

## High Concentrations of the Carcinogen 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) Occur in Chicken but Are Dependent on the Cooking Method<sup>1</sup>

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### Abstract

Heterocyclic aromatic amines (HAAs) are mutagenic and carcinogenic compounds found in meats cooked at high temperatures. Although chicken is consumed in large quantities in the United States, there is little information on its HAA content. The objective of this study was to measure the five predominant HAAs (IQ, MeIQ, MeIQx, DiMeIQx, and PhIP) in chicken cooked by various methods to different degrees of doneness. Chicken breasts were panfried, oven-broiled, or grilled/barbecued. Whole chickens were roasted or stewed. Skinless, boneless chicken breasts were cooked to three degrees of doneness: just until done, well done, or very well done. High levels of PhIP (ranging from 12 to 480 ng/g cooked meat) were found in chicken breasts when panfried, oven-broiled, and grilled/barbecued but not in whole roasted or stewed chicken. PhIP concentration increased in skinless, boneless chicken breast with longer cooking time, higher internal temperature, and greater degree of surface browning. PhIP concentration was also high in chicken breasts cooked with skin and bones. MeIQx and DiMeIQx levels increased with the degree of doneness, whereas IQ and MeIQ were not detectable in any of these chicken samples. Certain cooking methods produce PhIP, a known colon and breast carcinogen in rodents and possibly a human carcinogen, at substantially higher levels in chicken than has been reported previously in red meat.

### Introduction

HAAs,<sup>3</sup> a family of compounds known to be highly mutagenic *in vitro*, are formed in meats cooked at high temperatures (1–3). PhIP, one of the most abundant HAAs in cooked meat, produces colon and mammary gland tumors in rodents (4–6), especially in conjunction with diets high in fat (7, 8). The IARC concludes that “there is sufficient evidence in experimental animals for the carcinogenicity of PhIP” and that “PhIP is possibly carcinogenic to humans” (9). Furthermore, the results of several epidemiological studies suggest that consumption of well done or well-browned red meat, potential surro-

gates for HAAs, is associated with colorectal and breast cancers (10–13). To date, it has been widely assumed that red meat is the primary source of exposure to HAAs. In a preliminary study, however, Wakabayashi *et al.* (14) reported that broiled chicken contained twice the level of PhIP (38.1 ng/g cooked meat) when compared to broiled beef (15.7 ng/g cooked meat). Since over 20% of the meat eaten in the United States is poultry (15), we measured the levels of HAAs in chicken cooked by a variety of methods to different degrees of doneness.

### Materials and Methods

Three types of chicken were purchased from a local supermarket: skinless, boneless chicken breasts; breasts with skin and bones; and whole chicken. Chicken breasts were either panfried, oven-broiled, or grilled/barbecued. Whole chickens were roasted or stewed.

The chicken was cooked by nutritionists at the Human Nutrition Research Center, United States Department of Agriculture, Beltsville, MD. For panfried and oven-broiled chicken, one skinless, boneless breast was cooked in each of six sessions (Table 1). The number and cooking levels of skinless boneless chicken breast samples that were grilled/barbecued follows: three breasts were cooked in one session, just until done; three breasts were cooked in each of two sessions, well done; six breasts were cooked in one session, very well done. For chicken breasts with skin and bones, two pieces were cooked in each of three sessions. One whole chicken was either roasted or stewed in each of five sessions. The bones (from whole chicken and breasts with skin and bones) were removed manually and edible parts (flesh and skin) of the cooked chicken were minced finely in a Robot Coupe mixer (Jackson, MS) to form a composite sample for the specific chicken type, method of cooking, and degree of doneness.

The degree of doneness was intentionally varied. Skinless, boneless chicken breasts were cooked to three levels of doneness: just until done, well done, and very well done. Chicken breasts with bones and skin and whole chicken were cooked to only one degree of doneness, well done.

