February 6, 2017

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Re: Nominations to the National Toxicology Program for the Report on Carcinogens and Office of Health Assessment and Translation; Request for Information; FR Doc. 2016-21698; 81 *Fed. Reg.* 62513 (September 9, 2016)

Dear Drs. Lunn and Boyd:

The North American Meat Institute (NAMI or Meat Institute) submits these comments in response to the National Toxicology Program's (NTP) Request for Information (Request) concerning nominations for possible review for future editions of the Report on Carcinogens (RoC).[1] Formed from the merger of the American Meat Institute and North American Meat Association, the Meat Institute has a rich, century-long history and provides essential member services including legislative, regulatory, scientific, international, and public affairs representation. Together, NAMI members produce the vast majority of U.S. beef, pork, lamb, and poultry products – many of which are within three of the four "nominated substances."[2]

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[1] 81 *Fed. Reg*. 62513-14 (Sept. 9, 2016).
[2] NAMI's comments will focus on consumption of red meat, consumption of processed meat, and consumption of meat cooked at high temperatures.

The Request Should Examine Mechanisms that Present Legitimate Health Issues.

The Request is troubling for several reasons. First, rather than focusing on a food category, NTP should focus on specific components that could present legitimate health issues in a particular food and examine mechanisms to eliminate, reduce, or mitigate the potentially concerning components. NTP should not advise against eating whole foods that are a good source of required nutrients in the diet.

For example, when acrylamide was identified as a potential issue in baked and fried products, no one rationally suggested people not eat bread. Rather, science focused on developing ways to reduce acrylamide formation in foods during the cooking and production process. Scientists do not tell people not to eat potatoes because of solanine, the advice is not to eat green potatoes. Likewise, nutrition experts do not advise consumers not to eat spinach, even though it contains a reasonable amount of oxalic acid.

For this reason, the Request is misguided with respect to meat products. In the case of grilled meats the focus should not be to eliminate consumption, but an examination of how to reduce production of heterocyclic amines and polycyclic aromatic hydrocarbons, which can be accomplished through marinating the meat. For processed meat, concern about nitrosamines is largely eliminated if a source of Vitamin C is used, *e.g.* ascorbate. Finally, for red meat, the focus should be on eating a balanced diet including fruits and vegetables to aid absorption of critical nutrients required for health.

A Review of Red and Processed Meat Should not be done in a Vacuum Ignoring the Nutritional Benefits they provide.

Second, analyzing the impact of red meat or processed meat consumption cannot be done in a vacuum. Rather, that analysis must be done considering the context of a total diet. Meat consumption is one component of an individual's diet and there are countless nutrient and non-nutrient interactions that can enhance or inhibit the potential carcinogenicity of foods consumed, and their effect on the total diet. In addition, examining the "downside" to meat consumption without acknowledging the numerous nutritional benefits meat provides is a disservice to science and consumers.

Meat and poultry, which includes red and processed meats, are nutrient-dense foods and are part of a healthy dietary pattern. Nutrient-dense foods provide substantial amounts of vitamins and minerals (micronutrients) and relatively few calories compared to foods that have solid fat or added sugars. Diets are more likely to meet dietary recommendations if nutrient-dense foods, such as meat, are selected.[3] About 95 percent of Americans make meat and poultry products part of their diets, and for good reason.[4]

Meat and poultry products provide consumers with a convenient, direct, and balanced dietary source of all essential amino acids. These products are important sources of micronutrients, such as iron, selenium, vitamins B_{12}, B_6, thiamin, riboflavin, niacin, and potassium. Per serving, meat, poultry, and fish provide more protein than dairy, eggs, legumes, cereals, vegetables, or nuts. The iron and zinc in beef, pork, lamb, poultry, and fish are also more bioavailable than from other sources, meaning these minerals are more easily absorbed and utilized by the body.

Protein is critical for developing, maintaining, and repairing strong muscles; is vital for growth and brain development in children; and is essential to prevent muscle loss in the aged.[5,6] However, as many as 16 percent of U.S. adults and more than 20 percent of individuals over age 60 are marginally depleted in vitamin B_{12}, and this deficiency increases with age, as evidenced by the fact that six percent of adults age 70 and older are vitamin B_{12}-deficient.[7] Numerous studies demonstrate meat intake decreases bone fracture risk, which is crucial to the aging population because bone fractures can be a critical life event.[8,9,10] Meat and poultry play an integral role in ensuring adequate vitamin and mineral intake, and

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[3] Weaver, C.M, Dwyer, J., Fulgoni, V., King, J., Leveille, G.A., MacDonald, R.S., Ordovas, J. and Schnakenberg, D. 2014. Processed Foods: Contributions to Nutrition. Am J Clin Nutr DOI: 10.3945/ajcn.114.089284.
[4] 2012 Gallup Poll http://www.gallup.com/poll/156215/consider-themselves-vegetarians.aspx.
[5] Campbell, W. W., et al. (1999). "Effects of an omnivorous diet compared with a lactoovovegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men." Am J Clin Nutr 70(6): 1032-1039.
[6] Robinson, M. J., et al. (2013). "Dose-dependent responses of myofibrillar protein synthesis with beef ingestion are enhanced with resistance exercise in middle-aged men." Appl Physiol Nutr Metab 38(2): 120-125.
[7] Allen, L. H. (2009). "How common is vitamin B-12 deficiency?" Am J Clin Nutr 89(2): 693S-696S.
[8] Monma Y, Niu K, Iwasaki K, Tomita N, Nakaya N, Hozawa A, Kuriyama S, Takayama S, Seki T, Takeda T, Yaegashi N, Ebihara S, Arai H, Nagatomi R, Tsuji I. Dietary patterns associated with fall-related fracture in elderly Japanese: a population based prospective study. BMC Geriatr. 2010;10:31. PMID:20513246.
[9] Wosje KS, Khoury PR, Claytor RP, Copeland KA, Hornung RW, Daniels SR, Kalkwarf HJ. Dietary patterns associated with fat and bone mass in young children. Am J Clin Nutr. 2010;92(2):294-303. PMID:20519562.
[10] Samieri C, Ginder Coupez V, Lorrain S, Letenneur L, Alles B, Feart C, Paineau D, Barberger-Gateau P. Nutrient patterns and risk of fracture in older subjects: results from the Three-City Study. Osteoporos Int. 2013;24(4):1295-305. PMID:22976577.

recommendations that downplay this significance are counterproductive to the overall health of the American population.[11,12,13,14]

The high iron content in meat and poultry products is particularly important to certain subpopulations, including the 1.2 million children in America with anemia, and teenage girls and pregnant women who are at a higher risk of anemia.[15] Although iron supplementation can be an option, the heme iron present in meat is the most absorbable form of iron and continued deficiency could lead to long-term health effects, including decreased mood, shortness of breath, dizziness, and headaches, among others.[16] The natural presence of heme iron also aids absorption of non-heme iron.[17]

Lean meat's inclusion in a healthy dietary pattern is supported by the scientific evidence demonstrating its high nutritional value. The preponderance of the evidence, which affirms the healthful role lean meat and poultry, including red and processed meats, play in dietary patterns. In particular, followers of the Mediterranean-style diet, hailed as the "gold standard" by the 2015 Dietary Guidelines Advisory Committee, consume twice as many red and processed meats compared to amounts included in USDA Food Patterns.[18] Consuming a Mediterranean-style diet has shown to have positive effects on human health by reducing the incidence of cardiovascular disease and associated risk factors, having anti-inflammatory properties, and by reducing blood pressure and insulin resistance; all of which are also critical for reducing chronic disease. [19] Moreover, high-quality evidence from at least 12 randomized controlled trials shows that lean red meat is a beneficial component of a healthy dietary pattern, while other studies

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[11] Institute of Medicine, National Academy of Sciences. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press., Washington, DC. 2001. http://www.nap.edu/openbook.php?isbn=0309072794

[12] Institute of Medicine, National Academy of Sciences. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press. Washington, DC. 2000.

[13] National Academy of Sciences. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. National Academy Press. Washington, DC. 2000.

[14] Sharma, S., et al. (2013). Contribution of meat to vitamin B(12), iron and zinc intakes in five ethnic groups in the USA: implications for developing food-based dietary guidelines" J Hum Nutr Diet 26(2): 156-168.

