

September 10, 2015

Dr. Veronique Bouvard, Responsible Officer  
Kurt Straif, Head of the IARC Monographs Programme  
IARC  
Lyon, France

**Re: Volume 114: Red Meat and Processed Meat – Call for Data – Systematic review: Evidence is weak and inadequate in both humans and animals concerning the mechanistic relationship between dietary HCA exposure from red and processed meat and human prostate, breast, and colorectal cancer.**

Dear Drs. Bouvard and Straif:

Several heterocyclic amines (HCAs) associated with meat have been previously reviewed by IARC (IARC Monograph 56, 1993). However, population-based studies fail to demonstrate consistent and convincing evidence for a relationship between HCAs, when consumed as part of a diet, and cancer risk (Demeyer et al., 2015). Some methods of cooking create HCAs in foods, including red and processed meat, by condensation of creatine with amino acids and sugars, particularly at high-temperatures (Turesky, 2007). **Therefore, we used a systematic approach to determine whether consumption of HCAs in red and processed meat as the result of cooking could be mechanistically linked with cancer risk in humans.** Our interest focused on the four most predominant HCAs from cooked meat: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP); 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx); 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx); and 2-Amino-3-methylimidazo[4,5-f]quinolone (IQ). We also limited our scope of interest to prostate, breast, and colorectal cancer as the sites most often studied in relation to diet and HCAs.

**Systematic Review: Mechanistic Relationship between Dietary HCA Exposure from  
Red and Processed Meat and Human Prostate, Breast, and Colorectal Cancer**

**EXECUTIVE SUMMARY**

A search of the PubMed database for mechanistic evidence relating dietary HCAs with human cancer revealed the following:

**Results**

- Of 294 studies published since 2000, 29 publications met the full criteria for inclusion.
- Twenty-three of the included studies were case-control designs or nested case-control studies within a larger prospective cohort, and three utilized other types of designs in humans.
- Only 3 of the included studies utilized an animal model to investigate HCA intake from foods or as part of a well-described diet. PhIP was the only HCA evaluated in the included animal studies.
- In human studies there was near-exclusive use of a single, non-comprehensive database, rather than direct analytical measurement, for determination of dietary HCAs.
- Due to limitations of estimating dietary HCAs with existing databases and lack of in-study validation, any mechanistic links of dietary HCAs with cancer in humans are inconclusive.

## Conclusions

- Interactions between individual genetic factors, estimations of HCA intake from red and processed meat, and cancer outcomes were inconsistent.
- Due to limitations of estimating dietary HCAs with existing databases and lack of in-study validation, any mechanistic links of dietary HCAs with cancer in humans are inconclusive.
- **Evidence is weak and inadequate in both humans and animals concerning the mechanistic relationship between dietary HCA exposure from red and processed meat and human prostate, breast, and colorectal cancer.**

## **STUDY PROCESS AND STUDY QUESTION**

The use of a systematic approach for retrieval and selection of literature to investigate mechanistic links in health and disease are well defined (OHAT, 2015). We used this approach and adapted the process and selection criteria developed by Kushman et al., 2013 in their model case-study of Di(2-ethylhexyl)phthalate. For conducting the search, we used the following study question: **“what mechanisms link HCA exposure from a food, or as part of an overall diet, with human prostate, breast, or colorectal cancer?”**

## **DEVELOPMENT OF EVALUATION CRITERIA FOR DIETARY HCA AND MECHANISMS OF CARCINOGENESIS**

It was essential for this review, that included research accommodate exposure from a whole food or in the context of an overall, well-described diet. Our criteria allowed selection of only those studies with design and dietary detail sufficient for making mechanistic conclusions regarding human dietary HCA exposure and cancer of the prostate, breast, colon or rectum. Therefore, the following factors of nutrition research were considered in drafting the screening criteria. The complete list of criteria is presented in **Table 1 of the Appendix:**

### A. Selection of appropriate research models

#### *In vitro*

Monolayers of cells in culture do not replicate whole body metabolism of dietary components under investigation in this review. While many uses for cells in culture are appropriate, transformed cell lines and cells derived from tumors of any species do not reflect the impact of diet on normal metabolism at the earliest stage of carcinogenesis under investigation in this review (Eisenbrand et al., 2002). Therefore, studies involving experimentation exclusively in cell culture were excluded.

#### *In vivo*

Under normal conditions, non-mutated animal models reflect whole body metabolism of dietary components. Mutated animal models which increase the risk of tumors, however, do not detect more subtle diet interactions within normal physiology and were excluded from this review.

Human studies of various designs can provide important mechanistic data when the mechanism under investigation is relevant and the level of HCAs in the diet can be accurately determined. Therefore, this review included human studies of various designs.

## B. Consideration of dietary composition

### *Importance of the whole diet*

There are a large number of nutrients and compounds found within the diet that can interfere with processes required for carcinogenesis (Yerba-Pimentel et al., 2015). Such dietary components are known to influence cell proliferation and cell death, as well as carcinogen activation and detoxification (Zanini et al., 2015).

Intended or unintended modifications of these chemo-preventive compounds in study diets can invalidate diet-related results. The complete composition of the diet must be known and diet-related statistical analyses performed in order to determine the effect of a diet-related exposure on mechanistic pathways or events (Bidlack et al., 2009).

### *Relevance to human diets*

Animal diets should be described in detail sufficient for translation or interpretation with regard to a human diet. Traditional laboratory 'chow' diets vary greatly with regard to the type of food components used in their production and are not a constant variable. Even the more defined semi-synthetic diets, such as the rodent AIN-76 or AIN-93, contain only a limited number of nutrients in comparison with the wide variety of compounds found in a human diet (Reeves et al., 1997).

