EPIX ANALYTICS
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1643 Spruce Street, Boulder, CO, 80302, USA
Phone: 1 303 440 8524, Fax: 1 303 440 032

October 11, 2016

RE: Request for Public Comment on the Nomination of Meat-Related Exposures for possible review for future editions of the Report on Carcinogens (RoC)

Dear Dr. Lunn and Dr. Boyd,

The following comments are provided toward the nomination of meat-related exposures (i.e., consumption of red meat, processed meat, and meat cooked at high temperatures) for possible review for future editions of the Report on Carcinogens (RoC). In summary, the focus on a whole food rather than the chemical compounds ingested with a food product is misleading, and counter to the mechanism-based toxicology strategy identified as part of the NTP Vision. It also introduces confounding and misclassification of exposure which would limit the proper identification of any carcinogenic potential. Further, evaluation of a whole food which is highly prevalent in the American diet should be performed in the context of the dietary alternatives which might be consumed while also carrying potential health impacts, including cancer risks. A tradeoff analysis must be performed quantitatively and would benefit from a focus on potentially carcinogenic compounds rather than foods which may carry them at highly variable levels.

Jane Pouzou, MPH, PhD

Solenne Costard, DVM, MS, PhD

Francisco Zagmutt, DVM, MPVM, PhD

1643 Spruce Street, Boulder, CO, 80302, USA
Phone: 1 303 440 8524, Fax: 1 303 440 032

Specific comments and supporting references:

1) *Evaluation of the whole food rather than specific compounds can introduce biases, particularly when in presence of small effect sizes. Without assessment of individual compounds, potential causative exposures may be confused or ignored due to such confounding.*
   - **A.** The degree to which some compounds found in red, processed, and high-temperature-prepared meat (HCA, PAH, and potentially N-Nitroso and heme-iron) separately contribute to risk is difficult to estimate and confounded based on dietary estimations and observational studies.[1] The same food products may fall into multiple categories, or may be grouped in ways that disguise associations with health impacts.
   - **B.** Aggregating exposures to the separate compounds will continue this confounding, as exposures may be correlated with other compounds generated during preparation, and to diet and lifestyle differences in consumers.[2]
     - i. As estimated among these example studies, the major sources of consumed HCAs and BaP were not necessarily the same foodstuffs. Fish contributed somewhat more of MeIQx than other sources (up to 5.66 ng/g of MeIQx),[3] whereas PhIP and BaP were more often taken in with chicken (0.2-34.9 ng/g of PhIP and 2.34 ng/g or less of MeIQx), beef, or hamburger (0.33 or less ng/g PhIP, 2.19 or less ng MeIQx.[4] In contrast, beef contributed two-thirds of the dietary meat-derived BaP compared with chicken in the diets of American military.[5]
     - ii. Mollusks may contain higher levels of heme-iron per serving than red meat.[6] Participants in the Multi-Ethnic Study of Atherosclerosis, a multicenter study of cardiovascular disease, consumed similar amounts of heme-iron from fish and poultry in milligrams per day as from red meat.[7]
   - **C.** Associations between red and processed meat consumption and CRC in humans are heterogeneous and in general weak, with little evidence for dose-response.[8] Figure 3 from Alexander et al (reproduced at the end of this document) illustrates the heterogeneity in the estimation.[8] Confounding or a lack of true association may be the cause for discrepancies/differences in study findings, but overall the effect seems to be small or non-existent. For example:
     - i. A meta-analysis by Alexander et al (2015) estimated the relative risk (RR) of colorectal cancer associated with "high" red and processed meat consumption as 1.10 (95% CI 1.03-1.18) compared with low consumption, and for red meat only, 1.05 (95% CI 0.98-1.12).[8]
     - ii. A study found poultry consumption associated with colorectal cancer (RR of 1.59, 95% CI 1.04–2.44), where red meat was not (RR of 1.50, 95% CI 0.77–2.94).[9] Another study found a positive but non-significant association between poultry consumption and colon cancer.[10]

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iii. In another study, no association was found with increased meat consumption (RR of 1.05, 95% CI 0.96–1.15) or poultry consumption (RR of 0.96, 95% CI 0.86–1.07) and CRC. [11]
iv. In a third example study, red meat was associated with increased risk of CRC (RR of 1.32 ,95% CI 1.03-1.68) but a non-significant inverse effect was observed for poultry consumption (RR of 0.62, 95% CI 0.34-1.13).[12]