[15] Accessed July 2, 2010: http://www.anemia.org/patients/feature-articles/content.php?contentid=000338.

[16] Iron and Iron Deficiency. http://www.cdc.gov/nutrition/everyone/basics/vitamins/iron.html.

[17] National Academy of Sciences. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press. Washington, DC. 2001.

[18] Data presented at November 7 Meeting of the 2015 Dietary Guidelines Advisory Committee (DGAC).

[19] Casa, R., et al. (2014). The Immune Protective Effect of the Mediterranean Diet Against Low-grade Inflammatory Disease. Endocrine, Metabolic & Immune Disorders - Drug Targets, 14, 245-254

revealed that meat and poultry, when consumed in combination with vegetables, help the body absorb more nutrients from those vegetables.[20,21,22,23,24,25,26,27,28,29,30,31] Research has also shown that meat's high protein and low carbohydrate content offer benefits for both weight management and diabetes control.[32,33,34,35,36]

On average, a three-ounce serving of lean beef is about 150 calories and is an excellent source of six nutrients, including protein, zinc, vitamin B_{12}, vitamin B_6, niacin, and selenium, and a good source of four nutrients—phosphorous, choline, iron, and riboflavin.[37] Lamb is also nutrient dense and on average, a 3-ounce cooked portion, provides greater than 20 percent of the daily value of zinc, vitamin B_{12}, niacin, and protein in about 175 calories.[38] In addition, more than 65 percent of beef cuts sold at retail meet USDA standards for "lean," including 17 of the 25 most popular cuts.

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[20] Clara Lau. Comment #874. Submitted to 2015 DGAC December 11, 2014.
[21] Clara Lau. Comment #638. Submitted to 2015 DGAC August 27, 2014.
[22] Clara Lau. Comment #638. Submitted to 2015 DGAC July 23, 2014.
[23] Shalene McNeill. Comment #566. Submitted to 2015 DGAC July 11, 2014.
[24] Clara Lau. Comment #416. Submitted to 2015 DGAC March 14, 2014.
[25] Clara Lau. Comment #402. Submitted to 2015 DGAC March 10, 2014.
[26] Clara Lau. Comment #395. Submitted to 2015 DGAC March 5, 2014.
[27] Shalene McNeill. Comment #113. Submitted to 2015 DGAC September 23, 2013.
[28] Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: Human studies. Am J Clin Nutr 1997;65:1628S-44S.
[29] Kris-Etherton PM, Pearson TA, Wan Y, Hargrove RL, Moriarty K, Fishell V, et al. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr 1999;70:1009-15.
[30] Gilmore LA, Walzem RL, Crouse SF, Smith DR, Adams TH, Vaidyanathan V, Cao X, Smith SB. Consumption of high-oleic acid ground beef increases HDL-cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. J Nutr 2011;141:1188-1194.
[31] Gilmore LA, Crouse SF, Carbuhn A, Klooster J, Calles JAE, Meade T, Smith SB. Exercise attenuates the increase in plasma monounsaturated fatty acids and high-density lipoprotein cholesterol, but not high-density lipoprotien 2b cholesterol caused by high-oleic ground beef in women. Nutr Res 2013;33:1003-1011.
[32] Leidy, Mattes. Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity.* Obes Res. 2007; 15: 421-429.
[33] Layman, D. K., et al. (2009). A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. J Nutr 139(3): 514-521.
[34] Paddon-Jones, D., et al. (2008). Protein, weight management, and satiety. Am J Clin Nutr 87(5): 1558S-1561S.
[35] Leidy, H. J., et al. (2010). The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men. Obesity. 18(9): 1725-1732.
[36] Leidy, H. J., et al. (2011). The effects of consuming frequent, higher protein meals on appetite and satiety during weight loss in overweight/obese men. Obesity. 19(4): 818-824.
[37] U.S. Department of Agriculture, Agricultural Research Service. 2010. USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page.
[38] Carson, Jo Ann S., Hilton, G.G. and VanOverbeke. (2007) Lamb: It's place in the U.S. diet. http://leanonlamb.com/media/activity/lamb_in_US_Diet.pdf. Accessed May 7, 2015.

In addition to lean meat and poultry's important dietary role, processed meats also fit within a healthy dietary pattern and offer key nutrients. The Meat Institute commissioned a menu model analysis using the *Dietary Guidelines for Americans, 2010* requirements for macro- and micro- nutrients and food groups based on a 2,000-calorie per day diet. The results demonstrated that processed meats, even when consumed twice daily for one week, allow consumers to stay within daily calorie and nutrient goals, while also helping individuals meet or exceed recommended nutrient intakes. Processed meat and poultry product offerings are diverse and include luncheon meats, marinated roasts, and fully cooked meatloaf and turkey breast, among others. These products are also available in low-fat, low-sodium, and other formulations to fit consumer nutrition needs and taste preferences. Not only do processed meats have an exemplary safety record, but these products are also convenient, affordable, and provide crucial nutrients and protein to people on fixed incomes and to those groups with limited abilities to prepare food at home.[39]

<u>The International Agency for Research on Cancer's Review is flawed.</u>

In 2015, the International Agency for Research on Cancer (IARC) failed to consider that the above-discussed benefits of meat and poultry consumption far outweigh perceived negative health outcomes and NTP would be making a similar error if it elects to consider evaluating whole foods rather than chemicals or components of food.[40] This concern is particularly applicable given the inadequacy of much of the data IARC used to concoct its conclusions, *i.e.*, national survey data and research studies do not adequately separate or consistently define meat from red meat from processed meat, *etc*. Creating additional problems are the considerable challenges associated with designing studies with whole foods to determine an endpoint effect. Whole foods are generally not appropriate for traditional or standardized toxicology studies designed to find a dose-response effect. For red meat specifically, lack of a clearly defined, plausible mechanism by which red meat uniquely may influence cancer risk, limits the specificity needed in a cancer evaluation and does not offer the public with meaningful guidance.

NTP should not repeat IARC's mistakes. IARC erroneously drew sweeping conclusions about whole categories of products, treating all products in those categories as if they are the same and as if the same scientific results were found in all studies. They are not.

[39] American Meat Institute Comments. Submitted to the 2015 DGAC July 7, 2014.
[40] Indeed, NTP has recognized that the ability to evaluate the carcinogenicity of a single substance associated with the consumption of meat is limited. National Toxicology Program (NTP). 2014. Report on Carcinogens, Thirteenth Edition. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service.

IARC classified "processed meats" as Group 1, but conveniently ignored the fact that processed meat as a category includes thousands of products. Processed meat includes, pimento loaf, numerous types of bologna, countless styles of bacon, various hard salamis, numerous styles of soppresseta, cooked ham, cured ham, Iberian ham, turkey ham, cooked roast beef, hot dogs that include beef and pork, all beef hot dogs, turkey hot dogs, scrapple, goetta, liverwurst, liver pudding, pate, pastrami, bratwurst, mortadella, corned beef, beef and other types of jerky, as well as thousands of other processed products made around the world. IARC painted all of these products with the Group 1 classification in the absence of any scientific evidence examining most of these products and for those products. Of those products that were the subject of scientific review, there were conflicting results.

IARC's classification of red meat is not as flawed but only because the classification is 2A. Regarding red meat, again the scientific results are mixed with little, if any, recognition of the differences among the various red meat products. Red meat includes everything from ribeye steak to pork chops to veal cutlets to venison burgers and beyond. Yet, IARC throws a blanket classification over the category, notwithstanding the fact the scientific studies that are not applicable to most of the products in the category.

The Request makes the same flawed assumptions when it asks for information regarding product categories that are so vast they are almost immeasurable. NTP should not take a blunderbuss approach to examining these issues. Rather, NTP should be more focused and targeted in whatever, if any, analysis it undertakes.

