

July 29, 2015

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IARC  
Lyon, France

**Re: Volume 114: Red Meat and Processed Meat – Call for Data – Case Study: Mechanisms of Dietary BaP Exposure in Colorectal Cancer**

Dear Drs. Bouvard and Straif:

Consumption of polycyclic aromatic hydrocarbons (PAHs) has been proposed as a mechanism by which foods cooked via high temperature grilling could increase human risk for colorectal cancer. However, recent reviews have failed to find sufficient evidence directly linking PAH consumption from red meat with colorectal cancer (Demeyer et al., 2015; Trudo and Gallaher, 2015).

**Using benzo(a)pyrene (BaP) as a representative PAH, and the colon as the tumor site, we conducted a systematic review of the mechanistic evidence relating dietary exposure of BaP to human colon carcinogenesis.** Our methods followed the process outlined previously (Kushman et al., 2013).

**Case Study: Mechanisms of Dietary BaP Exposure in Colorectal Cancer**

**EXECUTIVE SUMMARY**

A search of the PubMed database for mechanistic evidence relating dietary BaP and colorectal cancer, revealed the following:

**Results**

- Of 51 studies published since 2000, **only 4 publications met the full criteria for inclusion.** Three of the studies were conducted in humans, one utilized a lacZ mouse model.
- The primary reason for study exclusion at Tier 1 was:
  - Failure to evaluate effects of BaP or a BaP metabolite administered orally or as part of a diet or cell culture media
- The primary reasons for study exclusion at Tier 2 was:
  - Failure of the study design to accurately represent non-cancerous colon physiology and metabolism
  - Failure to describe human or animal diets in detail
- No study in animals or in cell culture assessed BaP exposure from red and processed meat
- No study in humans provided a direct assessment of BaP exposure in the diet.

**Conclusion**

- A critical research gap exists regarding mechanistic evidence linking BaP exposure from the diet and colorectal cancer.
- **Evidence is weak and inadequate in both humans and animals concerning the mechanistic relationship between dietary BaP exposure and human colorectal cancer.**

## STUDY PROCESS AND STUDY QUESTION

The use of a systematic approach for retrieval and selection of literature to clarify scientific support for cancer mechanisms of action was described in a case study by Kushman et al. (2013). We used this approach and modified their general question “what are the mechanisms by which a chemical may cause carcinogenicity in the target tissue?” to the more specific question **“what are the mechanisms by which BaP exposure from a food, or as part of an overall diet, may cause carcinogenicity in colon and/or rectal tissue?”**

## DEVELOPMENT OF EVALUATION CRITERIA FOR DIETARY BaP EXPOSURE

Kushman et al. (2013) reported the evaluation criteria used for studies of mechanisms of action for the agent of interest in their case study. We used this criteria, with adaptations, to accommodate exposure from a whole food or as part of an overall diet. Our modified criteria allowed selection of only those studies with design and dietary detail sufficient for making mechanistic conclusions regarding human dietary BaP exposure and colorectal cancer.

Previous reviews have included few, if any, studies designed to mechanistically examine a relationship of colorectal cancer and BaP, when exposure originates in food or as part of a whole diet (IARC, 2010; IARC, 2012; Xue and Warshawsky, 2005).

In order to select literature specifically designed to answer such a question, the following factors of nutrition research were considered:

### A. Selection of appropriate research models

#### *In vitro*

- Cells in colonic crypts are structurally polar, with strict orientation toward either the lumen or circulatory system, and they receive carcinogen and anti-carcinogen exposure from both sides. Their life-span is short, and within the crypt structure are exposed to activated circulatory and gut-associated lymphocytes, chronic inflammation, endocrine and microbial perturbations (Casey et al., 2015). Monolayers of cells in culture, therefore, cannot replicate the colonic environment with regard to dietary exposure and/or metabolism (Eisenbrand et al., 2002).

#### *In vivo*

- Under normal conditions, colon cells respond to BaP-induced DNA damage with mechanisms such as cell cycle arrest and apoptosis (Lowe et al., 2004). While it is clear that BaP is mutagenic in some tissues, establishment of colon tumors requires the use of either a tumor promoter (Lasne et al., 1977; Miller et al., 2000; Mukherjee and Kumar, 2013), an agent which interferes with the cell’s protective mechanisms (Mukherjee et al., 2011; Price and Calderwood, 1993) or the use of a genetically modified animal which is inherently unable to activate the protective proteins (Yamada and Mori, 2007). Such models which forcefully produce tumors do not detect more subtle diet interactions within normal colon physiology.

## 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 prevent the occurrence of cancer (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 or cell culture media 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). *In vitro* models often include nutrient compositions which bear little resemblance to normal levels found in cells, serum, or diet.