D. The relationships between processed meat and cancers, and high-temperature meat consumption and cancers, may be similarly confounded, as formation of compounds such as HCA and PAH are dependent not only on food type but preparatory method.

i. An analysis of the MultiEthnic Cohort study data where processed meat included poultry and red meat products found association with pancreatic cancer.[13]
ii. Heinen et. al did not find a significant relationship between processed foods and pancreatic cancer, where processed foods included any foods which underwent some method of preservation, such as addition of nitrate salts.[14]
iii. A study of colorectal adenomas found no association with processed meats as a whole, but did find associations with "done-ness" (RR 1.21, 95% CI of 1.06-1.37 at the fifth quintile of consumption), and with bacon and sausage specifically (1.14, 95% CI 1.00-1.30). As with many such studies, no association was found with white meats; however, fried poultry and fish were not separated out, ignoring a potential effect of these preparations.[15] The differences in studies of meat and meat preparations, particularly in the dietary exposure classifications, makes comparison and synthesis difficult.[8]

E. A report by the National Food Institute at the University of Denmark summarized a number of American and European studies of meat consumption and CRC. Among the reviewed cohorts, approximately half showed a positive association between red or processed meat consumption and CRC.[1]

2) *While characterization of the vehicle of exposure is important in prevention and mitigation strategies, assessment of specific compounds' risk rather than focusing on that vehicle allows the data to be extrapolated to other exposure scenarios and vehicles, and to be used in cumulative, aggregate, and combined risk assessments.*

A. Mollusks, fish, and fats and oils may have on average higher or comparable concentrations of PAH to red meats, as do cocoa beans and butter (Table 1). Investigation of these food concentrations in conjunction with consumption rates is critical to investigation of dietary risks, particularly when subpopulations may have very different consumption patterns and/or preparation methods.[16]

B. Cereals contained the highest levels of PAH in a study of Spanish diet[17] and contributed the highest percentage of dietary intake of PAH in UK and the Netherlands,[18] see Table 2.

C. Smoking and cooking methods introduce significant variation in PAH and HCA levels even within the same food.[19]

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- **D.** Characterization of compounds which may be taken in through both inhalation during cooking and consumption after cooking, or as occupational inhalation exposures, may further confuse the categorization of exposures to a whole food, rather than a compound.
  - i. PAHs and HCAs may be generated in varying amounts (tables 2 and 3), and are known to have varying potencies as carcinogens among individual compounds within the group and depending on the exposure route.[18–20]
  - ii. The dermal and inhalation contact of barbecue fumes may contribute to PAH exposures (Wu et al estimated 2.8-27 ng exposure per day through inhalation, and 0.2-50 ng per day through dermal exposure from an hour of barbecuing).[21] Potential exposure differences to PAH may exist between those who cook/eat vs those who only eat.
  - iii. Workers in the catering and food preparation business or the main cook of a household may be exposed to airborne PAH/HCA and other compounds, adding to total dose but requiring differential toxicity information than if it were derived from red meat rather than individual compounds.[22]

3) *Given the presence of the suspected carcinogen compounds in other protein sources and foodstuffs in general, an exposure and risk substitution analysis is important in understanding the attributable risk of red meat consumption and various preparatory methods, as well as that of other meat substitute foods of which consumption might increase if meat consumption is reduced.*
   - **A.** The NTP Roadmap mentions the incorporation of alternatives assessment in evaluations, along with reduction and refinement of toxicological study models.[23] A comparison of dietary consumption of compounds of interest such as PAH and HCA among multiple alternatives would contribute to this goal and to understanding the relative hazards among diet choices.
   - **B.** Risk tradeoffs between alternatives are important to assess the net health effect of potential food substitutes.[24] Since red meat is a popular protein source, a decrease in its consumption would likely result in an increase in consumption of other protein sources. Tradeoffs are particularly important to evaluate where foods have the potential for high concentrations of PAH and other compounds due to environmental contamination, as is the case in Gulf fish following the BP spill.[25]
   - **C.** Red meat, fish, poultry, and shellfish among others potentially contain the suspected carcinogenic compounds in red meat.[20,26] Furthermore, some of these compounds, can also be found in grains, oils, and fruits and vegetables as reported in EFSA's summary (data reproduced in table 1). These differences in content among food types and preparation methods must be quantitatively evaluated to understand the net health effect of food substitutions.
   - **D.** To this end, EpiX Analytics is currently undertaking a quantitative comparative exposure assessment of American dietary consumption of PAH and HCA compounds to assess the

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relative exposure probabilities from various foods and preparatory methods. We will be glad to share our preliminary findings with NTP.