<u>There is no Definitive Link to Meat Consumption and Many Human Health Issues.</u>

NTP should also consider that while red meat consumption has decreased over time, no changes in negative health outcomes have been observed. Protein intake has remained relatively stable over the last 35 years but there has been a shift in the product category where consumers are getting their dietary protein. Attributable to nutritional recommendations to reduce red meat consumption and cost, consumption of all red meats, *e.g.* beef, veal, pork, lamb, and mutton, has decreased from 133.9 pounds (retail) in 1965 to 104.3 pounds in 2013 – a 22 percent decrease.[41] For the entire animal protein category, which includes all red meat, poultry, and fish, per capita consumption was 177.5 pounds in 1970. [42] Total animal protein consumption in 2013, the most recent year available, was 182.8 pounds, [43] a modest three percent increase over 43 years. In short, all red meat consumption declined markedly, while both chicken and turkey consumption nearly doubled and

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[41] North American Meat Institute and Sterling Marketing. *The 2014 Meat and Poultry Facts.*
[42] *Id.*
[43] *Id.*

fish consumption increased 17 percent. Given that obesity and adverse health outcomes have increased over that same time period, red meat consumption alone cannot be the root cause.

The Request Exceeds NTP's Statutory Charge.

Finally, this Request is an "overreach" by NTP. As discussed in the Request, the ROC evaluates substances "in our environment" that pose a cancer risk. Three topics identified in the Request, consumption of red meat, consumption of processed meat, and consumption of meat cooked at high temperatures, are not "substances in our environment" as contemplated by the law and therefore are outside the scope of what NTP is tasked by statute with reviewing.[44]

To assist NTP as it examines the questions posed in the Request, NAMI has attached to these comments numerous articles and other scientific literature that warrant careful consideration.

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The North American Meat Institute would be happy to discuss these comments, the Meat Institute's position regarding the Request, or questions you have. Thank you.

Respectfully submitted,

[redacted]

Mark Dopp
Senior Vice President, Regulatory and Scientific Affairs, & General Counsel

cc: Barry Carpenter
Norm Robertson
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[44] See Public Health Service Act 301(b)(4).

Scientific Studies By Topic

http://www.meatpoultrynutrition.org/content/scientific-studies-topic

General Nutrition Contributions of Meat

Composition of free and peptide-bound amino acids in beef chuck, loin, and round cuts, Journal of Animal Science, 2016
Dietary Protein Intake and Human Health, Food & Function, 2016
Research gaps in evaluating the relationship of meat and health, Meat Science, 2015
Inclusion of red meat in healthful dietary patterns, Meat Science, 2014
Vitamins and cognitive development and performance: Nutritional determinants of cognitive aging and dementia, Proceedings of the Nutrition Society, 2012
Meat Nutritional Composition and Nutritive Role in the Diet, Meat Science, 2012
Nutrient contribution of total and lean beef in diets of US children and adolescents: National Health and Nutrition Examination Survey 1999-2004., Meat Science, 2011
Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet?, Meat Science, 2004

Iron Deficiency Anemia and Iron Absorption

Iron We Consume, Iron Disorders Institute, 2009. http://www.irondisorders.org/iron-we-consume/ Accessed October 5, 2015
Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy, American Journal of Clinical Nutrition, 2015
Nutrition and Health – The Association between Eating Behavior and Various Health Parameters: A Matched Sample Study, PLoS One, 2014
Anemia and iron deficiency in children: association with red meat and poultry consumption, Journal of Pediatric Gastroenterological Nutrition, 2013
Iron and Zinc Nutrition in the Economically-Developed World: A Review, Nutrients, 2013
Iron bioavailability and dietary reference values, American Journal of Clinical Nutrition, 2010
Iron Deficiency Anemia, American Family Physician, 2007
Effect of beef and soy proteins on the absorption of non-heme iron and inorganic zinc in children, Journal of the American College of Nutrition, 2006
Iron deficiency anaemia: Effect on cognitive development in children: A review, Indian Journal of Clinical Biochemistry, 2005
Meat Protein Fractions Enhance Nonheme Iron Absorption in Humans, The Journal of Nutrition, 2006
Nutrition for Healthy Term Infants: Recommendations from 6 months to 24 months., Health Canada

B12 Deficiency
Brief Report: Childhood Disintegrative Disorder as a Likely Manifestation of Vitamin B12 Deficiency, Journal of Autism and Developmental Disorders, 2013
Delirium as a result of vitamin B12 deficiency in a vegetarian female patient, European Journal of Clinical Nutrition, 2013
Vitamin B-12 and homocysteine status among vegetarians: a global perspective, American Journal of Clinical Nutrition, 2009
Neonatal Vitamin B12 Deficiency Secondary to Maternal Subclinical Pernicious Anemia: Identification by Expanded Newborn Screening, Journal of Pediatrics, 2008
Vitamin B12 deficiency in infancy as a cause of developmental regression, Brain and Development, 2005
Vitamin B_{12} Deficiency and Depression in Physically Disabled Older Women: Epidemiologic Evidence From the Women's Health and Aging Study, Indian Journal of Clinical Biochemistry, 2005
Severe Vitamin B12 Deficiency in an Infant Associated With a Maternal Deficiency and a Strict Vegetarian Diet, Pediatric Hematology/Oncology, 2004
Vitamin B_{12} Deficiency and Depression in Physically Disabled Older Women: Epidemiologic Evidence From the Women's Health and Aging Study, The American Journal of Psychiatry, 2000
Serum vitamin B12 and blood cell values in vegetarians, Annals of Nutrition Metabolism, 1982

Bone Health
Vegetarian diets and bone status, American Journal of Clinical Nutrition, 2014
Protein intake and bone health, International Journal for Vitamin Nutrition Research, 2006

Brain Development/Cognitive Function
Vitamin B12 Deficiency: An Important Reversible Co-Morbidity in Neuropsychiatric Manifestations, Indian Journal of Psychological Medicine, 2015
Animal Protein Intake Is Associated with Higher-Level Functional Capacity in Elderly Adults: The Ohasama Study, Journal of the American Geriatrics Society, 2014
Delirium as a result of vitamin B12 deficiency in a vegetarian female patient, European Journal of Clinical Nutrition, 2013
Vitamin B12 Intake and Status and Cognitive Function in Elderly People, Epidemiologic Reviews, 2013
Vitamin B12 and cognitive function: an evidence-based analysis. Ontario Health Technology Series Assessment, 2013
Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology, 2012
Effects of vitamin B12 and folate deficiency on brain development in children, Food Nutrition Bulletin, 2008
Iron deficiency anaemia: Effect on cognitive development in children: A review, Indian Journal of Clinical Biochemistry, 2005
Vitamin B_{12} Deficiency and Depression in Physically Disabled Older Women: Epidemiologic Evidence From the Women's Health and Aging Study, The American Journal of Psychiatry, 2000
Could carnosine or related structures suppress Alzheimer's disease?, Journal of Alzheimer's Disease, 2007
Serum 25-hydroxyvitamin D levels and the risk of depression: a systematic review and meta-analysis.

D (Vitamin D)
Natural Vitamin D Content in Animal Products, Advances in Nutrition, 2013

Cancer and Meat
Stomach Cancer
Ingested nitrate and nitrite and stomach cancer risk: An updated review, Food and Chemical Toxicology, 2012
Dietary Patterns and Risk of Stomach Cancer Mortality: The Japan Collaborative Cohort Study, Annals of Epidemiology, 2010

Colon Cancer
The role of red and processed meat in colorectal cancer development: a perspective, Meat Science, 2014
Red meat and colorectal cancer: a critical summary of prospective epidemiologic studies, Obesity Reviews, May 2011
Meta-analysis of prospective studies of red meat consumption and colorectal cancer, European Journal of Cancer Prevention, 2011
Vitamins, minerals, essential fatty acids and colorectal cancer risk in the United Kingdom Dietary Cohort Consortium, International Journal of Cancer, 2011
Processed meat and colorectal cancer: a quantitative review of prospective epidemiologic studies, European Journal of Cancer Prevention, 2010
Low-Fat Dietary Pattern and the Risk of Colorectal Cancer, Journal of the American Medical Association, 2006
Dietary Fat and Fatty Acids and Risk of Colorectal Cancer in Women, American Journal of Epidemiology, 2004
Meat, Fat, and Their Subtypes as Risk Factors for Colorectal Cancer in a Prospective Cohort of Women, American Journal of Epidemiology, 2003
Diet and risk of colorectal cancer in a cohort of Finnish men, Cancer Causes and Control, 1999

Breast Cancer
Protein Intake and Breast Cancer Survival in the Nurses' Health Study, Journal of Clinical Oncology, 2016

