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Comments: Dear Dr. Boyd:

The Beef Checkoff appreciates the opportunity to submit scientific evidence to the Office of Health Assessment and Translation (OHAT) in response to the September 9, 2016, Federal Register (80 FR 62513-14) request for information regarding the possible evaluation of consumption of red meat, processed meat, and meat cooked at high temperatures for non-cancer health outcomes (e.g. development, reproductive, or immunological disorders).

Several scientific issues confound the ability to accurately assess these foods as potentially carcinogenic exposures (Oostindjer et al., 2014; Klurfeld, 2015). As described in the systematic reviews by Gibis (2016) and Alaejos and Afonso (2011), a myriad of reactions and compounds are generated during the cooking of meat (regardless of temperature) that need to be considered in isolation rather than grouped together as a single exposure, for example, meat cooked at high temperatures can result in the formation of heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs).

Observations by DeMeyer et al., (2015), Gibis (2016), Singh (2016) and those from additional studies regarding the available scientific evidence are listed below and indicate that evidence regarding PAHs, in particular benzo[a]pyrene (BaP), and HCA content in foods, is confounded by critical research gaps that call into question the ability to reliably assess exposure to dietary PAHs and HCAs from only one food group (e.g. meat) as mechanistic ally linked with increased cancer risk.

#### SUMMARY OF OBSERVATIONS

Data from Kazerouni and co-workers (2001) indicates that pan and deep fried meats are not significant sources of BaP compared to other sources of animal protein. When comparing a variety of animal proteins, across a variety of frying conditions, both in the lab and in restaurant/fast food settings, there is little difference in the reported BaP content (Kazerouni et al., 2001). The exception appears to be fried fatty fish and/or fish with added fat prior to frying (Olatunji et al., 2015) which can contain up to 70% of the mean BaP concentration calculated for all foods by the European Food Safety Authority (EFSA, 2007).

Data from Kazerouni and co-workers (2001) indicates that increased exposure to BaP from grilled food is not limited to red and processed meat. In fact, studies report the same or higher BaP contents in well-done grilled chicken (with skin) and fish, as compared to grilled beef (Kazerouni et al., 2001; Viegas et al., 2012).

Smoked fish is the greatest contributor of BaP among smoked animal protein regardless of smoking method in the studies by Duedahl-Olesen et al., 2010; Mohammadi et al., 2013; Kitts et al., 2012. Current data are limited regarding BaP content of foods (Yebra-Pimentel et al., 2015). In general, however, smoked fish is reported to contain more BaP when compared to other smoked foods (Duedahl-Olesen et al., 2010; Mohammadi et al., 2013; Kitts et al., 2012).

As reported by Kazerouni et al. 2001 BaP concentrations in plant-based foods, in particular, bread and/or toasted bread, are just as high as those reported for well-done grilled meats. Consistent with previous observations regarding the high PAH content of cereal grains (EFSA, 2007), reports in the current literature confirm the high level of BaP in bread products (Kazerouni et al., 2001; Alomirah et al., 2011; Al-Rashdan et al., 2010). Contamination of cereal grains likely occurs during technological processes such as direct fire drying where combustion products may come into contact with the grain used to formulate various bread products (EFSA, 2007).

The vast majority of foods, regardless of degree of doneness when cooked, fail to exceed established regulatory levels set for BaP in foods. EU (2011) has established regulatory limits for BaP for various food groups. Generally speaking, concentrations of BaP for most foods as reported by Kazerouni et al. (2001), and others, fall below these established limits.

One study reports data regarding inhaled BaP from cooking of red and processed meat (Jorgensen et al., 2014). Emissions from high temperature frying have been identified as probably carcinogenic to humans, however, data was considered insufficient to attribute risk to a specific chemical compound, to cooking oil alone, or a particular food being cooked (IARC, 2010). One study has reported BaP exposure from red and processed meat (bacon) cooking fumes (Jorgensen et al., 2013). Generally, current evidence suggests that the type of oil used in cooking, in addition to the temperature and cooking time, has the greatest effect on the

amount of BaP released in cooking fumes (Yao et al., 2015; Zhu and Wang, 2003; Chen and Chen, 2003).

