National Cattlemen's Beef Association
a contractor to the Beef Checkoff

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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 – Weak and limited evidence regarding heterocyclic amine (HCA) content in foods limits the ability to estimate human HCA exposure and calls into question the ability to assess HCAs as a mechanistic link associated with red and processed meat and cancer

Dear Drs. Bouvard and Straif:

Thank you for the opportunity to submit data for the upcoming monograph review of red and processed meat. The increased formation of heterocyclic amines (HCAs), also known as heterocyclic aromatic amines (HAAs), in various foods during cooking and the recognition of several HCAs by IARC as carcinogenic (IARC Monograph Volume 56, 1993) has led to the hypothesis that exposure to HCAs from cooked meats increases risk for various cancers. Interestingly, 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).

Nonetheless, in an effort to support determination of the relevance of HCAs in potential mechanisms for red and processed meat to promote human cancer, we have summarized the relevant data for the content of HCAs in the current food supply (**Appendix Tables 1-4**). To supplement our review, we are also providing a published comprehensive review of HCAs reported in various foods through 2009 (Alaejos and Afonso, 2011). Our review focused on the HCAs previously reviewed by IARC: 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 offer several key observations and identify critical research gaps that limit the credible understanding of the content of HCA in red and processed meat and call into question the reliability of estimations of HCA exposure, as reported in various observational studies linking red and processed meat to various cancer endpoints.

We recognize that any one food's content of HCAs is one aspect of human exposure, with frequency and amount of intake of a particular food also contributing. Therefore, in a series of separate submissions to IARC, The Beef Checkoff along with member countries of the International Meat Secretariat, will provide IARC current intake data for red and processed meat. The results of our current review further support mechanistic conclusions recently submitted to IARC (McNeill et al., September 11, 2015), that "evidence regarding HCA 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 ".

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Weak and limited evidence regarding heterocyclic amine (HCA) content in foods limits the ability to estimate human HCA exposure and calls into question the ability to assess HCAs as a mechanistic link associated with red and processed meat and cancer.

EXECUTIVE SUMMARY

Observations

- PhIP and MeIQx are the most commonly measured HCAs in foods despite the identification of over 25 HCAs in the food supply.
- PhIP is typically the highest HCA in foods and particularly so in pan-fried foods.
- Pan-frying increases HCA content in all meat, but the highest values are reported for PhIP in chicken.
- Marinated meat typically has lower HCA content than non-marinated meat but this is only obvious when both are cooked "very well done".

Research Gaps and Recommendations

- Despite the availability in the past decade of numerous quantitative reports of HCAs in various foods, the two most readily available HCA estimation databases have not been updated since at least 2003.
- Continuous updating of food HCAs analyses is required to reflect advancements in detection equipment and methodology and changes in the food supply.
- Terminology for cooking methods varies around the world. Standardization of cooking methods and terminology is needed in order to make comparisons between studies and across regions.

Conclusions

- Failure to update HCAs databases to reflect the current food supply, detection methodology and equipment, world-wide food availability and preparation techniques prevents the accurate assessment of human exposure to HCAs.
- **Significant evidence limitations call into question the relevance of HCAs exposure as a possible mechanism associated with cancer risk for red and processed meat intake.**

EVIDENCE IDENTIFICATION

A search of the PubMed database was conducted to identify studies published ≥ 2000 that measured HCAs formed during cooking in meats and other foods. Food preparation methods and levels of exposure that are applicable to the individual consumer, rather than a particular occupation, were of primary interest. Additional studies were identified from study bibliographies. A comprehensive review of reports of HCAs in foods published through 2009 was also considered (Alaejos and Afonso, 2011). Our tables contain reports published after 2009, along with reports published in 2000 or later, not included in the review by Alaejos and Afonso (2011). Studies published in or after 2000 were selected as they more likely reflect analysis of foods consistent with the current food supply as well as more recent analytical methods. Selection of this time period is particularly relevant for accurate representation of red and processed meat, which has steadily declined in fat and sodium content over the past several decades (McNeill et al., 2012; Higgs, 2000; Jacobson et al., 2013). Data were extracted from the resulting studies and reported in **Tables 1-4** of the accompanying Appendix.

SUMMARY OF OBSERVATIONS

1) Despite identification of over 25 HCAs in the food supply, most reports measure only PhIP and MeIQx. A true estimate of human exposure to HCAs depends on reliable documentation of all the HCAs available in the food supply. Reporting of only 2-4 common HCAs limits our understanding of total human HCAs exposure, potential interactions between HCAs, and the effect of various cooking methods on food total HCAs content.