Diabetes
Impact of Type 2 diabetes on lymphatic vessels identified, Amino acid found in red meat, poultry may improve lymphatic function in diabetes, Cardiovascular Research, 2015
Basic Meal Planning. Canadian Diabetes Association. Accessed September 21, 2015.
Control of blood glucose in type 2 diabetes without weight loss by modification of diet composition, Nutrition & Metabolism, 2006
Effect of a High-Protein, Low-Carbohydrate Diet on Blood Glucose Control in People With Type 2 Diabetes, Diabetes, 2004
Diet, nutrition and the prevention of type 2 diabetes, Public Health Nutrition, 2004

Isoflavones
USDA Database for the Isoflavone Content of Selected Foods. Release 2.0., US Department of Agriculture, Agricultural Research Service, 2008

Macronutrients
DRI for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids, Institute of Medicine, Food and Nutrition Board, 2005

Meat Consumption Data
Overview of Canadians' Eating Habits, Statistics Canada, 2007. Accessed September 22, 2015.
Do Canadian Adults Meet Their Nutrient Requirements Through Food Intake Alone?, Health Canada, 2012. Accessed September 22, 2015.
Food Statistics Analysis. Animal Products, Health Canada, last modified 2010. Accessed September 22, 2015.

Nitrite Safety and Benefits
Dietary inorganic nitrate: From villain to hero in metabolic disease?, Molecular Nutrition and Food Research, 2016
Sodium Nitrite Reduces Systolic Blood Pressure in Preeclampsia, Journal of the Federation of American Societies of Experimental Biology, April 2015
Potential Therapeutic Effects of Nitrate/Nitrite and Type 2 Diabetes Mellitus, International Journal of Endocrinology Metabolism, 2013
Ingested nitrate and nitrite and stomach cancer risk: An updated review, Food and Chemical Toxicology, 2012
Human safety controversies surrounding nitrate and nitrite in the diet, Nitric Oxide, 2012
Sodium Nitrite in Processed Meat and Poultry Meats: A Review of Curing and Examining its Risk Benefit Use, American Meat Science Association White Paper, 2011
Nutritional epidemiology in the context of nitric oxide biology: A risk–benefit evaluation for dietary nitrite and nitrate. Nitric Oxide, 2010
Food sources of nitrates and nitrites: the physiologic context for potential health benefits, American Journal of Clinical Nutrition, 2009
Sodium nitrite promotes regional blood flow in patients with sickle cell disease: a phase I/II study, British Journal of Haematology, 2008
The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics, Nature Reviews, 2008
Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury, Proceedings of the National Academy of Sciences, 2007
NO Generation From Nitrite and Its Role in Vascular Control, Arteriosclerosis, Thrombosis, and Vascular Biology, 2005
Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver, The Journal of Clinical Investigation, 2005
The nitric oxide/cyclic GMP pathway in organ transplantation: critical role in successful lung preservation, Proceedings of the National Academy of Sciences, 1994

Organic vs. Conventional Nutrition

Nutritional quality of organic foods: a systematic review, American Journal of Clinical Nutrition, 2009

Nutritional quality of organic food: shades of grey or shades of green? Proceedings of the Nutrition Society, 2002

Sarcopenia Prevention (Muscle Loss)

Protein-enriched diet, with the use of lean red meat, combined with progressive resistance training enhances lean tissue mass and muscle strength and reduces circulating IL-6 concentrations in elderly women: a cluster randomized controlled trial, American Journal of Clinical Nutrition, 2014

Nutrient-rich meat proteins in offsetting age-related muscle loss, 58th International Congress of Meat Science and Technology, 2012

Position of the Academy of Nutrition and Dietetics: Food and Nutrition for Older Adults: Promoting Health and Wellness. Academy of Nutrition and Dietetics, 2012

Dietary protein recommendations and the prevention of sarcopenia, Protein, amino acid metabolism and therapy, Current Opinion Clinical Nutrition and Metabolic Care, 2009

Relationship between animal protein intake and muscle mass index in healthy women, British Journal of Nutrition, 2009

Satiety (Satisfaction)

Inclusion of Pork Meat in the Diets of Young Women Reduces Their Intakes of Energy-Dense, Nutrient-Poor Foods: Results from a Randomized Controlled Trial, Nutrients, 2014

The contribution of gastrointestinal appetite hormones to protein-induced satiety, The Journal of the Federation of the American Societies for Experimental Biology, 2012

Pork, beef and chicken have similar effects on acute satiety and hormonal markers of appetite, Appetite, 2011

Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance, New England Journal of Medicine, 2010

The satiating power of protein—a key to obesity prevention?, The American Journal of Clinical Nutrition, 2005

A Satiety Index of Common Foods, European Journal of Clinical Nutrition, 1995

Saturated Fat and Coronary Heart Disease

Nutrition data review shows red meat has neutral effect on cardiovascular disease risk factors, American Journal of Clinical Nutrition, 2016.

Trans fats, but not saturated fats like butter, linked to greater risk of early death and heart disease, British Medical Journal, August 2015.

Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies, British Medical Journal, 2015

Dietary Intake of Saturated Fat Is Not Associated with Risk of Coronary Events or Mortality in Patients with Established Coronary Artery Disease, The International Journal of Nutrition, 2015

Association of Dietary, Circulating, and Supplement Fatty Acids With Coronary Risk: A Systematic Review and Meta-analysis, Annals of Internal Medicine, 2014

Dietary Protein Intake and Blood Pressure: A Meta-Analysis of Randomized Controlled Trials, American Journal of Epidemiology, 2012

Beef in an Optimal Lean Diet study: effects on lipids, lipoproteins, and apolipoproteins, American Journal of Clinical Nutrition, 2012

Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease, American Journal of Clinical Nutrition, 2010

Partial substitution of carbohydrate intake with protein intake from lean red meat lowers blood pressure in hypertensive persons, American Society for Clinical Nutrition, 2006

Sodium/Salt

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NORTH AMERICAN
MEAT INSTITUTE

September 11, 2015

Dr. Veronique Bouvard, Responsible Officer
Dr. Kurt Straif, Head of the IARC Monographs Programme
IARC
Lyon, France

Re: Volume 114: Red Meat and Processed Meat – Call for Data – Evidence Is Insufficient To Classify Dietary Nitrate, Nitrite, And Endogenous *N*-Nitrosation In The Stomach As Involved In Carcinogenic Mechanisms Related To Red And Processed Meat Consumption

Dear Drs. Bouvard and Straif:

The North American Meat Institute (NAMI or Meat Institute) is the leading voice for the meat and poultry industry. Formed from the merger of the American Meat Institute and North American Meat Association, the Meat Institute has a rich, century-long history and provides essential member services including legislative, regulatory, scientific, international, and public affairs representation. Together, the Meat Institute's members produce the vast majority of U.S. beef, pork, lamb, and poultry, in addition to the equipment, ingredients, and services needed to produce the safest and highest quality products. The Meat Institute appreciates the opportunity to provide scientific evidence for consideration by the International Agency for Research on Cancer (IARC) Expert Panel during its upcoming review of red and processed meats.

In Monograph Volume 94 (2010), IARC makes the following determination, "*Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).*" IARC notes that this distinction is due largely to the endogenous inter-conversion of nitrates and nitrites and the mitigating dietary factors which complicate the evaluation of nitrates and nitrites when consumed as part of a whole diet, rather than provided as isolated compounds. IARC conducted its monograph review meeting in June 2006 and published the final monograph in July 2010.

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New scientific evidence is available. In 2012, a group of prominent researchers requested that IARC reevaluate its conditional classification of nitrate and nitrite from Group 2A to Group 3.[1] Since 2012, scientific evidence published strengthens the justification of a lower classification of endogenous nitrosation as a carcinogenic mechanism. Consideration of this new scientific evidence by the Working Group for Monograph Volume 114 is critical as IARC evaluates red and processed meats. Specifically, the Working Group should:

- consider scientific evidence that distinguishes between *N*- and *S*-nitrosation, rather than broadly considering "nitrosation" a carcinogenic mechanism;
- recognize mechanistic evidence that refutes a role of nitrates, nitrites, and endogenous nitrosation in cancer development; and
- consider endogenous nitrosation in the context of a complete and varied human diet where mitigating factors nullify any consequence of endogenous *N*-nitrosation.