## **SEARCH RESULTS**

Results of a PubMed search with application of the dietary exposure evaluation criteria are shown in the table below. Studies published in or after 2000 were selected as they more likely reflect analysis of foods consistent with today's food supply as well as better analytical methods. This is a particularly relevant consideration for red and processed meat, which has seen steady declines in fat and sodium content over the past several decades (McNeill et al., 2012; Higgs, 2000; Jacobson et al., 2013).

**Appendix, Table 2** provides the full list of citations and primary reason for exclusion. At Tier 1 the most common reason for exclusion was failure to evaluate effects of HCA in one of the three tissues of interest, and provision of only review information with no new data. The most common reason for study exclusion at Tier 2 was failure to provide detailed information regarding the diet, including estimation of dietary HCA intake.

<b>Database</b>	<b>Search terms</b>	<b>Number of citations</b>
PubMed	((("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]) OR ("carcinogenesis"[MeSH Terms] OR "carcinogenesis"[All Fields])) AND ("meat"[MeSH Terms] OR "meat"[All Fields])) AND (heterocyclic[All Fields] AND ("amines"[MeSH Terms] OR "amines"[All Fields] OR "amine"[All Fields])) AND ("2000/01/01"[PDAT] : "2015/12/31"[PDAT])	Initial search = 294 Tier I screen (abstract/full text) Include = 83 Exclude = 211  Tier 2 quality criteria applied (full-text) Include = 29 Exclude = 54

Out of the original 294 articles located in this search, 29 studies were considered acceptable, meeting full inclusion criteria with sufficient study design and diet detail, for evaluation of mechanisms linking dietary HCA exposure to human cancer of the prostate, breast, colon or rectum. A list of the included studies is included in **Table 3 of the Appendix**.

## **Studies in Humans**

### Epidemiological Studies

The greatest number of included studies were case-control designs of data from a clinical setting, from a population-based database, or nested in a larger prospective cohort. Four of those studies addressed cancer of the breast (**Delfino et al., 2000; Lee et al., 2013; Mignone et al., 2009; Rabstein et al., 2010**). Four studies reported outcomes related to prostate cancer (**Joshi et al., 2012; Koutros et al., 2009; Rovito et al., 2005; van Hemelrijck et al., 2012**). Fifteen studies were related to colorectal cancer (**Barbir et al., 2012; Budhathoki et al., 2015; Butler et al., 2005; Butler et al., 2008; Fu et al., 2012; Gilsing et al., 2012; Ishibe et al., 2002; Le Marchand et al., 2002; Nothlings et al., 2009; Nowell et al., 2002; Shin et al., 2008; Steck et al., 2014; Tiemersma et al., 2004; Voutsinas et al., 2013; Ward et al., 2007**).

Methodology common among many of the studies included determination of genotypes which were, in some, used to predict phenotypes. Odds ratios were calculated by logistic regression and reported with corresponding 95% confidence intervals. Genes most often reported were those involved in HCA metabolism (CYP1A1, CYP1A2, CYP1B1, GSTA1, GSTM1, GSTM3, GSTP1, NAT1, NAT2, SULT1A1, SULT1A2, and UGT1A locus). These genes regulate pathways involved in the bio-activation and detoxification of HCAs and DNA repair and their related polymorphisms are thought to contribute to the mutagenicity of HCAs (Ho et al., 2015). Also measured was PTGS2, the gene encoding the COX-2 protein, which is inducible by HCAs and is overexpressed in prostate cancer cells (Joshi et al., 2012); and EPS8L3 which encodes proteins responsible for Ras signaling in the pathway of actin remodeling for cytoskeletal integrity (Koutros et al., 2009).

**Breast Cancer.** The analysis by Delfino et al., (2000) found no association of MeIQx and DiMeIQx with breast cancer and in fact after adjusting for the intake of well-done chicken, they found a protective effect of PhIP. There was no interaction between dietary HCAs and NAT2, and the authors conclude that their findings “do not support a role for [HCAs] from meat or NAT2 in the etiology of breast cancer”. Lee et al., 2013 reported no association of postmenopausal breast cancer risk with NAT2 acetylator genotype, the CYP1A2 polymorphism, or the intake of HCAs. They also found no interaction between NAT2 acetylator genotype or CYP1A2 polymorphism and HCA intake. These authors conclude “These results do not support the hypothesis that genetic polymorphisms of xenobiotic enzymes involved in the metabolism of HCAs may modify the associations between intakes of red meat or meat-related mutagens and breast cancer risk”. Likewise, Mignone et al., (2009) found no statistically significant interactions of HCAs from red meat with NAT2 genotype. And, the conclusion by Rabstein et al., (2010) states “the modifying role of NAT2 for receptor-defined breast cancer is difficult to interpret in the light of complex mixtures of exposure to [HCAs]”. As a group, those studies investigating the interaction of dietary HCA with various genotypes found no interactive effect for breast cancer.

