Received via email on October 11, 2016

Dominik D. Alexander, PhD, MSPH

Principal Epidemiologist
EpidStat Institute

Dear Dr. Lunn,

I am a Principal Epidemiologist with EpidStat Institute, which is a research and consulting organization. Our group of epidemiologists and other health scientists collaborate with academic and governmental organizations on complex scientific matters, provide expert consultation, perform comprehensive literature reviews and quantitative assessments, and design and conduct studies with national and international academic institutions and public and private industries.

I received a doctorate degree in epidemiology under a National Cancer Institute Fellowship in Cancer Prevention and Control. Thus, I am a trained cancer epidemiologist, and my expertise is in the area of systematic review methodology, meta-analysis, and disease causation. I serve on the editorial board of the American Journal of Clinical Nutrition and I am an associate editor for Frontiers in Nutrition Methodology. Over the past decade, I have performed extensive research on red meat consumption and cancer. Specifically, I have applied systematic meta-analysis methodology and disease causation principles to red and processed meat intake, including correlates of meat intake such as cooking methods and heterocyclic amine exposures, and numerous types of cancer, such as colorectal, prostate, and breast. I've published 12 systematic reviews and meta-analyses on this topic in peer-reviewed journals, and have given over 30 presentations on the complexity and interpretation of epidemiologic studies of red meat intake and cancer to a wide variety of professional audiences, including registered dietitians, epidemiologists, nutrition scientists, toxicologists, and computational biologists. In addition, I have given talks internationally on red meat and cancer at various academic institutions and to governmental health leaders in Australia, New Zealand, Denmark, Norway, Mexico, and Canada.

Therefore, given my extensive epidemiologic research in the area of red meat and cancer epidemiology, I kindly ask that you earnestly consider my forthcoming scientific comments to

the Office of the Report on Carcinogens (RoC) in response to its September 9, 2016, Federal Register (80 FR 62513-14). I would also like you to consider my services as a cancer epidemiologist with applied knowledge on the topic of red and processed meat for any NTP committees or sub-groups on this complex scientific area.

Overview of Methodological Challenges

The potential role that red meat or processed meat intake plays on cancer risk has been widely debated in scientific communities. Indeed, interpreting findings from epidemiologic studies of dietary factors, such as individual foods or food groups, involves numerous methodological considerations. These include, but are not limited to: clearly and specifically defining the food variables (i.e., exposure) and outcomes of interest, accurately measuring food intake (a foremost challenge in nutritional epidemiology), accounting for dietary pattern differences across populations, understanding the role of bias and confounding within and across studies, isolating the effects of a single food or food group from the countless foods and dietary constituents that individuals consume on a daily basis, assessing potential (and relevant) biological mechanisms and genetic variation in metabolizing enzymes, and interpreting results based on varying analytical metrics and statistical testing parameters. What makes interpretation even more challenging is the fact that prospective cohort studies generate associations between foods and cancer that are very weak in magnitude, with most relative risks ranging between 0.8 and 1.25. Given the considerable degree of exposure misclassification from self-reported dietary intake, correlation of certain foods with other dietary and lifestyle factors, and the impact of bias and confounding, there is significant uncertainty surrounding the epidemiologic evidence for foods and cancer. In fact, despite billions of research dollars and decades of research, few, if any, foods have been clearly causally associated with increasing or decreasing the risk of cancer. Red meat consumption fits into this methodologically complex paradigm because of the inherent challenges of interpretation; many of which are more pronounced when evaluating red meat because of the high colinearity with other dietary and lifestyle factors.

The Utilization of Systematic Qualitative and Quantitative (Meta-Analysis) Assessments of the Body of Literature on Red and Processed Meat and Cancer

As discussed above, determining whether the scientific evidence supports an association between intake of red and/or processed meat and risk of cancer is a highly complex and methodologically challenging undertaking for several important reasons. The interdependency of food consumption with other dietary and lifestyle factors, socioeconomic characteristics, clinical variables, and genetic traits makes it difficult to isolate the independent effects of a specific food or food group, such as meat intake, on disease risk. Interpretation of findings from nutritional epidemiology studies are further complicated by the fact that this research area is particularly prone to reporting bias because of the numerous types of foods, food combinations, nutrients, and cooking methods ascertained on a typical food frequency questionnaire. Furthermore, results of many studies fail to distinguish fresh red meat from processed meat or they do not parse out individual meat types (pork, beef, lamb, salami, ham, etc.), limiting the certainty with which specific conclusions can be made regarding cancer associations for red and processed meat.