Endogenous Nitrosation Represents *both S-* and *N*-nitrosation.

The distinction between *S*-nitrosation and *N*-nitrosation is critical as IARC considers "endogenous nitrosation" as a mechanism by which red and processed meats may cause cancer. Previously, IARC only considered scientific evidence related to endogenous nitrosation in the context of the *N*-nitrosation pathway and did not evaluate the fundamental role *S*-nitrosation plays in human physiology. IARC's limited focus on *N*-nitrosation does not accurately put into context the hazard-benefit role that endogenous nitrosation has in the human body. Had IARC evaluated the mechanistic evidence of both *S*-nitrosation and *N*-nitrosation, the scientific evidence would have overwhelmingly led to a lower classification or, at the very least, a more precise definition of endogenous nitrosation.

Bryan *et al*. provides an excellent summary of endogenous nitrosation, how physiologically it is critical to human health and its history as it relates to cancer.[2,3] Bryan *et al*. also discusses the important differences between *S*-nitrosation and *N*-nitrosation. *S*-nitrosation is the physiological process to convey nitric oxide (NO) biochemistry, which has critical functions in many health outcomes, such as cardiovascular disease,[4] metabolic syndrome,[5]

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hypertension,[6] inflammatory bowel disease,[7] lung disease including chronic obstructive pulmonary disease,[8] and osteoporosis.[9]

Vegetables are the major exogenous contributor of nitrate in the human diet accounting for 60-80 percent of ingested nitrate, followed by drinking water (15-20 percent) and, lastly, the contribution from the consumption of cured meats, which nitrate and nitrite are added for preservation (10-15 percent).[10] All exogenous sources, however, are limited in importance with respect to endogenous nitrite, of which 80-85 percent of human exposure occurs due to *in vivo* conversion of nitrate.[11] Humans excrete five times the nitrate they ingest.[12]

N-nitrosamines and *N*-nitrosamides comprise the group of substances known as *N*-nitroso compounds (NOC). NOC are the end result of endogenous *N*-nitrosation or they can be formed in heated foods and be consumed in the diet. However, it is the inability to effectively distinguish between endogenous versus exogenous NOC exposure, via validated biomarkers, and to thus confirm if either is directly linked to benefit or harm that limits the ability to establish the relationship between nitrates, nitrites, and overall NOC exposure and cancer risk.[13]

A recent expert panel organized by the Senate Commission on Food Safety of the German Research Foundation concluded that *"…there is a need for further research addressing potentially negative and positive health effects associate with dietary nitrate and nitrite exposure. The available evidence is inadequate to be used as a basis for a comprehensive and reliable assessment of positive as well as negative health effects, especially regarding long term effects*."[14] "*Future comprehensive studies need to take into consideration, among other parameters, nitrate/nitrite exposure and extent of NOC formation in vivo, the nature and relevance of* N*-nitrosatable precursors and the resulting NOC, the influence of individual dietary and physiologic factors, but also the individual health status, especially with respect to those conditions favoring endogenous NOC formation, including inflammatory and/or infectious diseases."*[15]

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[6] McNally et al., 2015.
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Evidence from Human Observational Studies Does Not Support a Link between Ingested Meat, Nitrate, Nitrite, or Endogenous Nitrosation and Cancer.

An important consideration in the formation of NOCs is the reaction's dependence on the pH of the fluid or medium *in vivo*, because at certain pH levels NOC formation is optimized. Nitrite under acidic conditions must first be converted to nitrous acid (HONO), an extremely unstable compound, which is in equilibrium with nitrous anhydride (N_2O_3), the required nitrosating species. Nitrous anhydride is produced from two molecules of HONO, hence the rate of the reaction is dependent on the square of the nitrite concentration as noted above.

The pKa value for the nitrous acid to nitrite anion equilibrium is 3.34, meaning that at a pH of 3.34, only 50 percent of a HONO nitrosating agent can exist in solution, such as in the stomach contents, while at a higher pH there is much less nitrosating potential as the nitrite anion will predominate. Given that endogenous nitrosation is facilitated by the acidic environment of the stomach, occurrence of gastric cancer in relation to dietary nitrite and nitrate exposure is a critical consideration. Since the 2010 publication of IARC's Monograph Volume 94, major epidemiological studies found no association with nitrite, nitrate, and/or NOC and stomach cancer:

- Loh *et al.* (2011) examined the relationship between dietary intake of exogenous and endogenous *N*-nitroso compounds and cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study.[16] This large prospective cohort with more than 23,000 participants found neither estimated intake of exogenous *N*-nitroso compounds nor endogenous *N*-nitroso compounds were significantly associated with an increased cancer risk. Importantly, Loh *et al*. (2011) came to this conclusion by factoring in the biological significance of the protective effect of vitamin C by inhibiting the endogenous nitrosation process, which reduced endogenous nitrosation as well as the relationship with *Helicobacter pylori.* This work built on the 2006 cohort EPIC-EURGAST study that also found no association between dietary intake of preformed *N*-nitroso compounds and stomach cancer.[17]
- Nitrite and nitrate were found by Cross *et al.* (2011) not to be associated with increased esophageal or stomach cancer.[18] Cross *et al.* studied the National Institutes of Health-AARP Diet and Health Cohort, a prospective cohort of more than 500,000 participants. This project was the largest study examining nitrite, nitrate and stomach cancer in a United States population.

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- Keszei et al. (2013) studied the relationship between nitrite, nitrate and NOC and gastric cancer subtypes in the Netherland Cohort Study of 120,852 men and women aged 55-69 years over a 16-year period.[19] After adjustment for diet-related variables, including fruit and vegetable intake, no associations were reported between nitrite, nitrate, and *N*-nitrosodimethylamine intake in men or women and gastric cardia and noncardia adenocarcinoma. The authors concluded there were no clear associations between NOC and gastric subtypes.
- One recent cohort publication, Xu *et al.* (2015), highlights the important interaction between gastric cancer risk and *H. pylori* infection.[20] The investigators conducted a nested case-control study within the Shanghai Cohort Study (n=18,244). The authors determined the association between urinary levels of nitrate, nitrite and NOC in *H. pylori* positive and negative subjects. The authors found that overall there was no significant difference in urinary levels of nitrate, nitrite or any NOCs between gastric cancer patients and controls. Researchers did report, however, a significant association between urinary nitrate and gastric cancer risk in *H. pylori* negative subjects (OR for highest tertile – 4.82; 1.05-22.17). The overall implications of these findings are difficult to discern. Importantly, the rate of *H. pylori* infection was over 80 percent in this population, leaving the observation of an association between urinary nitrate and gastric cancer based on only 20 cases and 94 controls. Additionally, based on urinary levels alone, it is not possible to discern the relationship between dietary sources of nitrate and this observation. Finally, the authors did not discern between gastric cancer subtypes (Gonzalez *et al.,* 2012)[21] nor consider *H. pylori* strain-specific infections using multiplex serology which distinguishes with greater sensitivity presence of *H. pylori* antibodies compared to methods that simply detect *H. pylori* positive or negative status (Epplein *et al.*, 2014).[22] This consideration is particularly relevant in Asian populations where rates of gastric cancer and *H. pylori* infection are high.

In summary, recent findings from large prospective cohorts demonstrate no consistent association with nitrite, nitrate or NOC and stomach cancer. Using IARC classification methodology, the total epidemiological scientific evidence would be classified as "inadequate evidence." Had the above-discussed epidemiological evidence been available and considered by IARC in 2006, the Group 2A classification related to endogenous nitrosation as a carcinogenic mechanism would not have been supportable.

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Mechanistic Evidence Does Not Support a Link between Ingested Processed Meat, Nitrites or Endogenous Nitrosation and Cancer

Multiple mechanistic studies conducted since IARC's June 2006 deliberations have shown no evidence that nitrite-only exposure has led to tumor formation.[23, 24, 25, 26, 27, 28] Evidence published more recently also fails to support a link between ingested meat, nitrites or endogenous nitrosation and cancer and also provides evidence of dietary nitrite health benefits.