In their review, Alaejos and Afonso (2011) recognize that despite identification of over 25 HCAs in the food supply, most reports measure only PhIP and MeIQx. 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 (Alaejos and Afonso, 2011).

Data from Puangsombat et al. (2012) and Viegas et al. (2012) indicate that increased exposure to HCAs from pan-frying is not limited to red and processed meat. In fact, studies report either similar or much higher levels of PhIP in pan-fried chicken and grilled chicken as compared to beef and pork (Puangsombat et al., 2012; Viegas et al., 2012).

Marinated meat typically has lower HCA content than non-marinated meat (Meurillon and Engel, 2016). Marinating is proposed as a strategy for limiting HCA in grilled foods but this appears most effective in foods that are very well done (Meurillon and Engel, 2016). There is also limited consistency with regard to the types of and ingredients in various marinades reported in the literature (Viegas et al., 2015) which makes it difficult to standardize a marinating strategy beneficial for all meats prepared via all methods and degrees of doneness (Meurillon and Engel, 2016).

## RESEARCH GAP

Continuous updating of food PAH/BaP and HCA analyses and database are required to reflect advancements in detection equipment and methodology and changes in the food supply (Alaejos and Afonso, 2011; Singh et al., 2016). Few studies of BaP/PAH quantitation are available and those that are, likely do not represent the current food supply or advances in detection methodology (Alaejos and Afonso, 2011). Databases used to estimate PAH/BaP and HCA exposure in humans may be limited by outdated analyses, thus calling into question links between exposure estimates and disease occurrence (Alaejos and Afonso, 2011; Oostindjer et al., 2014; Singh et al. 2016). Analysis of BaP in a variety of food types, as the result of a variety of cooking techniques, relies heavily on reports from only two sources (Kazerouni et al., 2001; EFSA, 2007). Both sources are nearly a decade old. Newer evaluations are also likely to benefit from improvements in accuracy afforded by advancements in detection equipment and

methodology (Alaejos and Afonso, 2011). 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 (Alaejos and Afonso, 2011; Gibis, 2016). These advancements have increased the ability to accurately separate and quantitate compounds and likely have contributed to observed variations in the HCA content of foods over time (Alaejos and Afonso, 2011; Gibis, 2016).

Information in the published literature is lacking detail to inform public health advice regarding preferred cooking methodologies to limit human exposure to PAHs and HCAs from foods (Alaejos and Afonso, 2011; Singh et al., 2016). A review by Alaejos and Afonso (2011) clearly articulates that failure to specify cooking conditions in published experiments and variations in terminology used globally for cooking are critical issues limiting comparison of studies reporting PAH/BaP and HCAs in foods. Strategies to limit human exposure to dietary PAHs and HCAs depend on complete and detailed reporting of experimental methodologies and replication of findings (Meurillon and Engel, 2016). Importantly, however, it should be noted that reported cooking times and temperatures in many studies are excessively high (i.e., up to 30 minutes and 300°C) and would likely not represent typical in-home preparation or result in an edible cooked meat (Alaejos and Afonso, 2011; Gibis 2016; RoC, 2002).

HCA estimation databases do not provide data reflective of the current food supply (Alaejos and Afonso, 2011). Despite availability of newer data, the CHARRED database appears to have had no recent food data updates other than correction of errors and database malfunctions (NCI, 2015). Continuous updating of food HCA analyses and databases linked to these analyses are required to reflect changes in the food supply (Alaejos and Afonso, 2011). Similarly, while a database created by Jakszyn and co-workers (2004) expands on values available from CHARRED, the methodology for HCA measurement is not uniformly reported or standardized across studies, and the database appears to not have been updated since 2003.

## CONCLUSIONS

As discussed in reviews by Alaejos and Afonso (2011), Gibis (2016), and Singh et al. (2016) critical research gaps indicate significant shortfalls and barriers to accurately assess PAH and

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 PAHs and HCAs from red and processed meat, as well as from other foods (Trudo and Gallaher, 2015; Alaejos and Afonso, 2011). Significant evidence limitations call into question the importance and relevance of PAHs and HCAs as a possible mechanism associated with cancer risk for red and processed meat intake (DeMeyer et al, 2015).

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