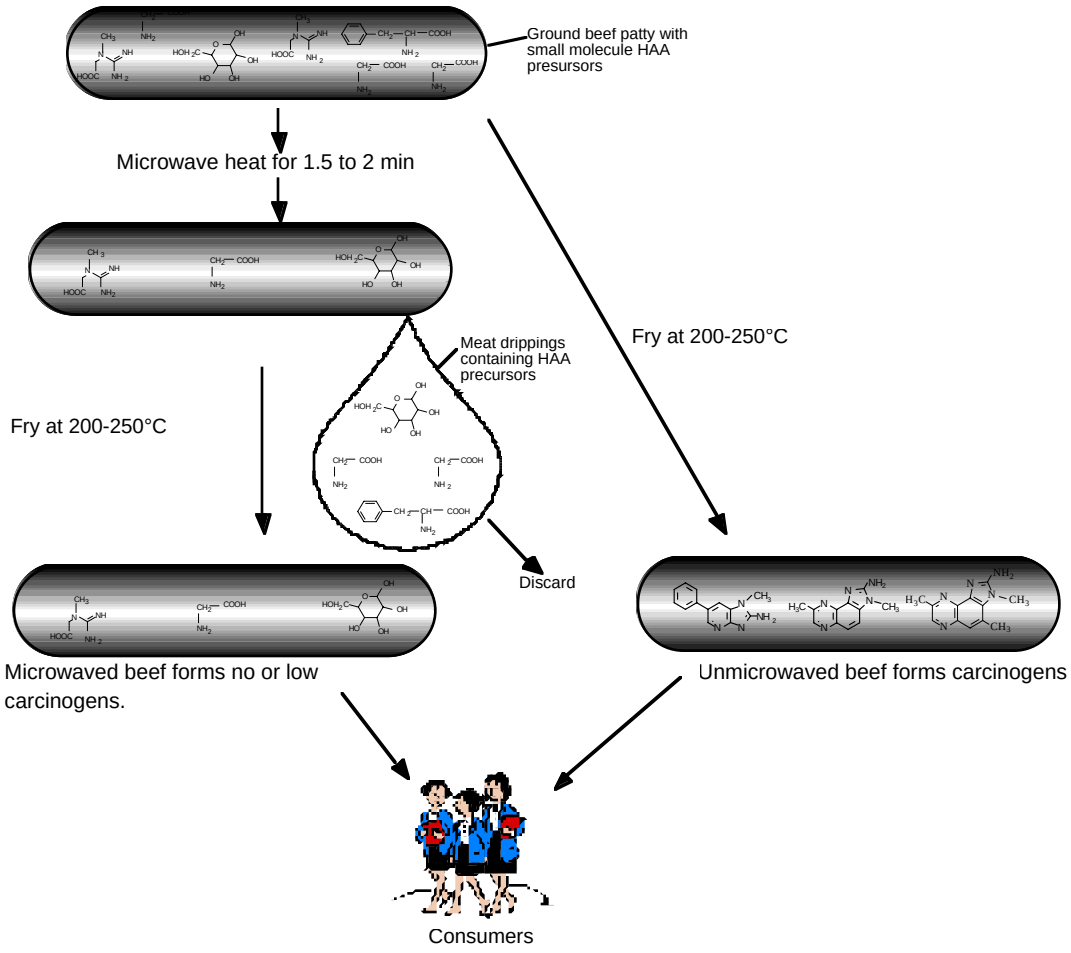
DBA



Indenopyrene


Turned over once
Turned every minute
Heterocyclic amines, ng/g
0
5
10
15
20
160
180
200
250
Pan Temperature, °C


Turned over once
Turned every minute
Cooking time, minutes
14
12
10
8
6
4
2
0
160°C
180°C
200°C
250°C
Pan temperature


Ground beef patty with small molecule HAA presursors
Microwave heat for 1.5 to 2 min
Fry at 200-250°C
Meat drippings containing HAA precursors
Fry at 200-250°C
Discard
Microwaved beef forms no or low carcinogens.
Unmicrowaved beef forms carcinogens
Consumers