**Prostate Cancer.** Rovito et al., (2005) found no association between prostate cancer and the interaction of MeIQx or PhIP intake from meat with NAT1 and NAT2 genotype. However, the interaction was reported as significant for the PTGS2 gene and estimated MeIQx from diet (Joshi et al., 2012) and for GSTM3/EP8L3 and GSTP1, variants in two genes known to detoxify HCAs (Koutros et al., 2009). The surprising results of Van Hemelrijck et al., (2012) showed that in subjects with less than 2 deletions of the GSTT1 and GSTM1 genes, higher intake of PhIP, MeIQx, or DiMeIQx was associated with a lower risk of prostate cancer as compared to those with 2 or more deletions. In contrast, there was an opposite effect on the risk of advanced prostate cancer in those having more than 2 deletions. These disparate results emphasize not only the metabolic differences of tumors as they progress, but also the importance of obtaining dietary data at a time point most relevant to the cancer stage under investigation. The authors explain the inconsistency as a potentially different mechanism of action linking dietary HCA with these SNPs and development versus the progression of prostate cancer. There was also an association between DiMeIQx intake, MnSOD polymorphism, and prostate cancer risk. Surprisingly, in subjects with the MnSOD polymorphism, there was a decreased risk of prostate cancer associated with higher DiMeIQx intake. Van Hemelrijck and colleagues concluded that these inconsistent data were not expected and “are not necessarily concordant with the underlying biological hypotheses”. As a group, therefore, these studies indicate human genetic data which are inconsistent and likely highly influenced by cancer stage.

**Colorectal Cancer.** Studies of outcomes related to colorectal cancer reported mixed results. Budhathoki et al., (2015) found no significant association between estimated intake of total HCA, or MeIQ specifically, and colorectal adenoma risk according to NAT2 acetylation genotype. Nothlings et al., (2009) also failed to find a significant association of HCA intake with the NAT2 genotype on the risk of colorectal cancer. And similarly, Butler et al., (2008) found no colon cancer association with HCA intake according to NAT2. Butler et al., also reported a colon cancer association with HCA intake that was modified by NAT1, but that the NAT1 genotype most at risk differed by race. Voutsinas et al., (2013) likewise reported a combined effects of HCA intakes and NAT2 activity on the risk of adenoma.

Gilting et al., (2012) reported a significant interaction between NAT1 (rs6586714) and MeIQx, specifically, on colorectal adenoma risk. However, no other associations were found with the over 300 single nucleotide polymorphisms in 18 genes, including GSTM3, UGT1A, AHR, EPHX, NAT1 and CYP2C9 as measured in the study. Fu et al., (2012) also found no statistically significant interactions between HCA dietary exposure and a HCA-metabolizing score that was constructed from 16 functional genetic variants. And, the report by Nowell et al., (2002) failed to detect interactions of dietary HCAs with the polymorphisms measured in their study which included hGSTA1 genotype, SULT1A1 genotype and the phenotype for CYP2A6. Similarly, Steck et al., (2014) reported that patterns of increasing colon cancer risk with higher HCA intake were not modified by nucleotide excision repair genotypes.

Shin et al., (2008) reported interactions for intake of HCAs with AhR, NAT1, and NAT2 genotypes, and the interactions were statistically significant for both adenomatous and hyperplastic polyps. However, no statistical adjustments were made for the multiple comparisons made in this study, making the statistical significance of these findings uncertain. In the study by Ishibe et al., (2002), subjects with MeIQx intake in the 80<sup>th</sup> centile showed an increased adenoma risk among both rapid and slow NAT1 acetylators. In contrast, NAT2 genotype and CYP1A2 and NAT2 hepatic activity were not associated with adenoma and not modified by MeIQx. The study by Tiemersma et al., (2004) provided analytical measurements verifying the presence of HCAs in cooked meat in one part of their study. In their

case-control analysis, the combination of NAT2 slow acetylation and frequent meat consumption increased adenoma risk. However, these authors did not investigate total or individual HCA intake and gene interactions on adenoma risk making any conclusions non-specific for HCA exposure.

Butler et al., (2005) reported that the association of DiMeIQx intake and colon cancer was modified by the UGT1A7 genotype, when comparing intakes above versus below the median. Barbir et al., (2012) determined that SULT1A1 phenotypes modified the effect of MeIQx on the risk of developing colorectal adenoma, with a stronger association of MeIQx intake for slow, rather than normal, phenotypes. The authors recognize that due to the number of comparisons made in their analysis “the modifying effect of SULT1A1 on the association of HCA intake with CRA risk may be due to chance”. No other HCA modifying effects by phenotypes were found.

The literature as a whole is inconsistent in the use of statistical adjustments needed for the often large number of comparisons made within each study. Even though a large amount of diet information must have been available to the researchers from food frequency questionnaire analysis, none of the studies made statistical adjustments for dietary components known to influence the metabolism of HCAs. Of particular concern is the almost exclusive use of a single database for estimating HCA intake. The limitation of the database to only three HCAs, in a limited number of foods, creates bias throughout the literature. As a group, therefore, these studies report conflicting and inconsistent results for several studied polymorphisms, and limited results for many polymorphisms of interest, making any mechanistic link between HCA intake and colorectal cancer risk based on genetic and related effects in humans speculative.

#### Human Studies – other design

Three studies conducted in humans utilized other methodological designs:

**Breast Cancer. Lightfoot et al., (2000)** examined the bioavailability of PhIP to human breast tissue and resulting binding with DNA. Patients undergoing breast surgery consumed a capsule of 14C PhIP by mouth prior to surgery. At surgery, normal and cancerous breast tissue was resected and subjected to liquid scintillation to trace the radioactively labeled PhIP. DNA adducts were counted using accelerator mass spectrometry. This tracer study demonstrated that orally administered PhIP was capable of reaching breast tissue and forming strong covalent bonds with DNA in this tissue. Due to the lack of correlation with actual tumor development, however, no direct mechanistic link can be made.