- Van Hecke *et al.* (2014) demonstrated certain *N*-nitroso compound formation was not supported by nitrite-cured meats.[29] The authors studied the involvement of nitrite curing of chicken, pork and beef in NOC-induced DNA damage before and after digestion in an *in vitro* model stimulating the mouth, stomach, duodenum and colon. The authors noted failure to observe higher formation of O^6-C-MeG, when meats were nitrite-cured, negates prior evidence that nitrite curing is important for NOC formation. The authors also noted "*nitrite-cured meats also contain far less residual nitrite than many vegetables, which are not associated with increased risk in epidemiologic studies*." Finally, the authors commented on prior work by Santarelli and co-workers,[30] which stresses the importance of anaerobic storage to reduce oxidation of lipids which may promote NOC formation.

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- Morcos *et al*. (2010) reported the inhibition of human bladder cancer cell replication by nitrite added to culture via sterilized, acidified, human urine.[31] The researchers reported the addition of nitrite to T24 cells, incubated in urine, resulted in a significant reduction in [^{3}H]-thymidine incorporation, and nitrite-mediated inhibition of replication was further enhanced in the presence of ascorbic acid. The authors concluded that nitrite, "*one of the most studied supposedly carcinogenic compounds, actually can produce inhibition of tumor cell replication under physiologically relevant conditions*."
- Finally, Ohtake and co-workers (2010) examined the role of oral nitrite as a protective agent against experimental colitis in mice.[32] Colitis is a precursor to inflammatory bowel diseases, which affect the entire digestive tract, including the stomach, small intestine and large intestine. In mice suffering from severe colitis, nitrite administration restored colonic nitrite levels and TNF-α expression to that of normal control mice. Colonic histology of mice with colitis, but not treated with nitrite, exhibited multifocal dropouts of entire crypts in all parts of the colon and marked infiltration of inflammatory cells into the mucosa. Histology scores of nitrite-treated mice were restored to those consistent with control mice. Authors concluded "…*considering the causative role of inflammation in the development of colon carcinogenesis, inhibiting the early inflammatory response by nitrite may be protective from inflammation-induced carcinogenesis, and possibly at least providing an opportunity to reconsider the risk of nitrite in cancer*."

Collectively, these results indicate that dietary nitrites and related NOCs, even those from cured processed meats, are not associated with increased cancer risk and nitrites may actually prove to be anti-carcinogenic in the future. Future studies should continue to explore physiologically relevant, mechanistic work to fully elucidate the roles that dietary nitrites, nitrates, NOCs and endogenous nitrosation may play in cancer risk.

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* * * * * * *

Given the weak and inconclusive evidence regarding cancer risk coupled with evidence of emerging benefits, it is not possible to consider nitrites, nitrates, NOCs and endogenous* N*-nitrosation in the stomach to be involved in carcinogenic mechanisms related to red and processed meat consumption.

Thank you for your consideration of these points. For your convenience, I have also included the referenced scientific evidence. Should you have any questions, please do not hesitate to contact me at bbooren@meatinstitute.org.

Respectfully submitted,

[redacted]

Betsy Booren, Ph.D.
Vice President, Scientific Affairs

In Cooperation with:
James R. Coughlin, Ph.D., CFS, Coughlin & Associates
Andrew L. Milkowski, Ph.D., University of Wisconsin - Madison

Attachments:
Zip file enclosures – Evidence re: nitrates, nitrites, NOC, and endogenous *N*-nitrosation

NORTH AMERICAN MEAT INSTITUTE

September 11, 2015

Dr. Veronique Bouvard, Responsible Officer
Dr. Kurt Straif, Head of the IARC Monographs Programme
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Re: Volume 114: Red Meat and Processed Meat – Call for Data – Considerations Regarding Dietary Heme and Formation of Endogenous N-Nitrosamines in Relation to Colorectal Cancer

Dear Drs. Bouvard and Straif:

The North American Meat Institute (NAMI or Meat Institute) is the leading voice for the meat and poultry industry. Formed from the merger of the American Meat Institute and North American Meat Association, the Meat Institute has a rich, century-long history and provides essential member services including legislative, regulatory, scientific, international, and public affairs representation. Together, the Meat Institute's members produce the vast majority of U.S. beef, pork, lamb, and poultry, in addition to the equipment, ingredients, and services needed to produce the safest and highest quality products.

The Meat Institute appreciates the opportunity to provide scientific evidence for consideration by the International Agency for Research on Cancer (IARC) Working Group during its upcoming review of red and processed meats.

Mechanisms Related To Heme From All Dietary Sources Must Be Evaluated

The Volume 114: Red Meat and Processed Meat Working Group must consider a broad set of relevant data and a wide range of interpretations regarding published literature, and request that the enclosed evidence and related comments be shared with the Monograph Working Group members prior to the meeting. In addition, IARC will be considering the "High Priority" evaluation and classification of "Dietary iron and iron used as supplements or for medical purposes" (IARC, 2014) as recommended by their Advisory Group, which "haeme" (heme), the iron-containing protoporphyrin, will be a part of this evaluation. It is critical for IARC to <u>first systematically evaluate heme mechanisms</u> from all dietary sources in this future monograph process and to <u>avoid considering the mechanistic role of heme in red and processed meat in your current Volume 114 process</u>**.**

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Mechanistic And Regulatory Aspects Of Intestinal Iron Absorption

The Working Group should consider the complexities and uncertainties surrounding mechanisms and regulation of intestinal iron absorption during their deliberations on the possible role of iron and heme iron in the etiology of colorectal cancer. Intestinal iron absorption and homeostasis was comprehensively addressed in a recent review by several experts in this field (Gulec *et al.,* 2014). The key parameters reviewed were:

(1) iron absorption in the duodenum and proximal jejunum;
(2) regulation of iron absorption by cellular and systemic factors;
(3) forms of iron in the diet, its bioavailability and effects of other dietary constituents;
(4) how whole-body iron levels are maintained and where iron is lost in the body; and
(5) perturbations in intestinal iron transport associated with various pathological conditions, including inflammatory bowel diseases, intestinal bleeding and cancer.

In addition, several other key review articles and new discoveries published over the past 25 years offer very important considerations and advances that have developed in this field, specifically on heme iron absorption (Frazer and Anderson, 2005; Hunt, 2005; Hurrell and Egli, 2010; Sharp, 2010; Shayeghi *et al.*, 2005; West and Oates, 2008; Young *et al.*, 1989). While this vast body of research findings is beyond the scope of the comments herein, significant consideration for the below key concepts and issues is needed by the Working Group in their evaluation of the possible role of heme-catalyzed nitrosation in human colorectal cancer.

<u>Key Concepts of Intestinal Iron Absorption</u>

Iron is an essential trace metal for all organisms and in humans plays a crucial role in a number of biochemical functions, including the transport of oxygen in the blood and energy production in the mitochondria. Daily turnover of iron is a dynamic process; the body recycles some 20 - 30 mg iron per day from senescent red blood cells via the reticuloendothelial macrophage system. However, as part of this process, approximately 0.5 - 2 mg of iron, depending on the age and gender of the individual, is lost each day due to normal minor blood loss and the constant shedding of iron-containing cells (*e.g.*, skin, hair and the gastrointestinal and urinary tracts). Thus an equivalent amount of iron must be absorbed each day from dietary sources to replace these endogenous losses in order to maintain iron homeostasis.

The lack of a process for ridding the body of iron demonstrates the critical requirement for this essential nutrient and likely reflects the fact early humans did not have consistent, readily available sources of highly bioavailable iron. As a result, humans and other mammals have developed complex regulatory mechanisms to control body iron content at the level of absorption in the proximal small intestine. As described in Gulec *et al.* (2014), iron absorption by the proximal small bowel is a critical regulatory point in the maintenance of whole-body iron levels since, unlike most other essential nutrients, no regulated excretory systems exist for iron in humans. Exquisite regulatory mechanisms have evolved to modulate how much iron is acquired from the diet, whether from heme or non-heme dietary sources. Body iron levels are principally controlled by modulation of iron absorption in the duodenum and proximal jejunum, which allows absorption to be precisely matched to unregulated losses. These mechanisms regulating iron absorption also allow for appropriate increases or decreases according to physiological demand. An important additional point made by these authors, however, is that while tight regulatory control of iron levels is maintained in healthy individuals, those individuals with several inherited disorders and in other pathophysiological states have perturbed iron sensing and dysregulated intestinal iron absorption.