Because of the numerous aforementioned methodological and analytical challenges on this topic, the most scientifically appropriate approach to make informed decisions on whether red or processed meat may cause cancer is to perform systematic qualitative and quantitative meta-analysis assessments. This approach facilitates a critical examination of the totality of the available epidemiologic evidence. Specifically, a meta-analysis can be used to create informative sub-group stratifications, to evaluate potential dose-response relationships, to examine potential sources of heterogeneity and to evaluate the consistency of results by partially controlling for some of the study-specific characteristics. In addition, a meta-analysis improves the precision of summary estimates of association, which is especially important when attempting to demonstrate patterns of relative risks across subgroups. To this end, my research team and I have conducted systematic reviews and quantitative meta-analyses of red and processed meat intake and all types of cancer. Many of these assessments can be found in the included reference list. In addition, we have also evaluated correlates of meat intake, such as cooking methods and potential heterocyclic amine exposures from cooking and ingesting meat.

Our systematic evaluations of red meat and processed meat consumption and cancer risk involved conducting comprehensive literature searches using several scientific databases, including PubMed, EMBASE, the Cochrane Library, and hand searches of individual articles to

identify studies that reported data for red meat or processed meat intake and all types of cancer. Several hundred epidemiologic studies have been reviewed carefully for quality parameters and patterns of relative risk data. Based on our systematic appraisal of the studies, we performed quantitative meta-analysis modelling to estimate the relative strength of summary associations, and the consistency of findings across studies. In addition, we have evaluated potential dose-response relationships using categorical intake stratifications and continuous meta-regression methodology. Bias, namely misclassification bias, is a global concern in studies of dietary factors and cancer, and was considered in the assessment of findings. Confounding, which can be evaluated more quantitatively because of the reporting of covariates in each study, was appraised in the evaluation of the epidemiologic studies. The Sir Bradford Hill criteria for judging causality across observational studies was utilized. Specifically, the strength of association, potential dose-response patterns, and consistency of findings within and across studies were emphasized as these factors may be the most relevant when assessing the current state of the epidemiologic science on this topic. Importantly, an association should be established before causal criteria are applied. However, upon review of the epidemiologic evidence, an independent association (i.e., free from chance, bias, and confounding) is lacking for the red meat – cancer relationships. The associations between processed meat and some cancer types are slightly stronger in magnitude and more consistent than for fresh red meat, but suffer from the same methodological deficiencies. Despite this, the epidemiologic evidence was interpreted in the context of strength, dose, and consistency, while the role of chance, bias, and confounding were carefully considered.

The Epidemiologic Evidence Does Not Support a Causal Role Between Consumption of Red Meat or Processed Meat and Risk of Any Type of Cancer

In over a decade of research, my review and interpretation of the epidemiologic evidence of red and processed meat and cancer has included a critical examination of several key factors to consider when evaluating statistical associations in nutritional epidemiology. These have included the potential impact of bias across studies, the level of statistical adjustment for relevant risk factors by cancer site, the observation of statistically significant relative risks, the patterns of associations across sub-groups and study characteristics, and the variability in red and processed meat definitions. In addition to synthesizing and summarizing the epidemiologic associations,

three fundamental criteria from the Sir Bradford Hill guidelines for causality should be considered. These include the strength of association, dose-response patterns, and the consistency of relative risk associations within and across studies. In general, the stronger the magnitude of relative risk, the more likely the relationship is to be causal. Furthermore, when there are strong statistical associations, sources of uncertainty, such as confounding, exposure misclassification, and other biases may not be sufficient to obscure a valid association. Second, potential dose-response trends and patterns of associations by varying red meat and processed meat intake levels must be considered, particularly because red and processed meat are intertwined with other dietary factors and behavioral characteristics. A monotonically increasing relationship between an exposure and risk estimate is more likely to represent a causal relationship than other patterns. However, and as indicated, dietary behaviors are highly correlated and there is variability in how researchers define and analyze red and processed meat variables. Thus, dose-response patterns should be evaluated using different methodologies, such as stratified intake groupings based on uniform consumption levels and by continuous meta-regression modelling on each cancer site by increasing intake levels to determine if there is a positive linear trend. It would be expected that if an exposure was associated with increasing the risk of an outcome, then similar findings would be observed using varying analytical techniques, all of which have their own assumptions and strengths and weaknesses. Third, the consistency of associations across the individual studies and through meta-analysis methodology should be reviewed. The criterion of consistency refers to the extent to which the study results are similar (e.g., direction and magnitude of associations) across the entire body of evidence. The more consistent the findings are across different study populations, research groups, and time periods, the more likely the relationship is to be causal.