**Colon Cancer. Ho et al., (2014)** conducted a cross-sectional study of 342 patients who recently underwent colonoscopy. Genetic differences related to HCA metabolism (CYP1B1) and DNA repair (XPD) were determined and PhIP, MeIQx and DiMeIQx intake from cooked meat was estimated using the CHARRED database. There were no relationships between dietary HCAs, meat mutagen exposure and colorectal adenoma risk in the population as a whole. However, there was positive association between dietary exposure to HCAs/meat mutagens and adenoma risk in males. In addition, gene - diet interactions were observed for dietary PhIP and polymorphisms in CYP1B1 and XPD and for dietary DiMeIQx and XPD polymorphisms. The authors speculated that these genetic differences confer increased risk due to changes associated with biotransformation pathways and DNA repair.

**Mutagenicity. Shaughnessy et al., (2011)** conducted a 2 week crossover feeding study in sixteen subjects randomly assigned to one of two experimental groups. One group consumed a diet containing meat cooked at either low (100<sup>o</sup> C) or high-temperature (250<sup>o</sup> C). The other group a diet containing the high temperature meat diet alone or in combination with three putative mutagen inhibitors (inhibitor foods): cruciferous vegetables, yogurt and chlorophyllin tablets. The Salmonella assay was used to analyze the meat, urine and feces for mutagenicity, and the comet assay rectal biopsies and peripheral blood lymphocytes was used to quantify DNA damage. The high-temperature meat diet increased the mutagenicity of hydrolyzed urine and feces compared to the low-temperature meat diet. The diet with the inhibitor foods increased the mutagenicity of the hydrolyzed urine by nearly twofold, indicating that the inhibitor foods increased conjugation and elimination in the urine. Inhibitors decreased significantly the mutagenicity of un-hydrolyzed and hydrolyzed feces. The inhibitor diet also decreased by nearly twofold the DNA damage detected in colorectal cells. These results point to the importance of studying the whole diet in relation to mutagenicity of any single compound.

### **Studies in Animals**

Only three studies using animal models met the criteria for inclusion. Our primary interest was determining mechanistic links with HCA exposure as it would occur from a food source or within the context of a complete diet. The majority of animal studies, however, provided only a minimal description of diet, reported use of an undefined chow diet, and offered no statistical analyses or adjustments for dietary variables. All the animal studies meeting the inclusion criteria provided PhIP as the HCA exposure. Most animal studies meeting the inclusion criteria were related to colorectal cancer risk, one considered breast cancer risk, none evaluated mechanisms related to prostate cancer.

**Parasramka et al., (2012)** studied the tumor-suppressive effects of dietary spinach in PhIP-initiated Fisher 344 rats. The research team used MicroRNAs (miRNA) profiles in several tissues, including the colon, to quantify the mitigating effects of dietary spinach. The study was conducted over a 52-week period after initiation using PhIP via oral gavage in three cycles. The feeding of spinach was introduced 18 weeks after the post-initiation phase of the study. Tumors were enumerated in the colon and several other target organs. A microarray analysis was conducted on colon tumor samples and it was determined by computational analysis that the aberrant miRNA profiles were reduced by 58% in the colon tumors of rats fed the diet containing added spinach. It is important to note that the dosing protocol used in this study is designed to maximize the tumor-inducing potential. The authors hypothesized that spinach, fed at 10% (wt/wt) in the AIN-93M diet, appeared to correct PhIP-induced cellular dysregulation associated with colon carcinogenesis.

**Purewal et al., (2000)** investigated the ability of PhIP to induce aberrant crypt foci (ACF), an intermediate biomarker, in two different species of rats, the Fisher 344 (rapid acetylator) and the Wistar Kyoto (slow acetylator). The theory behind this research is that the enzyme N-Acetyltransferase-2 (NAT2) catalyzes the conversion of PhIP and other HCAs to a DNA-reactive form. The rapid NAT2 genotype has been associated with an increased colorectal cancer risk, in some, but not all, human epidemiological studies. Both species of animals were fed on an AIN-76 diet supplemented with either zero (control), 0.01% or 0.04% PhIP for eight weeks. PhIP induced ACF in both rapid and slow-acetylator rats in a dose-dependent manner. There was no difference in the number of ACF at the 0.01% dose level, but, at the higher dose level, the rapid acetylator F344

rats developed ACF at twice the rate of the slower Wistar Kyoto rats. The authors concluded that this may support previous epidemiological findings related to the importance of genetic variations and the frequent consumption of very well done meats in carcinogenesis. Nonetheless, it should be noted that a four-fold increase in the PhIP dose level was required to provoke an increase in the ACF response.

**Schut and Yao (2000)** utilized female Fisher 344 rats in order to study the ability of green and black teas as chemopreventive agents following administration of PhIP via gavage at a dosage of 1 mg/kg/day during weeks 3, 4 and 5 of the study. The animals received the teas at a level of 2% wt/vol as their sole source of fluid for six weeks. Adduct formation was measured in the colon and mammary epithelial cells (MECs), as well as several other tissues, on days 1 and 8 following the termination of carcinogen exposure. The PhIP-DNA adducts were found to be inhibited in the colon and MEC's of female F344 rats who consumed green tea as their sole source of fluids on both days that were tested. Black tea inhibited adduct formation in the colon on day 1 only, but had no effect for either days measured related to the MECs. The authors hypothesize that the observed inhibition of PhIP-DNA adduct formation might be due to the effects of the green and black teas on carcinogen-metabolizing enzymes, such as induction of phase II detoxifying enzymes. Thus, the induction of these enzymes by the two teas might provide an inhibitory action on DNA adduct formation in this animal model. These results point to the importance of the whole diet in relation to mutagenicity of any single compound.