### *Significance of human exposure from a dietary source*

- A review of pharmacokinetic and detoxification mechanisms of BaP report the daily oral dose of BaP in mouse studies as 750 to 7,500 times the known human exposure level (Nebert et al., 2013). There is insufficient data at present to know the mouse response at levels more typical of human dietary intake. In addition, carcinogen exposure in small doses, over a longer period of time may better mimic human dietary exposure than fewer but larger bolus, gavage administrations.

## **INCLUSION/EXCLUSION CRITERIA FOR MECHANISTIC STUDIES OF DIETARY BaP EXPOSURE**

The resulting inclusion/exclusion criteria, based on the above considerations, are shown in **Appendix A, Table 1**. These criteria differ from those used in the Kushman case study with regard to: the inclusion of dietary BaP as the agent of interest; designation of colon/rectum as tissues of interest; and, limitation to *in vivo* and *in vitro* designs which model non-cancerous colon metabolism. The criteria also require studies to report diet composition in detail and statistically analyze outcomes according to dietary variables. In the modified criteria, both Tier 1 (at the abstract level) and Tier 2 (at the full text level) are applied to each study. While the Kushman criteria required that studies employ a dose-/concentration-response design, our criteria allowed the inclusion of studies with only one level of BaP exposure.

## SEARCH RESULTS

Results of a PubMed search following application of the dietary exposure evaluation criteria are shown in the table below. **Appendix A, Table 2** provides the full list of citations and primary reason for exclusion. At Tier 1 the most common reason for exclusion was study failing to evaluate effects of BaP or a BaP metabolite administered orally or as part of a diet or cell culture media. The most common reason for study exclusion at Tier 2 was use of study design which did not accurately represent non-cancerous colon physiology and metabolism.

Database	Search terms	Number of citations
PubMed	(colorectal cancer) AND benzo[a]pyrene Filters: From 2000/01/01 to 2015/06/27	Initial search = 51  Tier 1 screen (abstract/full text) Include = 27 Exclude = 24  Tier 2 quality criteria applied (full-text) Include = 4 Exclude = 23

**Out of the original 51 articles located in this search, only four studies were considered acceptable, meeting full inclusion criteria with sufficient study design and diet detail, for evaluation of dietary BaP exposure related to colorectal cancer.**

### A. Results from screening *in vivo* studies

- Seven *in vivo* studies were included following Tier 1 screening (Appendix A, Table 2).
- Six of those studies were excluded at Tier 2 screening (Appendix A, Table 2).
  - Each excluded study was conducted in the Apc Min/+ mouse. This strain has a point mutation at the Apc (tumor suppressor) gene, predisposing the animal to multiple intestinal neoplasms (Yamada and Mori, 2007). The Apc mutation prevents normal tumor suppression, thus masking many diet-related mechanisms of human colon tumor prevention, promotion, or progression.
  - The excluded studies describe the animal diet simply as ‘chow’ or a ‘basal’ diet.
- One *in vivo* study met the full criteria for inclusion (Guttenplan et al., 2001). The animal model used included a BALBc transgenic mouse engineered to detect mutations *in vivo* (Myhr, 1991). The study described the animal diet in detail and discussed potential diet-related confounders.

All of the animal studies administered BaP orally, but via gavage in large doses, not administered as part of the diet. Exposure occurred over a limited period of time and at total exposure levels which varied >100-fold between studies.

### B. Results from screening *in vitro* studies

- Search results included fifteen *in vitro* studies using the Tier 1 criteria (Appendix A, Table 2). All were excluded at Tier 2. Most were conducted in cell lines derived from human adenomas or adenocarcinomas, or in cell lines modified with aberrant tumor suppressive genes. These cell lines bear little resemblance to normal human colon either in morphology or metabolism.

### C. Results in humans

- Five studies in exposed humans were included at Tier 1 (Appendix A, Table 2).
- Two of the studies were excluded at Tier 2, for failing to report any measurement of BaP exposure in the diet. (Appendix A, Table 2)
- Three studies were included following the Tier 2 screening (Burnett-Hartman et al., 2011; Steck et al., 2014; Girard et al., 2008).

#### **EVIDENCE REGARDING DIETARY BaP EXPOSURE FROM INCLUDED STUDIES**

Evidence from the four included studies is presented in **Appendix A, Table 3**.

From the results of these four studies, it is not possible to conclude any potential mechanistic connection relating dietary BaP exposure with colorectal cancer. Each investigated a different potential mechanism including protection against DNA damage as well as its repair, BaP activation and detoxification. None found a clear relationship.