The degree of doneness for the chicken breasts was defined primarily by internal temperature, irrespective of cooking surface temperature, and secondarily by surface browning. Meat was also monitored subjectively for interior and juice color after it was cooked. The internal temperature recommended for cooked poultry by cookbooks and public health agencies is 80°C (16). This temperature is considered the minimum internal temperature necessary to reduce chances of consuming undercooked poultry and to reduce the potential for gastrointestinal infection by food-borne pathogens. In this study, 80°C was designated as just until done. For well done and very well done chicken, the internal temperature was higher. Internal temperature was taken using a tissue implantable thermocouple microprobe (Type 1T-18; Physitemp Instruments, Inc., Clifton, NJ) connected to a base thermocouple digital thermometer (Model # 08500–40; Cole-Parmer, Chicago, IL). The level of surface browning was judged to be one of the following categories: not browned, moderately browned, or well browned or charred. After the chicken samples were cooked, they were checked for color of the meat and the presence of clear juices with no trace of pink. Thus, the chicken pieces in the just until done category were

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<sup>3</sup> The abbreviations used are: HAAs, heterocyclic aromatic amines; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline; QC, quality control.

Table 1 Characteristics of chicken cooked by different methods to varying degrees of doneness

Chicken samples, cooking method, and degree of doneness	No. of samples (session <sup>a</sup> × no. of pieces)	Weight loss during cooking (%)	Surface temperature (°C)	Internal temperature (°C)	Total cooking time (min)	Surface browning/charring
<b>Chicken breast without skin and bone</b>						
<b>Pan-fry</b>						
Just until done	6 (6 × 1)	25	197	76	14	None
Well done	6 (6 × 1)	35	202	82	28	Moderate
Very well done	6 (6 × 1)	43	211	90	36	Dark brown
<b>Oven-Broil</b>						
Just until done	6 (6 × 1)	31	86	80	9	None
Well done	6 (6 × 1)	42	79	91	14	Moderate
Very well done	6 (6 × 1)	51	83	98	17	Dark brown
<b>Grill/Barbecue</b>						
Just until done	3 (1 × 3)	28	180	78 <sup>c</sup>	10	None
Well done	6 (2 × 3)	45	177	93	40 <sup>d</sup>	Moderate
Very well done	6 (1 × 6)	59	260	99	43	Charred
<b>Chicken breast with skin and bone<sup>b</sup> well done</b>						
Pan-fry	6 (3 × 2)	30	190	78	62	Moderate
Oven-broil	6 (3 × 2)	53	180	96	43	Moderate
Grill/Barbecue	6 (3 × 2)	42	191 (500 during flashing) <sup>e</sup>	83 <sup>c</sup>	63	Moderate
<b>Whole chicken</b>						
Roast	5 (5 × 1)		175	85		Moderate
Stew	5 (5 × 1)		100			

<sup>a</sup> A session was when the whole cooking procedure was started anew with clean pans, etc.

<sup>b</sup> Chicken with skin and bones were larger pieces; therefore, they were cooked for longer times to achieve the same level of doneness as skinless, boneless chicken.

<sup>c</sup> The internal temperature of grilled/barbecued samples was taken just after the chicken pieces were removed from the grill.

<sup>d</sup> Well done skinless and boneless chicken breasts were the first samples to be grilled/barbecued. With the lid of the grill open, the meat cooked slowly; the grill was, therefore, closed after cooking for 27 min, and samples were cooked for an additional 13 min. As such, there was minimal cooking during a large proportion of the 40-min cooking time. Using the cooking experience from the well done chicken, the just until done and very well done categories were cooked with the lid of the grill closed.

<sup>e</sup> There was flashing of flames due to dripping fat during the cooking of chicken breasts with skin and bones; therefore the lid was left open during the preparation of these samples. The temperature at the grilling surface was recorded throughout cooking as well as the maximum temperature reached when fat dripped on the hot briquettes with flashing of flames.

cooked until the internal temperature reached 78 to 81°C, there was little or no browning on the surface, with no traces of pink meat or juice. For well done and very well done categories, the internal temperature was at least 5°C higher for each subsequent doneness category. The surface appearance for well done chicken breast was moderately browned *versus* well browned or charred for the very well done category.