## RESEARCH GAPS

As reported in the above studies, the predominance of evidence is weak and inconclusive with regard to mechanistic links between diet HCA and human cancer. The following identified research gaps likely contribute to bias and confounding of the literature previously recognized by others (Demeyer et al., 2015; Ho et al., 2015).

**Inaccurate or biased measurements of dietary HCAs.** The majority of epidemiological studies included in this review estimated dietary content of HCAs based on the CHARRED database (Sinha et al., 2005; NCI website) and due to its limitations discussed below, the results of human genetic and related effects and dietary HCA exposure, as reported in the studies in this review must be considered inconclusive. Furthermore, any meta-reviews of this literature must consider this overriding bias. Generally, investigators estimate HCA exposure using a food frequency questionnaire (FFQ) specific to the intake of certain kinds of meat, cooked by predetermined techniques, to predetermined degrees of doneness. Photographs of such cooked meat are offered to aid completion of the FFQ. Use of CHARRED allows investigators to assign a corresponding quantity of PhIP, MeIQx and DiMeIQx to the meat items. While the database is useful, its nearly universal application to food intake data by investigators worldwide has the potential to impact the evidence as a whole in a negative and biased way. For instance, the database does not include values for fish. Investigators who independently measure HCA production in fish as the result of cooking, report significant levels in this food group. A study by Puangsombat et al., (2012), compared the effect of different cooking methods on HCA levels and found MeIQx and PhIP to be similar if not lower in beef and pork when compared to poultry and fish. Similarly, Viegas et al., (2012) quantitated HCAs (and PAHs) in barbecued beef and salmon under identical methods of charcoal grilling and found overall higher levels of HCAs and PAHs in the samples of salmon. The HCA content of a number of other foods, not reported in the CHARRED database, may also be significant.

There are 15 -25 HCAs known to accumulate in cooked meat that have been identified (Sugimura et al., 2004; Alejos and Afonso, 2011) and only a few can be estimated by use of the CHARRED database. Widespread use of the database as a surrogate for direct HCA measurement encourages a gap in knowledge with regard to the impact of all HCAs and possibly delays the discovery of new ones. In addition, the over-reliance on one database has led to an assumption that the intake of only certain meats cooked at high temperature is an acceptable surrogate for total dietary HCA estimation or direct measure of HCA content in various foods. This is evidenced by over 25 studies excluded in this review specifically for that reason (**Appendix Table 2**). It is important to note that only two studies of the 26 human studies included here, performed any type of analytical testing in order to validate database estimates. This is definitely a significant research gap and critical methodological shortcoming.

The use of one master database is premature for this literature. By definition, the database was created by analyzing a single meat source from one locale, using cooking methodology pertinent to one region of the world. Wider reports of direct measurements by a variety of investigators, in a variety of foods, in the ever-changing food supply is needed. In addition, the composition of red and processed meat has changed significantly in the past 15 years (McNeill et al., 2012; Higgs, 2000; Jacobson et al., 2013) and many of the meat samples were obtained and tested prior to that time (Sinha et al., 2005). In their review, Demeyer et al., (2015) point to the apparent disconnect between estimated HCA intake based on application of database values to food frequency data and actual HCA content. The correlation of data from studies using photographs as a means to indirectly estimate HCA intake with actual intake is not clear (Skog and Solyakov, 2002; Alaejos and Afonso, 2011). Similarly, estimates for dietary HCA exposure obtained from FFQs were poorly correlated with urinary levels of PhIP in a study by Deziel and others (2012). According to Demeyer, “[m]ore consistent cancer risk estimates of dietary HCA exposure may therefore require improved HCA assessment tools”.

**Cancer stage and undetermined latency period between HCA exposure and development of cancer.** The time between exposure to a carcinogen and development of overt tumors varies greatly depending on the carcinogen and the tumor site, but the process can occur over decades. It is not clear whether intake data from decades prior to diagnosis is more relevant than more recent intake data, or whether consumer memory regarding historical food intake is accurate. In fact, results from one of the included studies indicated that HCA intake differentially impacts genetic risk for colorectal cancer based on cancer stage (Van Hemelrijck et al., 2012). To evaluate possible options for analyzing historical intake data in relation to meat intake and colorectal cancer, Bernstein et al., (2015) applied four models of food intake. In their analysis, they chose to report outcomes related to a cumulative average intake model. The model calculated the mean intake from many available FFQs, beginning at the earliest baseline in 1986 and including periodic follow-up questionnaires at four year intervals. Many of the epidemiological studies included in this review did not include intake data from multiple time points, but rather included data from one FFQ collected at undetermined periods in the process of carcinogenesis. It is clear that research on the effectiveness of collecting multiple FFQ to more accurately associate diet and disease development is warranted.

**Our review shows a preponderance of human observational data and a limited number of relevant animal studies.** Available human studies report inconsistent results and potentially biased HCA dietary exposure data due to an absence of analytical validation of HCA content in foods and the universal use of a surrogate database. In addition, our search retrieved a limited number of animal studies where whole foods were used as the source of these compounds. The combination of sufficient, consistent, human and animal data is a critical aspect of IARC classifications. The gaps in research regarding HCAs from red and processed meat and promotion of various cancers are significant.