- Guttenplan and colleagues quantified mutagen formation in the colon of mice exposed and unexposed to BaP (Guttenplan et al., 2001). Mice were fed different doses of a lycopene-rich tomato oleoresin (LTO) in AIN-76A diets modified to eliminate synthetic antioxidants and to contain 20% corn oil to promote absorption of LTO. The level of vitamin E, another lipid soluble antioxidant and potential confounder, was also modified in light of the addition of LTO. The researchers reported a non-significant inhibition of spontaneous mutagenesis in colon by lycopene. In BaP-treated mice, mutagenesis was enhanced in the colons of animals fed higher levels of lycopene. This result was in contrast to findings in prostate, indicating that antioxidant/BaP metabolism is organ specific. These results indicate that dietary factors mitigate carcinogenesis and emphasize the need to select appropriate organ/tissue models.
- Burnett-Hartman et al. (2011) investigated a potential association between “charred meat” consumption (as a surrogate for BaP/PAH exposure), microsomal epoxide hydrolase (mEH) polymorphisms and colorectal adenomas and hyperplastic polyps (proximal and distal) in humans. Microsomal epoxide hydrolase activity is thought to metabolically convert BaP to BPDE, potentially increasing the risk of DNA adduct formation. However, there was no association of mEH genotype and colorectal polyps in the study. The intake of “charred meat” was associated with increased distal hyperplastic polyps but not adenomas in the distal colon. However, there was no interaction of mEH genotype with “charred” meat-intake on polyp occurrence, perhaps indicating no mechanistic association between dietary BaP and polyp occurrence. Furthermore, the authors used an inaccurate definition of “charred” to include panfrying, broiling, grilling, or barbecuing and inconsistently applied the terms “browned” and “charred” to their results. In addition, the authors concluded that “...there is still little consensus on the role that variations in mEH activity play in colorectal carcinogenesis, either directly or as a cofactor with environmental exposures to BaP.”
- In a case/control design, Girard et al., 2008 investigated the relationship of genetic variations in UGT1A1 and UGT1A9 genes with estimated dietary BaP exposure in colon cancer. Glucuronidation by the UDP-glucuronosyltransferase enzymes (UGTs) is thought to play a role in detoxification and elimination of dietary PAHs. In this study, the authors found that UGT1A1-53 and -3156 genotypes modified the association between dietary BaP and colon cancer. Unexpectedly, the strongest association was observed

for those with the lower level of estimated dietary BaP exposure (<7.7 ng/day) and the low activity genotypes when compared to the higher level of estimated dietary BaP ( $\geq 7.7$  ng/day) and combined high/intermediate genotypes. These results indicate that genotype modulates the association between dietary BaP exposure and colon carcinogenesis. The authors concluded that "Overall, the results of the present study and of Butler et al...support the hypothesis that UGTs may play a role in carcinogens elimination and, as a result, influence colon cancer risk."

- In a similar design, Steck et al., 2014 investigated the relationship between estimated dietary BaP exposure and nucleotide excision repair (NER) pathway polymorphisms in colon cancer. The authors reported a non-statistically significant increased risk of colon cancer for higher BaP intake with  $\geq 4$  high risk alleles. In contrast to the results from Girard et al. above, these results may indicate the lack of mechanistic association between these polymorphisms, dietary BaP exposure, and an increased risk for colon cancer. In fact, the authors note that the activity of the NER pathway may be protective stating "...some studies suggest that polymorphisms in xenobiotic-metabolizing enzymes (XME) may play significant role in colon cancer by detoxifying meat-related carcinogens, thereby reducing their bioavailability and deleterious effects..."

Results from the included studies indicate the unique metabolism of dietary exposures by colorectal tissue and provide indications that cancer risk may be dependent upon individual genotype. A significant weakness of the three included studies in humans should be noted. The terms 'meat' and 'red meat' are poorly defined and inconsistent among the studies. None directly assessed the level of BaP in the diet. Rather, the studies relied on multiple layers of estimations with regard to the types of foods consumed, the quantity of foods consumed, the prevalence of cooking methods used, and the level of BaP previously assayed in such foods.

## CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

**A critical research gap exists** regarding mechanistic evidence linking BaP exposure from the diet and colorectal cancer.

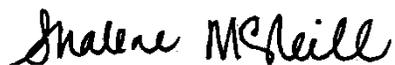
A lack of available mechanistic data from study designs sufficient for evaluating exposures from diet in the colon, is evident.

- Of 51 studies published since 2000, **only four publications met the full criteria for inclusion**. The primary reasons for study exclusion included:
  - Failure to evaluate effects of BaP or a BaP metabolite administered orally or as part of a diet or cell culture media
  - Failure of the study design to accurately represent non-cancerous colon physiology and metabolism
  - Failure to describe human or animal diets in detail
- The four included studies are inconclusive with regard to mechanistic connections between dietary BaP exposure and colorectal cancer.

Further research is needed utilizing study designs which are models of intact colon metabolism. In order to assess mechanisms related to dietary exposure, study designs are needed which statistically analyze outcomes according to relevant dietary components. In addition, more precise quantification of exposure from food and scientific standardization of food and cooking terminology is needed.

**Evidence is weak and inadequate in both humans and animals concerning the mechanistic relationship between dietary BaP exposure and human colorectal cancer.**

Sincerely,



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Attachments:

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

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