4) *Evaluation of whole food rather than a specific compound in food groups is counter to the strategy identified in the NTP Roadmap Vision of mechanism-based evaluation.*
    - **A.** According to the *NTP Roadmap for the 21st Century*, mechanistic endpoints will provide a guiding target for research and investigation of nominations.[23]
    - **B.** Understanding the mechanisms of red meat associated health impacts requires investigation into the specific pharmacokinetic pathways by which cancer or non-cancer outcomes are induced.
    - **C.** Mechanistic studies of PAH, N-nitroso compounds, HCA, and heme-iron suggest variable mechanisms by which colorectal cancer is initiated and promoted,[27] therefore dietary sources of mixtures of these compounds may not have a single mechanism of toxicity as a food, but might act through many different mechanisms such as cytotoxicity, epoxide formation, and competitive receptor interactions.[8]
    - **D.** Evaluating the whole food instead of compounds or compound mixtures is a step back from the goal of mechanistic toxicity and may prove relatively insensitive to exposure-risk and dose-response relationships in general, as smaller effect sizes can inhibit demonstration of dose-response, especially at human-relevant doses.[28]

Disclaimer: the comparative exposure assessment aforementioned is partly funded by the beef checkoff.

References

1. Mejborn, H. *et al*. *Mechanisms behind cancer risks associated with consumption of red and processed meat*. 73 (National Food Institute, Technical University of Denmark, 2016).

2. Fogelholm, M., Kanerva, N. & Männistö, S. Association between red and processed meat consumption and chronic diseases: the confounding role of other dietary factors. *Eur. J. Clin. Nutr.* 69, 1060–1065 (2015).

3. Oz, F. & Seyyar, E. Formation of Heterocyclic Aromatic Amines and Migration Level of Bisphenol-A in Sous-Vide-Cooked Trout Fillets at Different Cooking Temperatures and Cooking Levels. *J. Agric. Food Chem.* 64, 3070–3082 (2016).

4. Iwasaki, M. *et al.* Heterocyclic amines content of meat and fish cooked by Brazilian methods. *J. Food Compos. Anal.* 23, 61–69 (2010).

5. Sinha, R. *et al.* 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. *Cancer Res.* 55, 4516–4519 (1995).

6. Taniguchi, C. N. Evaluation of molluscs as dietary sources of iron: Heme and non-heme-iron content of clams and oysters consumed in the Asia-Pacific region. (University of Hawai'i at Manoa, 2015).

7. de Oliveira Otto, M. C. *et al.* Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. *Am. J. Clin. Nutr.* 96, 397–404 (2012).

8. Alexander, D. D., Weed, D. L., Miller, P. E. & Mohamed, M. A. Red Meat and Colorectal Cancer: A Quantitative Update on the State of the Epidemiologic Science. *J. Am. Coll. Nutr.* 34, 521–543 (2015).

9. Järvinen, R., Knekt, P., Hakulinen, T., Rissanen, H. & Heliövaara, M. Dietary fat, cholesterol and colorectal cancer in a prospective study. *Br. J. Cancer* 85, 357–361 (2001).

10. Hsing, A. W. *et al.* Risk factors for colorectal cancer in a prospective study among U.S. white men. *Int. J. Cancer* 77, 549–553 (1998).

11. Brink, M. *et al.* Meat consumption and K-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study. *Br. J. Cancer* 92, 1310–1320 (2005).

12. Larsson, S. C., Rafter, J., Holmberg, L., Bergkvist, L. & Wolk, A. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: The Swedish Mammography Cohort. *Int. J. Cancer* 113, 829–834 (2005).

13. Larsson, S. C., Håkanson, N., Permert, J. & Wolk, A. Meat, fish, poultry and egg consumption in relation to risk of pancreatic cancer: A prospective study. *Int. J. Cancer* 118, 2866–2870 (2006).

14. Heinen, M. M., Verhage, B. A. J., Goldbohm, R. A. & van den Brandt, P. A. Meat and fat intake and pancreatic cancer risk in the Netherlands Cohort Study. *Int. J. Cancer* 125, 1118–1126 (2009).

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15. Sinha, R. *et al.* Meat, Meat Cooking Methods and Preservation, and Risk for Colorectal Adenoma. *Cancer Res.* 65, 8034–8041 (2005).