## CONCLUSIONS

According to results from these studies, there is no consistent and convincing mechanistic link between dietary HCAs and human cancer. **Evidence is weak and inadequate in both humans and animals concerning the mechanistic relationship between dietary HCA exposure from red and processed meat and human prostate, breast, and colorectal cancer.**

Sincerely,



Shalene McNeill, PhD, RD  
Executive Director, Human Nutrition Research,  
National Cattlemen's Beef Association, a contractor to the Beef Checkoff

In Cooperation with:

Connye Kuratko, PhD, RD, Kuratko Nutrition Research  
George Dunaif, PhD, DABT, Toxpro Solutions, LLC  
Mary E. Van Elswyk, PhD, RD, Van Elswyk Consulting, Inc.  
Charli A. Weatherford, MS, Weatherford Consulting Services

Attachments:

Zip file enclosure #1 – Mechanistic Evidence re: HCAs and cancer; Appendix A  
Zip file enclosure #2 – Evidence Supporting Modified Evaluation Criteria for Dietary Carcinogens

## References

- Alaejos, M. S. and Afonso, A. M. (2011). Factors that affect the content of heterocyclic aromatic amines in foods. *Comprehensive Reviews in Food Science and Food Safety*. 10(2):52-108.
- Barbir, A., Linseisen, J., Hermann, S., Kaaks, R., Teucher, B., Eichholzer, M., and Rohrmann, S. (2012). Effects of phenotypes in heterocyclic aromatic amine (HCA) metabolism-related genes on the association of HCA intake with the risk of colorectal adenomas. *Cancer Causes Control*. 23:1429-1442.
- Bernstein, A. M., Song, M., Zhang, X., Pan, A., Wang, M., Fuchs, C. S., Ngoan, L., Chan, A. T., Willett, W. C., Ogino, S., and Giovannucci, E. L. (2015). Processed and unprocessed red meat and risk of colorectal cancer: analysis by tumor location and modification by time. *PLoS ONE*. 10(8): e0135959. doi:10.1371/journal.pone.0135959.
- Bidlack, W. R., Birt, D., Borzelleca, J., Clemens, R., Coutrelis, N., Coughlin, J. R., Dunaif, G. E., Ebert, A., Heimback, J. T., McColl, D. B., McQuate, R. S., Munro, I., Petersen, B., Roberts, A., Scimeca, J., Slayne, M., and Trautman, T. (2009). Expert report: making decisions about the risks of chemicals in foods with limited scientific information. *Comprehensive Reviews in Food Science and Food Safety*. 8(3):269-303.
- Budhathoki, S., Iwasaki, M., Yamaji, T., Sasazuki, S., Takachi, R., Sakamoto, H., Yoshida, T., and Tsugane, S. (2015). Dietary heterocyclic amine intake, NAT2 genetic polymorphism, and colorectal adenoma risk: the colorectal adenoma study in Tokyo. *Cancer Epidemiology, Biomarkers and Prevention*. 24(3):613-620.
- Butler, L. M., Duguay, Y., Millikan, R. C., Sinha, R., Gagne, J. F., Sandler, R. S., and Guillemette, C. (2005). Joint effects between UDP-glucuronosyltransferase 1A7 genotype and dietary carcinogen exposure on risk of colon cancer. *Cancer Epidemiology, Biomarkers and Prevention*. 14(7):1626-1632.
- Butler, L. M., Millikan, R. C., Sinha, R., Keku, T. O., Winkel, S., Harlan, B., Eaton, A., Gammon, M. D., and Sandler, R. S. (2008). Modification by N-acetyltransferase 1 genotype on the association between dietary heterocyclic amines and colon cancer in a multiethnic study. *Mutation Research*. 638(1-2):162-174.
- Delfino, R. J., Sinha, R., Smith, C., West, J., White, E., Lin, H. J., Liao, S. Y., Gim, J. S. Y., Ma, H. L., Butler, J., and Anton-Culver, H. (2000). Breast cancer, heterocyclic aromatic amines from meat and N-acetyltransferase 2 genotype. *Carcinogenesis*. 21(4):607-615.
- Demeyer, D., Mertens, B., De Smet, S., and Ulens, M. (2015). Mechanisms linking colorectal cancer to the consumption of (processed) red meat: a review. *Critical Reviews in Food Science and Nutrition*. 15:0. Epub ahead of print.
- Deziel, N. C., Buckley, T. J., Sinha, R., Abudaker, S., Platz, E. A., and Strickland, P. T. (2012). Comparability and repeatability of methods for estimating the dietary intake of the heterocyclic amine contaminant 2-amino-1methyl-6-phenylimidazo[4,5b]pyridine (PhIP). *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment*. 29(8):1202-1211.
- Eisenbrand, G., Poo-Zobel, B., Baker, V., Balls, M., Blaauboer, B. J., Boobis, A., Carere, A., Kevekordes, S., Lhuguenot, J. C., Pieters, R., and Kleiner, J. (2002). Methods of in vitro toxicology. *Food and Chemical Toxicology*. 40:193-236.