After careful evaluation and consideration of these fundamentally important factors for making causal inferences, collectively, the epidemiologic evidence either does not support a causal relationship between red or processed meat and any type of cancer, or the epidemiologic evidence is not sufficient to make a determination of causation because of limited and inadequate evidence.

Addressing the question of whether intake of red meat or processed meat is associated with cancer risk is complex, involving biological mechanisms, food definitions, intake measurements, outcome classifications, statistical testing, colinearity of red meat intake with other food items, and many lifestyle and behavior characteristics. Furthermore, making the extension from an association to making a determination of causation requires even greater scrutiny of the scientific evidence. Although positive associations between red meat or processed meat intake and different types of cancer have been reported in the literature, numerous cohort studies and meta-analyses have observed null findings as well. Despite some positive associations for certain types of cancer, namely colorectal cancer, the epidemiologic evidence does not support or is not sufficient to support a causal relationship. The primary reasons why the epidemiology does not support causation or why the evidence is insufficient to make a causal inference between red meat or processed meat consumption and cancer are as follows:

- Statistical summary associations are weak in magnitude (typically ranging between 0.80 and 1.25), making it difficult to differentiate an association that is not impacted by chance, bias, or confounding.
- There is no clear pattern of a dose-response relationship when coalescing different ways of evaluating potential trends, such as by categorical intake groupings, meta-regression, and continuous linear and/or non-linear patterns.
- Results between studies (and even within studies in many cases) are relatively inconsistent.
- A relatively small proportion of individual study relative risks, as well as summary relative risks in meta-analyses, are statistically significant.
- Heterogeneity is present across the literature, and several sources of between-study variation have been identified, such as by gender, study country, and tumor site.
- Definitions of red and processed meat vary considerably in the literature, rendering it difficult to harmonize exposure categories.
- The role of bias, namely misclassification bias, and confounding, coupled with the high degree of colinearity between red/processed meat intake and other dietary and lifestyle factors, warrants a cautious interpretation of the epidemiologic literature.

- There is a limited volume of human epidemiologic studies to make an informed decision for many red/processed meat – cancer relationships, such as for thyroid tumors and lymphohematopoietic malignancies.
- Carcinogenesis commonly takes several years to decades to progress and cancer comprises a heterogeneous array of specific types of malignant disease, each with their own set of etiological factors. The majority of epidemiologic studies analyzed participants over an insufficient duration to adequately account for cancer induction and latency. It is not just accounting for intake of red meat or processed meat over this time period, but also accounting for lifelong changes in other dietary factors, behavioral characteristics, socio-economic factors, and co-morbidity.

In 2015, my research team and I conducted systematic quantitative meta-analyses of red and processed meat intake and risk of all types of cancer. Based on this work, we have submitted for publication meta-analyses for two cancer sites, colorectal and prostate. The meta-analysis for colorectal cancer was published in the Journal of the American College of Nutrition (Alexander et al. 2015), and was the "most read" article in this journal for 2015. Our meta-analysis on prostate cancer was published in Nutrition Journal (Bylsma et al. 2015). Meta-analysis manuscripts for other cancer sites are ongoing. The following is a brief summary of findings from our collective body of work in this area:

Colorectal cancer. Based on our recently published meta-analysis (Alexander et al. 2015), the overall summary association between red meat and colorectal cancer is 1.10 (1.03-1.10) with statistically significant heterogeneity ($p = 0.010$). Out of 43 reported associations (high vs. low intake) for red meat intake at the individual study level, only seven are statistically significant. Of these, four were adjusted for a minimal amount of relevant confounding factors, and two were from the same study population. After removing studies that included processed meat items with fresh red meat items, the summary association dropped in half and became non-statistically significant with increased homogeneity. Furthermore, no association was found in the meta-analysis of red meat intake among women. Finally, there is no clear evidence of a dose-response pattern based on review of relative risks by increasing levels of intake. Based on the evidence from the epidemiologic studies, it is inconceivable that red meat could be considered a cause of

colorectal cancer. Although associations are slightly stronger in magnitude and consistency, the same challenges of interpretation are present for processed meat and colorectal cancer (see for example, Alexander et al. 2010). In fact, interpretation may be even more complex for processed meat than for fresh red meat because those who consume the highest amounts of processed meat tend to consume foods that may be associated with unhealthy dietary patterns, and they also tend to engage in behavioral activities that have chronic disease health implications.

Prostate cancer. In 2015 (Bylsma et al. 2015), we updated a prior meta-analysis publication of prospective cohorts of red and processed meats and prostate cancer with the inclusion of new and studies, as well as evaluate meat cooking methods, heme iron, and heterocyclic amine (HCA) intake exposure data. A total of 26 publications representing 19 different cohort studies were included in the analyses. The summary relative risk associations for total prostate cancer and total red meat consumption, fresh red meat consumption, and processed meat consumption were 1.02 (95% CI: 0.92-1.12), 1.06 (95% CI: 0.97-1.16), and 1.05 (95% CI: 1.01-1.10), respectively. Analyses were also conducted for the outcomes of non-advanced, advanced, and fatal prostate cancer when sufficient data were available, but these analyses did not produce significant results. No significant SRREs were observed for any of the meat cooking methods, HCA, or heme iron analyses. Dose-response analyses did not reveal significant patterns of associations between red or processed meat and prostate cancer. Clearly the epidemiologic evidence does not support an association between red meat or processed consumption and prostate cancer.

Breast cancer. Several epidemiologic studies have analyzed the relationship between red meat and processed meat and breast cancer, and overall, the findings do not support an independent positive association. All summary associations are null or weakly elevated, and there is no clear pattern of dose-response. Furthermore, analyses of heterocyclic amines and cooking methods have not produced significant results.

Pancreatic cancer. A modest, yet adequate, number of studies have been published on red or processed intake and cancer of the pancreas. Collectively there is no epidemiologic evidence to support an increased risk of pancreatic cancer based on consumption of red and/or processed meat across the prospective cohort studies.

Kidney cancer. A limited number of epidemiologic cohort studies have evaluated the relationship between red meat and processed meat and kidney cancer. There is no association between fresh red meat and kidney cancer, but there is a weakly elevated association for processed meat. The currently available epidemiologic evidence is considered limited and inadequate to formulate a conclusion on the relationship between red meat or processed meat consumption and kidney cancer.

Stomach cancer. The epidemiologic evidence base on red meat and processed meat and stomach cancer risk is relatively limited. Intake of fresh red meat has not been found to be associated with increasing the risk of stomach cancer, while processed meat has been associated weakly with stomach cancer. Stomach cancer development is clearly associated with certain factors, such as H. pylori infection, however, only one study accounted for this variable. Given the relatively modest number of studies, the fact that no association between red/processed meat was found in a large cohort of 500,000 US men and women, and the likely impact of H. pylori infection, the currently available studies provide limited evidence suggesting that processed meat intake is associated positively with stomach cancer risk.

Lung cancer. Historically, lung cancer has not been widely considered a malignancy with dietary etiology (aside from some trials of beta-carotene). Thus, modestly few studies providing limited epidemiologic evidence have analyzed red meat and processed meat intake and lung cancer. Interestingly, fresh red meat (although based on limited evidence from few studies) has been associated positively (albeit weakly) with lung cancer risk but processed meat (with several more data points than for fresh red meat) has not been associated with lung cancer. A foremost concern when interpreting associations with lung cancer is adequate control for smoking. Although studies may statistically adjust for smoking history, residual confounding by level of smoking is known to impact findings.