Gulec *et al.* (2014) noted that any large variations in iron status and stores among individuals, whether healthy or diseased, might be misconstrued as indicative of imprecise regulation of intestinal iron absorption; however, this might be true if one assumed that iron status/stores were directly related to the capacity of the small intestine to absorb iron. But this is not the case, according to the authors, since the amount of bioavailable iron is often limiting, which can partially explain variations in iron stores.

Moreover, various pathologies are known to influence the relationship between the rate of iron absorption and body iron levels. Importantly, iron balance is controlled in part by the liver-derived, serum-borne, peptide hormone hepcidin (HEPC), which functions to block intestinal iron absorption and inhibit iron release from stores. In sum, absorption of dietary iron by the proximal intestine is accurately regulated by cellular and systemic factors to ensure that overall body iron levels are maintained at adequate levels.

Forms of Iron in the Diet and Bioavailability

Iron in foods exists principally as heme and non-heme (or inorganic) iron, and heme iron is derived predominantly from hemoglobin and myoglobin in meats. Heme accounts for 5-10 percent of dietary iron intake, while approximately 90-95 percent of iron in all diets is non-heme iron, present in most foods including cereals, vegetables and meat as either simple ferric oxides and salts or more complex organic chelates. Some diets, largely though not exclusively in western countries, will also contain heme, and the heme content of meat varies dramatically and is more abundant in red meat compared with fish or poultry. However, those foods with the highest heme content, *e.g.* calves' liver, may not be as nutritionally important as other foods with lower heme content that are eaten more frequently.

Despite this relatively low dietary abundance of heme, it is the most bioavailable source of iron, and average absorption from a meal is approximately 20-30 percent. In contrast, only 1-10 percent of the dietary load of non-heme iron is absorbed, which reflects the significant influence of other dietary components on non-heme iron bioavailability. Sharp (2010) demonstrated prolonged cooking of meat at high temperatures will degrade the porphyrin ring, thus allowing the iron center to be removed and join the non-heme iron pool. Sharp also noted that few dietary components, with the exception of calcium, influence heme bioavailability, but meat also stimulates the absorption of non-heme iron from single meals. Furthermore, meat-based meals are known to enhance gastric acid secretion, which in turn is known to promote iron solubility in the intestinal lumen and thus increase bioavailability.

The absorption of both heme and non-heme iron takes place almost exclusively in the duodenum, and the key determinants of iron absorption are food bioavailability, dietary composition and systemic signals indicating iron storage and metabolic requirements for iron. Heme is absorbed as an intact porphyrin and iron is liberated from heme by the action of heme oxygenase 1 (HO1) in intracellular endosomes. Iron from heme then enters a common intracellular pool with the iron absorbed via the non-heme transport pathway. Heme iron absorption is efficient and largely uninfluenced by other dietary constituents, but in addition to dietary factors, body iron status is a major determinant of iron absorption. While heme is absorbed intact, the bioavailability of non-heme iron varies greatly depending on dietary composition, and a number of dietary components are capable of interacting with iron to regulate its solubility and oxidation state. Interestingly, there are important influences of both nutrient-nutrient and nutrient-gene interactions on iron intestinal iron absorption and bioavailability, as well as an emerging body of evidence suggesting that some nutrients also have direct effects on the expression and function of enterocyte iron transporters. In addition, the genes and proteins that control the rate of dietary iron absorption by enterocytes are themselves subject to tight regulation.

Colonic Blood Loss Occurs in Healthy Humans, and This Blood Loss is Exaggerated in Pathological Conditions

In healthy individuals, iron absorption is precisely matched to iron losses, but in numerous clinical conditions this balance is disrupted, resulting in the pathological consequences of iron overload and iron deficiency. One common cause of inefficient iron absorption (and consequent anemia) relates to a reduction in the absorptive surface area of the gut, as commonly occurs in Celiac disease, inflammatory bowel diseases and short bowel syndrome. Blood loss associated with various gastrointestinal disorders and diarrhea may also result in anemia. In a seminal review published over 25 years ago on the fate of hemoproteins and the absorption of heme, Australian researchers pointed out that in the gut lumen there are both exogenous dietary sources as well as endogenous sources of heme (Young *et al.*, 1989, Table 2). Among the main endogenous sources are: (1) the desquamation or shedding of mucosal cells containing catalase, certain peroxidases and certain cytochromes, a major source; (2) hemoglobin from “physiological” bleeding, also a major source; and (3) protoporphyrin from bile, a minor source.

As described by Young *et al.* (1989), human blood loss independent of cell shedding has been determined analytically, and it was found that in normal subjects, physiological blood loss ranges up to 1.5 mL/day or higher. The existence of this physiological blood loss was further supported by the HemoQuant assay, which demonstrated a fecal excretion of heme and heme-derived porphyrins from all sources of less than 2 mg Hb equivalent/g feces, equating to approximately 2 mL blood loss/day in normal subjects. In an accompanying table (Table 1), Young *et al*. (1989) also expressed the hemoprotein contents of various foods as hemoglobin equivalents (mg/g). Hamburger beef was shown to have a hemoglobin equivalent of 4.4-4.8 mg/g, whereas beef steak was 5.5-9.6 mg/g. With these measurements in mind, the authors calculated that a blood sample of 10 mL contains about as much heme as 400 g of hamburger beef, *i.e.*, nearly a pound of beef.

It is important to note, the normal "physiological" blood loss of approximately 2 mL/day equates to the heme equivalent contained in a dietary intake of about 80 g of beef, *i.e. ca.* 3 ounces. And for patients who may be suffering from various gastrointestinal conditions and diseases, such as colorectal polyps, hemorrhoids, ulcerative colitis, Crohn's disease and other inflammatory bowel diseases, where colonic and rectal bleeding may occur, the colon and rectum will be exposed to even more blood and heme content than normal subjects. In fact, if patients with these conditions and diseases experience blood losses approaching even 10 mL/day, this exposure to endogenously produced blood in the colon and rectum would be equivalent to the heme content of nearly a pound of beef consumed per day.

Consequently, epidemiologists and toxicologists investigating various dietary intakes of heme in relation to the incidence and potential biochemical mechanisms of colorectal cancer need to take into account and adjust for normal intestinal blood exposure in controls and for the higher blood exposures in cases with bleeding-related conditions. Failure to consider these endogenous, blood-related exposures to heme in both normal and diseased intestines will confound any results that have been ascribed to date for heme contained in red and processed meat.

Nitrosation Chemistry And Biochemistry: The Nitric Oxide Cycle

IARC Monograph Volume 94 (IARC, 2010) (Section 4.1.7, pages 274-277) contains important information on the endogenous formation of N-nitroso compounds in animals and humans. However, very little information was included in this Monograph Volume 94 on the important role of *S*-nitrosation *in vivo* or on the critically important "Nitrate–Nitrite–Nitric Oxide Pathway" in physiology, which was subsequently summarized by Bryan *et al.* (2012) in response to the publication of the IARC Monograph Volume 94. The details of nitrosation chemistry/biochemistry and the nitric oxide (NO) pathway occurring *in vivo* will not be reiterated here except to emphasize some key points for consideration, including sites of nitrosation, chemical formation mechanisms and bacterial formation mechanisms. This information is fully supported by extensive literature reviews, but not discussed within the enclosed comments Bryan *et al.,* 2012; Bryan and Ivy, 2015; Gladwin *et al*., 2003, 2005; Habermeyer *et al.,* 2015; Hord *et al.,* 2009; Lundberg *et al*., 2008; Machha and Schechter, 2011; McNally *et al.,* 2015; Mensinga *et al.,* 2003; Milkowski *et al.,* 2010; Sindelar and Milkowski, 2012; Tricker, 1997; Weitzberg and Lundberg, 2013).