- Fu, Z., Shrubsole, M. J., Li, G., Smalley, W. E., Hein, D. W., Chen, Z., Shyr, Y., Cai, Q., Ness, R. M., and Zheng, W. (2012). Using gene-environment interaction analyses to clarify the role of well-done meat and heterocyclic amine exposure in the etiology of colorectal polyps. *American Journal of Clinical Nutrition*. 96:1119-1128.
- Gilsing, A. M. J., Berndt, S. I., Ruder, E. H., Graubard, B. I., Ferrucci, L. M., Burdett, L., Weissfeld, J. L., Cross, A. J., and Sinha, R. (2012). Meat-related mutagen exposure, xenobiotic metabolizing gene polymorphisms and the risk of advanced colorectal adenoma and cancer. *Carcinogenesis*. 33(7):1332-1339.
- Higgs, J. D. (2000). The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science and Technology*. 11:85-95.
- Ho, V., Peacock, S., Massey, T. E., Ashbury, J. E., Vanner, S. J., and King, W. D. (2014). Meat-derived carcinogens, genetic susceptibility and colorectal adenoma risk. *Genes and Nutrition*. 9(6):430.
- Ho, V., Peacock, S., Massey, T. E., Godschalk, R. W. L., vanSchooten, F. J., Chen, J., and King, W. D. (2015). Gene-diet interactions in exposure to heterocyclic aromatic amines and bulky DNA adduct levels in blood leukocytes. *Environmental and Molecular Mutagenesis*. 56:609-620.
- Ishibe, N., Sinha, R., Hein, D. W., Kulldorff, M., Strickland, P., Fretland, A. J., Chow, W. H., Kadlubare, F. F., Lang, N. P., and Rothman, N. (2002). Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. *Pharmacogenetics*. 12:145-150.
- Jacobson, M. F., Havas, S., and McCarter, R. (2013). Changes in sodium levels in processed and restaurant foods, 2005 to 2011. *JAMA Internal Medicine*. 173(14):1285-1291.
- Joshi, A. D., Corral, R., Catsburg, C., Lewinger, J. P., Koo, J., John, E. M., Ingles, S. A., and Stern, M. C. (2012). Red meat and poultry, cooking practices, genetic susceptibility and risk of prostate cancer: results from a multiethnic case-control study. *Carcinogenesis*. 33(11):2108-2118.
- Koutros, S., Berndt, S. I., Sinha, R., Ma, X., Chatterjee, N., Alavanja, M. C. R., Zheng, T., Huang, W. Y., Hayes, R. B., Cross, A. J. (2009). Xenobiotic metabolizing gene variants, dietary heterocyclic amine intake, and risk of prostate cancer. *Cancer Research*. 69(5):1877-1884.
- Kushman, M. E., Kraft, A. D., Guyton, K. A., Chiu, W. A., Makris, S. L., and Rusyn, I. (2013). A systematic approach for identifying and presenting mechanistic evidence in human health assessments. *Regulatory Toxicology and Pharmacology*. 67:266-277.
- Le Marchand, L., Hankin, J. H., Pierce, L. M., Sinha, R., Nerurkar, P. V., Franke, A. A., Wilkens, L. R., Kolonel, L. N., Donlon, T., Seifried, A., Custer, L. J., Jones, A. L., and Chang, W. (2002). Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. *Mutation Research*. 506-507:205-214
- Lee, H. J., Wu, K., Cox, D. G., Hunter, D., Hankinson, S. E., Willett, W. C., Sinha, R., and Cho, E. (2013). Polymorphisms in xenobiotic metabolizing genes, intakes of heterocyclic amines and red meat, and postmenopausal breast cancer. *Nutrition and Cancer*. 65(8):1122-1131.

Lightfoot, T. J., Coxhead, J. M., Cupid, B. C., Nicholson, S., and Garner, R. C. (2000). Analysis of DNA adducts by accelerator mass spectrometry in human breast tissue after administration of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and benzo[a]pyrene. *Mutation Research*. 472:119-127.

McNeill, S. H., Harris, K. B., Field, T. G., Van Elswyk, M. E. (2012). The evolution of lean beef: identifying lean beef in today's U.S. marketplace. *Meat Science*. 90:1-8.

Mignone, L. I., Giovannucci, E., Newcomb, P. A., Titus-Ernstoff, L., Carbone, P. P., Hampton, J. M., Oray, E. J., Willett, W. C., and Egan, K. M. (2009). Meat consumption, heterocyclic amines, NAT2, and the risk of breast cancer. *Nutrition and Cancer*. 61(1):36-46.

Nothlings, U., Yamamoto, J. F., Wilkens, L. R., Murphy, S. P., Par, S. Y., Henderson, B. E., Kolonel, L. N., and Le Marchand, L. (2009). Meat and heterocyclic amine intake, smoking, NAT1 and NAT2 polymorphisms and colorectal cancer risk in the multiethnic cohort study. *Cancer Epidemiology, Biomarkers and Prevention*. 18(7):2098-2106.

Nowell, S., Coles, B., Sinha, R., MacLeod, S., Ratnasinghe, D. L., Stotts, C., Kadlubar, F. F., Ambrosone, C. B., and Lang, N. P. (2002). Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: contribution of metabolic variation to risk. *Mutation Research*. 506-507:175-185.

Office of Health Assessment and Translation (OHAT). (2015). Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration. Division of the National Toxicology Program National Institute of Environmental Health Sciences.

Parasramka, M. A., Dashwood, W. M., Wang, R., Abdelli, A., Bailey, G. S., Williams, D. E., Ho, E., and Dashwood, R. H. (2012). MicroRNA profiling of carcinogen-induced rat colon tumors and the influence of dietary spinach. *Molecular Nutrition and Food Research*. 56(8):1259-1269.

Puangsoombat, K., Gadgil, P., Houser, T. A., Hint, M. C., and Smith, J. S. (2012). Occurrence of heterocyclic amines in cooked meat products. *Meat Science*. 90:739-746.