16. Gossard, M. H. & York, R. Social Structural Influences on Meat Consumption. *ResearchGate* 10, (2003).

17. Falcó, G. *et al.* Polycyclic aromatic hydrocarbons in foods: human exposure through the diet in Catalonia, Spain. *J. Food Prot.* 66, 2325–2331 (2003).

18. European Food Safety Authority (EFSA). Findings of the EFSA Data Collection on Polycyclic Aromatic Hydrocarbons in Food: Findings of the EFSA Data Collection on Polycyclic Aromatic Hydrocarbons in Food. *EFSA J.* 5, 33r (2007).

19. Turesky, R. J., Taylor, J., Schnackenberg, L., Freeman, J. P. & Holland, R. D. Quantitation of Carcinogenic Heterocyclic Aromatic Amines and Detection of Novel Heterocyclic Aromatic Amines in Cooked Meats and Grill Scrapings by HPLC/ESI-MS. *ResearchGate* 53, 3248–58 (2005).

20. Sugimura, T., Wakabayashi, K., Nakagama, H. & Nagao, M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci.* 95, 290–299 (2004).

21. Wu, C.-C., Bao, L.-J., Guo, Y., Li, S.-M. & Zeng, E. Y. Barbecue Fumes: An Overlooked Source of Health Hazards in Outdoor Settings? *Environ. Sci. Technol.* 49, 10607–10615 (2015).

22. Vainiotalo, S. & Matveinen, K. Cooking Fumes as a Hygienic Problem in the Food and Catering Industries. *Am. Ind. Hyg. Assoc. J.* 54, 376–382 (1993).

23. National Toxicology Program. *A National Toxicology Program for the 21st Century: Roadmap to Achieve the NTP Vision*. (National Institute of Environmental Health Sciences, 2004).

24. Jacobs, M. M., Malloy, T. F., Tickner, J. A. & Edwards, S. Alternatives Assessment Frameworks: Research Needs for the Informed Substitution of Hazardous Chemicals. *Environ. Health Perspect.* 124, (2015).

25. Rotkin-Ellman, M., Wong, K. K. & Solomon, G. M. Seafood Contamination after the BP Gulf Oil Spill and Risks to Vulnerable Populations: A Critique of the FDA Risk Assessment. *Environ. Health Perspect.* 120, 157–161 (2012).

26. Jakszyn, P. *et al.* Development of a food database of nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. *J. Nutr.* 134, 2011–2014 (2004).

27. Oostindjer, M. *et al.* The role of red and processed meat in colorectal cancer development: a perspective. *Meat Sci.* 97, 583–596 (2014).

28. Lucier, G. W. Mechanism-based toxicology in cancer risk assessment: implications for research, regulation, and legislation. *Environ. Health Perspect.* 104, 84 (1996).

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Supporting figures and tables