Esophageal cancer. Collectively, the epidemiologic data from prospective cohort studies are limited with highly variable results that seem to differ by histology (squamous v. adenocarcinoma), however, there are too few data points to examine sources of tissue type

heterogeneity. Another important consideration when interpreting findings for esophageal cancer is the fact that certain conditions, such as gastroesophageal reflux disease and genetic syndromes, have been strongly associated with increasing the risk of this malignancy. Conversely, high intake of fruits and vegetables has been associated inversely with esophageal cancer. Additional analyses that fully account for these important risk factors should be conducted before a conclusion can be drawn for red meat or processed meat and esophageal cancer.

Ovarian cancer. Although based on limited evidence from epidemiologic cohort studies, there is no association between intake of red or processed meat and risk of ovarian cancer.

Bladder cancer. The available epidemiologic evidence pertaining to red meat and processed meat consumption and bladder cancer is limited and may be considered inadequate to draw an informative conclusion. There are weak positive associations between red meat and processed meat intake and bladder cancer in dose-response analyses, but the data are sparse and high v. low intake analyses show no association.

Liver cancer. There is limited and inadequate evidence (owing largely to an incomplete consideration of potential confounding factors) to formulate a conclusion for red meat or processed meat intake and liver cancer. Summary associations are widely variable with no associations observed for intakes of processed and total red meat, while fresh red meat intake has been associated weakly and positively with liver cancer. These analyses were based on few studies.

Non-Hodgkin lymphoma. The relationship between red meat and processed meat has been evaluated in a small number of, albeit large, cohort studies. Overall, there is limited evidence suggesting an association between red meat or processed meat intake and non-Hodgkin lymphoma, with generally null associations reported in large cohort studies.

Other types of cancer. Although limited by relatively sparse data, the currently available epidemiologic evidence does not appear to support an independent positive association between

red meat or processed meat consumption and endometrial cancer, skin cancer, brain or thyroid cancer, Hodgkins lymphoma, multiple myeloma, cervical cancer, testicular or penile cancer, or the leukemias.

Although the human health epidemiologic data do not support a causal relationship between red meat or processed meat and any type of cancer, there are several postulated mechanisms as to why or how meat consumption may contribute to carcinogenesis. However, no mechanism has been established as being responsible for increasing the risk of cancer in human studies. Of these, heterocyclic amines exposure from cooking muscle meats have been studied most extensively, however, no single heterocyclic amine has been clearly associated with increasing the risk of human cancer in epidemiologic studies of meat intake. In addition, many studies have evaluated meat cooking methods and doneness of consumed meat but no clear indication of increased risk of cancer is supported by the epidemiologic evidence. There are numerous challenges to evaluating secondary exposures, such as heterocyclic amines. First, virtually all observational epidemiologic studies on this topic have ascertained dietary information from a food frequency questionnaire (FFQ). There is not a question on an FFQ that asks respondents about their usual exposure to dietary mutagens. Thus, self-reported food data are translated into secondary exposures using various sources, such as the CHARRED database. The limitations of using data from food frequency questionnaires have been well-documented. Thus, researchers are using self-reported dietary intake data (with underlying biases), and applying this information to a database that also has issues with exposure misclassification. This is a major limitation in these types of epidemiologic assessments. Second, many secondary exposures, such as heterocyclic amines, are not included in food composition databases, since they are not a natural component of food and have no nutritional value. Third, measurement is complicated by reporting of consumption of mixed meat dishes, whereby the specific level and type of meat is difficult to estimate. Fourth, exposure to the same mutagenic compounds in dietary studies may also result from tobacco smoke and other sources, as they may be present in the environment and ambient air.

Summary

The purpose of these comments are to highlight many of the study design, methodological, and analytical challenges of interpreting epidemiologic studies of red meat and processed meat consumption and cancer. These challenges are not restricted to studies of red meat intake, rather these issues pervade the field of nutritional epidemiology. Red/processed meat consumption should not be viewed as exempt to the shortcomings of interpreting data from observational epidemiologic studies of diet and cancer.

Based upon my extensive experience in conducting systematic evaluations of the epidemiologic evidence on red meat and processed meat consumption and cancer, and applying causal framework methodology to make informed decisions on potential hazards of red/processed meat intake and cancer, the totality of the available epidemiologic evidence is not supportive of an independent association between red meat or processed meat and any type of cancer.