We used the most common cooking methods used in the United States: pan-fry, oven-broil, grill/barbecue, roast, or stew [we have reported deep fat-fried chicken elsewhere (17)]. Pan-fried chicken breasts (with or without skin and bones) were cooked in a Teflon-coated frying pan with 2 tablespoons of oil. The temperature on the pan surface was monitored with a surface thermometer (PTC; Pacific Transducer Corp., Los Angeles, CA). Oven-broiled chicken breasts (with or without skin and bones) were cooked in a commercial gas range broiler with the meat placed 5 inches away from the heat source. The surface temperature was monitored with a thermocouple probe near the surface of the broiling pan. Grilled/barbecued chicken breasts (with or without skin and bones) were prepared on a home propane gas barbecue unit with ceramic briquettes (Sunbeam Model 44M39, 27 1/2" × 15", 44,000 BTU). The surface temperature was recorded with a surface thermometer on the grill surface. Whole chicken was roasted in a gas oven heated to 175°C and cooked until the internal temperature reached 85°C in the thickest part of the breast. Gravy was made from the collected drippings, and the five sets of gravy were combined and assayed for HAAs. Stewed whole chicken was covered with water in a large pot and simmered until the leg joint became soft/moveable and the skin started to pull away from the leg bone.

Detailed information on the cooking methods and the criteria used to define different degrees of doneness is shown in Table 1. Other information gathered to further define the cooking methods included weight of chicken before and after cooking to calculate the percentage loss of weight with cooking and the total cooking time. The cooking time for just until done chicken was similar to cooking times recommended for various recipes (18).

The levels of IQ, MeIQ, MeIQx, DiMeIQx, and PhIP were measured in each of the composite samples extracted by solid phase extraction and HPLC according to the procedure of Gross and Gruter (19), which has been described in detail by Knize *et al.* (17). The samples were measured by investigators without the knowledge of cooking method or degree of doneness. Replicate samples containing relatively low and high HAAs were used as QC and were interspersed throughout the analysis to check for reproducibility. The HAA QC samples were made from hamburger cooked at low temperature (containing

low levels of HAAs—low QC) or hamburger patties cooked at high temperature (containing high levels of HAAs—high QC). The average concentrations of MeIQx, PhIP, and DiMeIQx found in the high QC ( $n = 13$ ) were: 7.2 (coefficient of variation: 0.36), 10.9 (0.24), and 1.7 (0.40) ng/g, respectively (17). IQ and MeIQ were not detectable in the QC samples.

## Results

Chicken breasts without skin and bones when pan-fried, oven-broiled, or grilled/barbecued contained MeIQx, DiMeIQx, and PhIP but no detectable levels of IQ or MeIQ. Both the cooking method and degree of doneness determined the type and amount of HAAs formed, as shown in Fig. 1. PhIP levels were the highest of the three HAAs formed in all samples. Pan-fried chicken breasts without skin and bones contained substantial levels of PhIP, even when cooked to just until done (12 ng/g), which increased to 70 ng/g for very well done meat. Broiled chicken breasts without skin and bones contained higher levels than pan-fried chicken with 150 ng/g in the very well done category. Grilled/barbecued chicken breasts without skin and bones contained the highest levels of PhIP, ranging from 27 ng/g in the just until done sample, to 140 ng/g in the well done sample, and 480 ng/g in the very well done sample.

Pan-fried chicken breasts without skin and bones contained low levels of MeIQx at every degree of doneness. Broiled chicken breasts without skin and bones in the just until done and well done categories did not contain detectable MeIQx, but the very well done samples contained a small amount. The just until done grilled/barbecued chicken breasts without skin and bones contained nondetectable levels of MeIQx, well done breasts had 2 ng/g, while the very well done sample contained 9 ng/g (Fig. 1).