Purewal, M., Velasco, M., Fretland, A. J., Hein, D. W., and Wargovich, M. J. (2000). 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine induces a higher number of aberrant crypt foci in Fischer 344 (rapid) than in Wistar Kyoto (slow) acetylator inbred rats. *Cancer Epidemiology, Biomarkers and Prevention*. 9:529-532.

Rabstein, S., Bruning, T., Harth, V., Fischer, H. P., Haas, S., Weiss, T., Spickenheuer, A., Pierl, C., Justenhoven, C., Illig, T., Vollmert, C., Baisch, C., Ko, Y. D., Hamann, U., Brauch, H., and Pesch, B. (2010). N-acetyltransferase 2, exposure to aromatic and heterocyclic amines, and receptor-defined breast cancer. *European Journal of Cancer Prevention*. 19:100-109.

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *Journal of Nutrition*. 127(5 Supplement):838S-841S.

Rovito Jr., P. M., Morse, P. D., Spinek, K., Newman, N., Jones, R. F., Wang, C. Y., Hass, G. P. (2005). Heterocyclic amines and genotype of N-acetyltransferases as risk factors for prostate cancer. *Prostate Cancer and Prostatic Diseases*. 8:69-74.

- Schut, H. A. J. and Ruisheng, Y. (2000). Tea as a potential chemopreventive agent in PhIP carcinogenesis: effects of green tea and black tea on PhIP-DNA adduct formation in female F-344 rats. *Nutrition and Cancer*. 36(1):52-58.
- Shaughnessy, D. T., Gangarosa, L. M., Schliebe, B., Umbach, D. M., Xu, Z., MacIntosh, B., Knize, M. G., Matthews, P. P., Swank, A. E., Sandler, R. S., DeMarini, D. M., and Taylor, J. A. (2011). Inhibition of fried meat-induced colorectal DNA damage and altered systemic genotoxicity in humans by crucifera, chlorophyllin, and yogurt. *PLoS ONE*. 6(4):e18707. doi: 10.1371/journal.pone.0018707.
- Shin, A., Shrubsole, M. J., Rice, J. M., Cai, Q., Doll, M. A., Long, J., Smalley, W. E., Shyr, Y., Sinha, R., Ness, R. M., Hein, D. W., and Zheng, W. (2008). Meat intake, heterocyclic amine exposure, and metabolizing enzyme polymorphisms in relation to colorectal polyp risk. *Cancer Epidemiology, Biomarkers Prevention*. 17(2):320-329.
- Sinha, R., Cross, A., Curtin, J., Zimmerman, T., McNutt, S., Risch, A., and Holden, J. (2005). Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. *Molecular Nutrition and Food Research*. 49:648-655.
- Skog, K. and Solyakov, A. (2002). Heterocyclic amines in poultry products: a literature review. *Food and Chemical Toxicology*. 40(8):1213-1221.
- Steck, S. E., Butler, L. M., Keku, T., Antwi, S., Galanko, J., Sandler, R. S., and Hu, J. J. (2014). Nucleotide excision repair gene polymorphisms, meat intake and colon cancer risk. *Mutation Research*. 762:24-31.
- Sugimura, T., Wakabayashi, K., Nakagama, H., and Nagao, M. (2004). Heterocyclic amines: mutagens/carcinogens produced during cooking of meat and fish. *Cancer Science*. 95(4):290-299.
- Tiemersma, E. W., Voskuil, D. W., Bunschoten, A., Hogendoorn, E. A., Witteman, B. J. M., Nagengast, F. M., Glatt, H. R., Kok, F. J., and Kampman, E. (2004). Risk of colorectal adenomas in relation to meat consumption, meat preparation, and genetic susceptibility in a Dutch population. *Cancer Causes and Control*. 15:225-236.
- Turesky, R. J. (2007). Formation and biochemistry of carcinogenic heterocyclic aromatic amines in cooked meats. *Toxicology Letters*. 168:219-227.
- Van Hemelrijck, M., Rohrmann, S., Steinbrecher, A., Delbrueck, M., Kaaks, R., Teucher, B., and Linseisen, J. (2012). Heterocyclic aromatic amines [HCA] intake and prostate cancer risk: effect modification by genetic variants. *Nutrition and Cancer*. 64(5):704-713.
- Viegas, O., Novo, P., Pinto, E., Pinho, O., Ferreira, I. M. P. L. V. O. (2012). Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. *Food and Chemical Toxicology*. 50(6):2128-2134.
- Voutsinas, J., Wilkens, L. R., Franke, A., Vogt, T. M., Yokochi, L. A., Decker, R., and Le Marchand, L. (2013). Heterocyclic amine intake, smoking, cytochrome P450 1A2 and N-acetylation phenotypes, and risk of colorectal adenoma in a multiethnic population. *Gastroenterology*. 62(3):416-422.

Ward, M. H., Cross, A. J., Divan, H., Kulldorff, M., Nowell-Kadlubar, S., Kadlubar, F. F., and Sinha, R. (2007). Processed meat intake, CYP2A6 activity and risk of colorectal adenoma. *Carcinogenesis*. 28(6):1210-1216.

Yebra-Pimentel, I., Fernandez-Gonzalez, R., Martinez-Carballo, E., and Simal-Gandara, J. (2015). A critical review about the health risk assessment of PAHs and their metabolites in foods. *Critical Reviews in Food Science and Nutrition*. 55:1383-1405.

Zanini, S., Marzotto, M., Giovinazzo, F., Bassi, C., and Bellavite, P. (2015). Effects of dietary components on cancer of the digestive system. *Critical Reviews in Food Science and Nutrition*. 55:1870-1885.