I was an invited observer for the International Agency for Research on Cancer (IARC) deliberations on the hazards of red meat and processed meat and cancer in Lyon, France. I am aware that the IARC monograph on this topic may be published later this year or in 2017. In addition, the World Cancer Research Fund (WCRF) report on diet and cancer will be released in 2017. If the NTP decides to conduct a cancer hazard evaluation for red and processed meat, perhaps such an evaluation may be best served after the release of these two reports.

I am grateful for the opportunity to submit comments on this important and scientifically challenging topic, and I sincerely hope that you scrutinize the methodological and analytical complexities summarized herein when you review the patterns of associations across the epidemiologic studies. I would also like you to consider my services as a cancer epidemiologist with applied knowledge on the topic of red and processed meat for any NTP committees or sub-groups on this scientific area.

These comments were submitted independently. I am very active in the field in epidemiology, and I serve as principal investigator on a wide variety of research projects, such as studies of occupational and environmental factors, pharmacoepidemiology, cancer survival, and dietary factors. I receive research funding from numerous organizations, including the National

Institutes of Health, and public and private organizations. Some of my work on the topic of red and processed meat epidemiology has been supported by the Beef Checkoff. All of my research projects, regardless of funding support, are conceptualized, designed, conducted, and interpreted independently.

Sincerely,

Dominik D. Alexander, PhD, MSPH

Principal Epidemiologist
EpidStat Institute
Seattle, WA and Ann Arbor, MI
dalexander@epidstat.com
630-390-8190

My Red and Processed Meat Publications

Alexander DD, Weed DL. On the need for improved methodologic quality of published reviews. Am J Clin Nutr. 2016 Mar;103(3):683-4.

Bylsma LC, Alexander DD. A review and meta-analysis of prospective studies of red and processed meat, meat cooking methods, heme iron, heterocyclic amines and prostate cancer. Nutr J. 2015 Dec 21;14(1):125.

Alexander DD, Weed DL, Miller PE, Mohamed MA. 2015. Red Meat and Colorectal Cancer: A Quantitative Update on the State of the Epidemiologic Science, J Am Coll Nutr. 2015 Nov-Dec;34(6):521-43.

Miller PE, Alexander DD, Weed DL. 2014. Uncertainty of Results in Nutritional Epidemiology. Nutrition Today 49:147-152.

Alexander DD. 2013. No association between meat intake and mortality in Asian countries. Am J Clin Nutr 98:865-866.

Maki KC, Van Elswyk ME, Alexander DD, Rains TM, Sohn EL, McNeill S. 2012. A meta-analysis of randomized controlled trials that compare the lipid effects of beef versus poultry and/or fish consumption. J Clin Lipidol 6:352-361.

Bryan NS, Alexander DD, Coughlin JR, Milkowski AL, Boffetta P. 2012. Ingested nitrate and nitrite and stomach cancer risk: an updated review. Food Chem Toxicol 50:3646-3665.

Alexander DD, Weed DL, Cushing CA, Lowe KA. 2011. Meta-analysis of prospective studies of red meat consumption and colorectal cancer. Eur J Cancer Prev 20:293-307.

Alexander DD, Cushing CA. 2011. Red meat and colorectal cancer: a critical summary of prospective epidemiologic studies. Obes Rev 12:e472-e493.

Alexander DD, Morimoto LM, Mink PJ, Lowe KA. 2010. Summary and meta-analysis of prospective studies of animal fat intake and breast cancer. Nutr Res Rev 23:169-179.

Alexander DD, Miller AJ, Cushing CA, Lowe KA. 2010. Processed meat and colorectal cancer: a quantitative review of prospective epidemiologic studies. Eur J Cancer Prev 19:328-341.

Alexander DD, Mink PJ, Cushing CA, Sceurman B. 2010. A review and meta-analysis of prospective studies of red and processed meat intake and prostate cancer. Nutr J 9:50. doi: 10.1186/1475-2891-9-50.:50-59.

Alexander DD, Cushing CA. 2009. Quantitative assessment of red meat or processed meat consumption and kidney cancer. Cancer Detect Prev 32:340-351.

Alexander DD, Cushing CA, Lowe KA, Sceurman B, Roberts MA. 2009. Meta-analysis of animal fat or animal protein intake and colorectal cancer. Am J Clin Nutr 89:1402-1409.