In these same pan-fried samples, DiMeIQx increased with degree of doneness with the lowest amount found in the just until done category and highest level in the very well done category. Oven-broiled breast meat did not contain detectable levels of DiMeIQx. Grilled/barbecued chicken breasts had no DiMeIQx in just until done samples, while

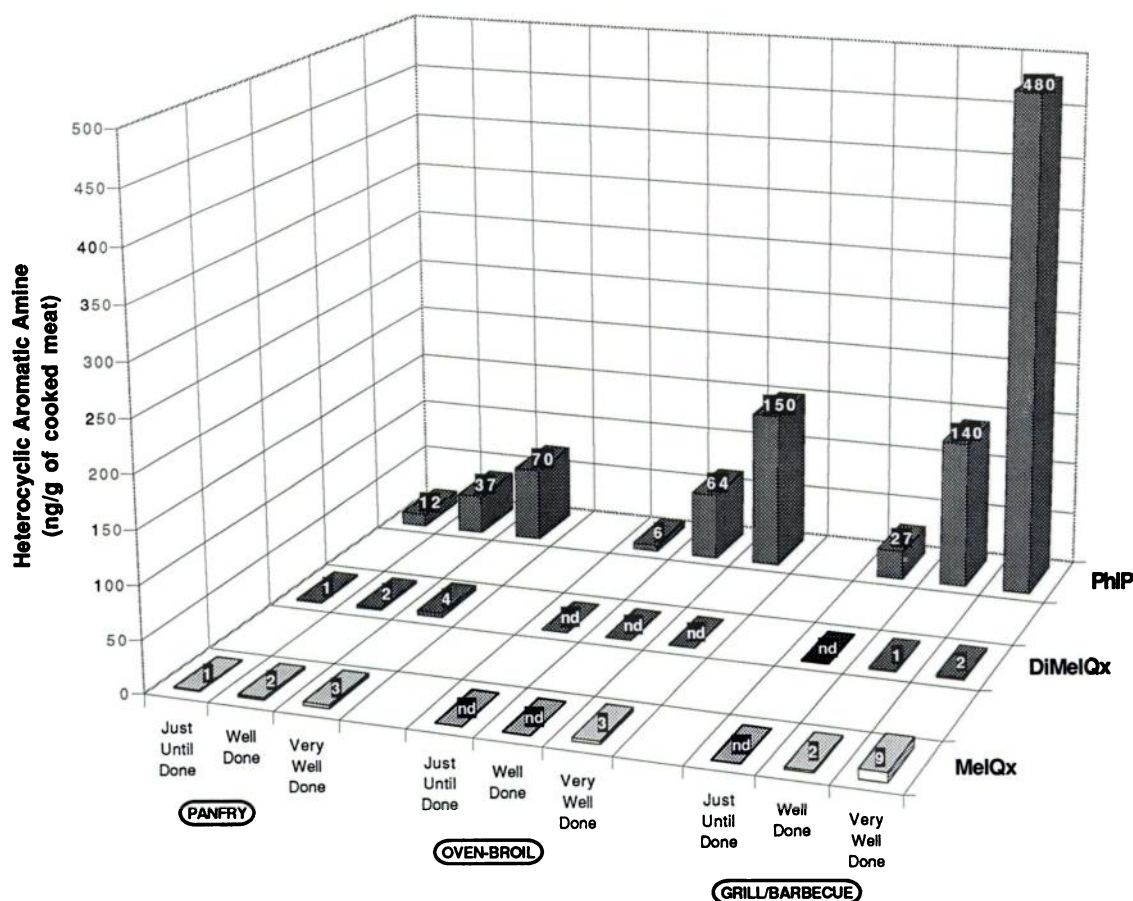


Fig. 1. MeIQx, DiMeIQx, and PhIP content of panfried, oven-broiled, and grilled/barbecued skinless, boneless chicken breasts cooked to varying degrees of doneness.

well done and very well done chicken breasts contained small amounts (Fig. 1).

Chicken breast with skin and bones cooked well done also contained appreciable levels of PhIP but had undetectable levels of both MeIQx and DiMeIQx. The PhIP content of these chicken samples by cooking method was 25 ng/g for panfried, 131 ng/g for oven-broiled, and 36 ng/g for grilled/barbecued.

Roasted chicken, stewed chicken, and chicken gravy contained no detectable levels of HAAs.

## Discussion

High levels of PhIP were found in chicken that was panfried, oven-broiled, or grilled/barbecued but not in roasted or stewed chicken. In the former samples, PhIP levels were much higher than amounts reported previously in red meats (3). In our earlier study, very well done panfried hamburgers cooked to maximize the production of these compounds contained 32.8 ng/g of PhIP (20). The comparable preparation of panfried, very well done chicken breast contained more than double the amount of PhIP at 70 ng/g meat. In this study, when steak and chicken samples were grilled/barbecued to very well done, the steak contained 30 ng/g,<sup>4</sup> while chicken contained 480 ng/g of PhIP.