2) Increased exposure to HCAs from pan-frying is not limited to red and processed meat. In fact, studies report either similar (Appendix Table 1) or much higher levels of PhIP in pan-fried chicken (Alaejos and Afonso, 2011) and grilled chicken (Appendix Tables 2 and 3) as compared to beef and pork.

3) Marinated meat typically has lower HCAs content than non-marinated meat but this is only obvious when both are cooked "very well done". Marinating is proposed as a strategy for limiting HCA in grilled foods but this appears most effective in foods that are very well done. There is also limited consistency with regard to the types of and ingredients in various marinades reported in the literature (Appendix Table 4), which makes it difficult to standardize a marinating strategy beneficial for all meats prepared via all methods and degrees of doneness.

CRITICAL RESEARCH GAPS IDENTIFIED

1) Despite the availability in the past decade of numerous quantitative reports of HCAs in various foods, the two most readily available HCAs estimation databases have not been updated since at least 2003. Continuous updating of food HCAs analyses and databases linked to these analyses are required to reflect changes in the food supply. The CHARRED database (NCI, 2015) is used to estimate exposure to HCAs in a multitude of epidemiologic observational studies of human diet and cancer risk. Unfortunately, the CHARRED database reports only three of the 25 HCAs identified in the food supply and then only in a one-time sample of meat collected from a local grocery in the USA in the late 1990's (Sinha et al., 1998a; Sinha et al., 1998b). In addition, the meat selected for quantitation of HCAs was limited, for beef – one fat level of hamburger, 2 steak cuts, and one roast were considered (Sinha et al., 1998a). Fresh pork was represented solely by a pork chop. Processed pork was represented by one brand of hot dog, a ham slice, and several types of breakfast sausage (Sinha et al., 1998b). Jakszyn and co-workers (2004) created a database of HCAs, polycyclic aromatic hydrocarbons (PAHs), and nitrosamines based on quantitative reports in the literature. While this database expands on values available from CHARRED, the methodology for HCA measurement is not uniformly reported or standardized across studies. In addition, no additional food data has been included since 2003. Despite availability of newer data, the CHARRED database also appears to have had no data updates but rather has only corrected errors and fixed database glitches (NCI. 2015). The limited scope of HCA compounds and representative foods used in the available HCAs estimation databases calls into question the reliability of any estimates of human exposure to HCAs reported since 2003.

2) Continuous updating of food HCAs analyses and databases linked to these analyses are required to reflect changes and advancements in detection equipment and methodology. The progression detection methodology for HCAs over time is evident in reports of HCAs quantitation reviewed by Alaejos and Afonso (2011). Many early studies rely on gas chromatography, with later studies relying heavily on high-performance liquid chromatography, and most recently GC-mass spectrophotometry. These advancements have increased the ability to accurately separate and quantitate compounds and likely have contributed to observed variations in the HCAs content of foods over time. The outdated detection equipment used in the quantitation of HCAs in the two most available HCAs estimation databases calls into question the reliability of any estimates of human HCAs exposure reported beyond 2003.

3) Standardization of cooking methods and terminology are needed in order to make comparisons between studies and across regions. Failure to specify cooking conditions and variations in terminology for cooking methods used around the world has been recognized as a critical issue limiting comparison of studies reporting HCAs (Alaejos and Afonso, 2011). For example, in some countries "grilling" may or may not expose foods to direct flame, but limited or unspecified cooking conditions reported in publications prohibits the ability to discern these differences. Limited methodologic details regarding cooking temperature, cooking duration, internal temperature reached at end of cooking, procurement procedures for food products, etc. limit the ability to replicate and compare results from study to study across the globe and even within the same laboratory reporting results over time. Sound advice regarding preferred cooking methodologies designed to limit human exposure to HCAs depends on complete and detailed reporting of experimental methodologies and replication of findings.

CONCLUSIONS

Critical research gaps indicate significant shortfalls and barriers to accurate assessment of HCA exposure from all foods tested globally. Consequently, there is a critical need to update existing databases, through an assessment of the current food supply and typical in-home preparation methods, using recent methodology, in order to better evaluate exposure to HCAs from red and processed meat, as well as from other foods, including white meats. Significant evidence limitations call into question the importance and relevance of HCAs as a possible mechanism associated with cancer risk for red and processed meat intake.

Sincerely,

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Attachments:
Zip file enclosure #1 – Quantitative Reports of HCA in Food; Appendix of Data Summary Tables
Zip file enclosure #2 – Publications Supporting Critical Research Gaps Regarding Measurement of HCAs in Foods

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