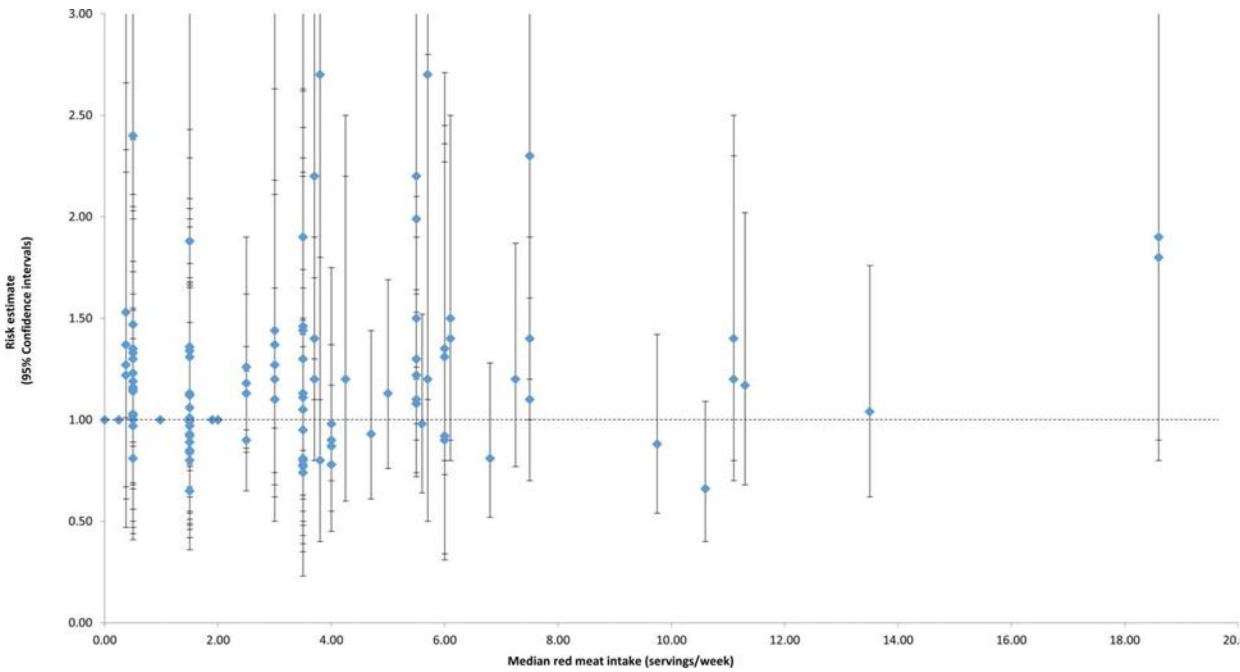

Figure 1: Relative risk estimates of colorectal cancer for each study included in the analysis stratified by servings per week. Reprinted from Figure 3 of Alexander et al 2015.[8]

Table 1: Percent of dietary intake of polycyclic aromatic hydrocarbons contributed by the food groups of the highest contributions. Modified from the European Commission Scientific Committee on Food.[18]

	UK (Σ11 PAH)	UK (Σ19 PAH)	Netherlands (Σ17 PAH)	Sweden (Σ11 PAH)
Oils and Fats	34%	-	14%	16%
Cereals	31%	35%	27%	34%
Vegetables	12%	13%	-	18%
Sugar and Sweets	-	-	18%	-
Meat	-	13%	-	-
Fruit and Sugar	6%	13%	10%	-

'-' represents unevaluated categories in individual studies

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Table 2: Levels in parts per billion of select polycyclic aromatic hydrocarbon per mass of food product in a collection of European studies. Modified from EFSA.[18]

	Benzo[a]pyrene		Benz[a]anthracene		Chrysene		Benzo(b)fluoranthene	
	Mean	95th percentile	Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Cocoa beans	0.007		0.13		0.06		0.07	
Fats & Oils	1.03	3.82	0.89	3.1	3.13	8.4	1.1	5.2
Meat & Smoked Meat	0.75	2.19	0.78	3.19	0.79	2.57	0.19	0.52
Fish & Smoked Fish	0.81	2.29	1.29	4.6	1.64	5.69	0.5	1.4
Fresh Fish	0.08	0.5	0.5	0.5	0.12	0.34	0.09	0.25
Canned smoked fish	2.97	34.1	9.62	34.1	8.66	34.7	3.41	16.8
Fresh mollusks	1.97	5.13	3.24	5.25	8.33	17.8	5.07	11.2
Cocoa butter	1.93	7.67	2.9	11.1	3.74	15.7	3.21	15.5
Cereal			0.08	0.37				

Table 3: Levels of various heterocyclic amines for assorted foods and preparatory methods in parts per trillion of food mass, modified from Sugimura et al Table 5[20] and Turesky et al. Table 1.[19]

	pan-fried beef (300C/6min)	**pan-fried beef (150-180C/10 min)**	**pan-fried beef scrapings**	**barbecued beef**	**barbecued chicken**	**Salmon (grilled)**	**Salted fish (grilled)**
IQ	260±43	<30	1,940±95	129±22	118±12		
IQ[4,5b]	210±48		3,300±404	172±8			
Iso-IQx	1,420±288		12,500±2570	1,070±80			
IQx	1,420±288	115±4	6,380±450	200±150	<30		
Iso-MeIQx	390±80		119,000±19400	6,490±790			
8-MeIQx	13,800±2230	1450±51	62,600±3420	1,600±95	335±65	600	670
4,8-DiMeIQx	5,310±715	175±20	15,000±176	431±17	276±47	0	90
7,8-DiMeI*g*Qx	1,020±345	84±61	7,670±3690	569±40	90±26	4,140	4,460
PhIP	15,200±2900	161±10	82,500±560	13,900±440	10,000±60	6,220	7,370
2-AαC	3,320±900	<30	2,890±596	7,750±621	8,700±1290		
MeAαC	143±60	<30	757±244	285±170	225±82		

Values represent Mean +/- Standard deviation (n=6)