PhIP concentration increased in chicken breasts with greater degree of doneness. People may routinely eat chicken well done, samples which contain substantial quantities of PhIP as compared to other types of cooked meat samples (20–22). The amino acid, sugar, creatinine, or moisture content of the chicken breast may have con-

tributed to the high levels of PhIP found in this study. The cooking conditions that lead to enhanced formation of PhIP need to be further investigated since PhIP has been implicated in the etiology of colon and mammary gland tumors in the rodent model (4, 7) and may contribute to risk of these cancers in humans.

These findings are important for epidemiological studies of HAAs and cancer. White meat (chicken and fish) consumption, in contrast to red meat consumption, has not been consistently associated with excess colon cancer risk (23) and, in some cases, has even been found to be protective (24, 25). Thus, if cooked chicken contains high levels of PhIP, then PhIP may not be a human carcinogen, or its association with cancer may have been attenuated because chicken preparation techniques have not been considered in detail by previous studies. Based upon our data, people who consume chicken cooked by roasting/baking, stewing/braising, or deep frying (17) receive little PhIP exposure, while those who consume chicken cooked by panfrying, oven-broiling, and grilling/barbecuing may have substantial exposure. Furthermore, chicken and fish consumption may be confounded with other healthy aspects of diet such as higher consumption of fruit/vegetables, fiber, and lower intake of fat.

Red meat, as noted above, has been associated with an increased risk of colon cancer in many studies (10, 11, 24, 25). People who eat predominantly red meat may cook it by methods which produce high levels of HAAs such as panfrying, oven-broiling, or grilling/barbecuing.<sup>4</sup> In addition, red meat consumption may be associated with other factors which increase cancer risk such as fat. For example, animal studies show that the effect of HAAs on the development of colon and mammary gland tumors may be modified in the presence of a high fat diet (7, 8). Since a diet high in fat is more strongly associated with red

<sup>4</sup> Unpublished data.

meat intake than with the intake of chicken or fish, the effect of HAAs may be manifested only among individuals consuming predominantly red meat.

To investigate the association of HAAs and cancer, all sources of these compounds should be used to create an exposure index that can accurately categorize subjects in epidemiological studies. To date, only surrogates of red meat HAA exposure (doneness or brownness) have been used in these studies. All types of meat, *i.e.*, beef, pork, chicken, and fish, need to be incorporated in a matrix with cooking method and level of doneness. To this end, we are currently developing a database for meats cooked by different methods to varying levels of doneness to link with a meat cooking module within a food frequency questionnaire.<sup>5</sup>

Although the study presented here is the most comprehensive to date on chicken, further studies are needed to better evaluate human dietary exposure to HAAs. Multiple chicken samples were cooked and made into a single composite sample, which was then analyzed. Thus, data on variability in HAA content within samples cooked by the same technique to the same degree of doneness are not available. In this study, we examined the production of HAAs in white chicken meat only. Further studies are needed to investigate the effect of cooking and doneness on HAA production in the dark meat portions of chicken (legs, thighs, and wings). Moreover, cooking conditions could not always be tightly controlled so that the internal temperature, external appearance, and total cooking time did not increase consistently from one degree of doneness to the next. Conditions for panfrying were the easiest to control, while those for grilling/barbecuing were the most difficult. Finally, chicken samples were cooked to different degrees of doneness and browning in a nutrition research facility. To judge the representativeness of these samples, there is a need to document usual home-cooking conditions.

In summary, we found that certain cooking methods produce relatively high levels of PhIP in chicken. Although the link between consumption of HAAs and excess cancer risk in humans has yet to be demonstrated, these compounds are established animal carcinogens (9). As such, it may be prudent to minimize exposure to PhIP when eating chicken. This can be achieved by roasting or stewing chicken and by avoiding overcooking or overbrowning when panfrying, ovenbroiling, and grilling/barbecuing.

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<sup>5</sup> R. Sinha *et al.*, manuscript in preparation.