Key learnings from this comprehensive literature relate to how *N*-nitroso compounds (NOC) are actually produced in the body. Certainly one of the most important considerations in the formation of NOC is the reaction's dependence on the pH of the fluid or medium *in vivo*, because there are certain pH's levels needed to optimize formation of NOCs. It has been known for over 60 years that neither the nitrate nor nitrite anion can possibly nitrosate amines or amides, even though the rate of nitrosation of a secondary amine/amide is well known to be proportional to the square of the nitrite concentration. Instead, nitrite under acidic conditions must first be converted to nitrous acid (HONO), an extremely unstable compound, which is in equilibrium with nitrous anhydride (N_2O_3), the required nitrosating species. N_2O_3 is produced from two molecules of HONO, hence the rate of the *N*-nitrosation reaction is dependent on the square of the nitrite concentration as noted above.

The pKa value for the nitrous acid to nitrite anion equilibrium is 3.34, meaning that at a pH of 3.34, only 50 percent of the HONO nitrosating agent can exist in solution, such as in the stomach contents, while at a higher pH there is much less nitrosating potential because the nitrite anion will predominate. The latter will be the situation in the neutral to more alkaline pHs found lower in the gastrointestinal tract (small and large intestines). Under these more neutral or alkaline conditions of the intestines, the nitrite anion will be the exclusive form in solution and will not act as a nitrosating agent.

A second important requirement for *N*-nitrosation reactions is the amine or amide must be in the unprotonated or uncharged, free amino state in order to react with HONO. Therefore, the ease of nitrosation of an amine increases as the basicity of the amine decreases, *i.e.*, as the pKa of the amine decreases. Thus, at a given pH, weaker amine bases will be nitrosated more rapidly because of the higher relative concentration of free unprotonated amines. For example, it is known that the rate of nitrosation at pH 3.0 is about 20,000 times faster for the weak base piperazine (pKa = 5.57) compared to the much stronger base piperidine (pKa = 11.2) (Mirvish, 1975). Since most of the dialkylamines, *i.e.* dimethylamine and diethylamine, have pKa values near or above 11, they will essentially be fully pronated (*i.e.,* positively charged) in the intestines and will therefore not undergo N-nitrosation there. In addition, any nitrite in the intestines will be in the form of the nitrite anion and thus be unable to act as a nitrosating agent.

In addition, a significant share of the nitrite escapes gastric passage and enters the systemic circulation and tissues, where it can be further metabolized to NO and other bioactive nitrogen oxides. A part of the dietary nitrate and nitrite may also proceed into the small intestine and cecum where it can be absorbed or further utilized by bacteria to produce NO, and a substantial portion of ingested nitrate and nitrite is absorbed or consumed even before reaching the colon (Sobko *et al.*, 2005, 2006). The Working Group must recognize these scientific facts during their deliberations regarding the possible role of nitrite as a reactant in the hypothesized heme-catalyzed *N*-nitrosation mechanism in the colon, which will be discussed below. There may be a very low concentration of nitrite anions in the colon and very limited amounts of heme iron to be available for reaction to produce *N*-nitroso compounds.

Jon Lundberg, Professor of Nitric Oxide Pharmacologics and research group leader for Pharmacological Nitric Oxide Research at Sweden's Karolinska Institute, and his colleagues have recently published studies on the preventive and therapeutic effects of nitrite supplementation in experimental inflammatory bowel disease (Jadert *et al*., 2014). These NO experts outlined a concise summary of the key facts on the "Nitrate–Nitrite–Nitric Oxide Pathway" in physiology, a discussion also summarized in Bryan *et al*. (2012) in response to IARC's Monograph Volume 94 on Ingested Nitrate and Nitrite. It is important to reiterate some of these key learnings as the Working Group deliberates and evaluates red and processed meat.

Endogenous production of NO by the nitric oxide synthases (NOS) is essential for physiological regulation of gastrointestinal function, and NO also plays a role during inflammatory conditions, *i.e.* ulcerative colitis and Crohn's disease, which are collectively included in the chronic intestinal disorders of inflammatory bowel diseases (IBD). In addition to the NOS, an alternative pathway for NO generation in mammals has been described, involving sequential reduction of dietary-derived nitrate and nitrite. Vegetables, in particular green leafy plants, contain high amounts of nitrate, which is efficiently absorbed in the gastrointestinal tract. About 25 percent of the systemic circulating nitrate is accumulated in the salivary glands, and salivary nitrate is then converted into nitrite by efficient oral commensal bacteria, resulting in high concentrations of salivary nitrite. When swallowed into the acidic environment of the stomach, a part of the salivary-derived nitrite is protonated and converted to NO and other nitrogen oxides, including *S*-nitrosothiols.

Heme-Catalyzed Nitrosation Hypothesis - Human Studies

Several factors have been suggested for involvement in mechanisms behind the reported weak positive epidemiological associations between red and processed meat intake and colorectal cancer. Red meat and heme were linked to increased production of "apparent total nitroso compounds" (ATNC) in the large intestine (Bingham *et al.,* 1996, 2002; Cross *et al.,* 2002, 2003, 2006; Hughes *et al.,* 2001; Jakszyn *et al.,* 2006; Joosen *et al.,* 2009; Kuhnle *et al*., 2007; Lewin *et al.,* 2006; Lunn *et al.,* 2007), and this hypothesis continues to be actively studied. Notable features in these studies were: (1) Human volunteers were placed on diets with an extremely high intake of greater than 400 grams of raw or processed meat per day versus a contrasting low meat or vegetarian diet; (2) measurement of fecal total nitroso compounds was performed; and (3) in some studies DNA adducts were measured, with the inference that their formation is catalyzed by heme from red meat or processed meat.

These putative mechanistic discussions are not supported by any measurement of specific *N*-nitrosamines or known carcinogenic compounds. In early studies, data for production of ATNC show four of 12 individuals on a high red meat diet (420 g/day) having fecal ATNC in the same range as when they were on a vegetarian diet (Cross *et al.,* 2003), and this contradiction to the conclusions was not discussed by the researchers. Notably, in one study where the high meat diet was a still an excessive 325 g red meat/day for males (Joosen *et al.,* 2010), increasing fish intake and reducing the intake of red meat did not have an effect on inflammation and fecal water-induced (oxidative) DNA damage. Additionally, the role of an interaction of colonic bacterial populations was acknowledged to exist, but was not accounted for in any of these experiments in favor of a simple focus only on heme and the formation of *N*-nitroso compounds.

However, as early as 2000, O'Brien *et al*. (2000) examined K-ras mutations in human patients with left-sided colon cancer and found no support for a relationship with meat consumption, although there was limited data presented in the report.

Animal Experiments On Heme-Catalyzed Nitrosation Fail To Establish An Adequate Cancer Mechanism For Colorectal Cancer

<u>No Effect of Dietary Heme and Nitrite on Colonic Lesions</u>

Various researchers have used animal models in an attempt to further elucidate the potential for colon tumor formation. A research group in Toulouse, France has used a rat model with a colon carcinogenesis initiator and a variety of dietary treatments for the animals, including heme and cured meat in studies of aberrant crypt foci (ACF) and ATNC (Allam *et al.,* 2011; Bastide *et al.,* 2011, 2015; Chenni *et al.,* 2013; Corpet, 2011; Corpet *et al.,* 2014; Davis *et al.,* 2012; Pierre *et al.,* 2008, 2010, 2013; Santarelli *et al.,* 2008, 2010, 2013). The term ATNC has been used as a proxy measurement of carcinogenic *N*-nitrosamines in many studies seeking to find a mechanistic basis for the reported epidemiologic associations (Bingham *et al.,* 1996, 2002; Cross *et al.,* 2003; Hughes et *al.,* 2002; Kuhnle *et al.,* 2007). However, this is a broad category of compounds comprised of a complex mixture of nitrite-derived products, including *N*-nitrosamines, *S*-nitrosothiols, *N*-nitrosamides, nitrosoguanidines and iron-nitrosyl species (Bryan *et al.,* 2012; Feelisch *et al.,* 2002; Hogg, 2007). These compounds, all of which are captured in ATNC assays, have a broad range of metabolic activities.

Bryan *et al.* (2012) suggested that a number of improvements are needed to further discriminate these compounds into groups that would have more biological specificity of action and careful discrimination between carcinogenic and non-carcinogenic *N*-nitrosamines. The latter group includes *N*-nitrosoproline and protein *N*-nitrosamines (Bryan *et al.,* 2012). Therefore, the measurements of total ATNC is not useful indicator of carcinogenic potential in studies of meat and colorectal cancer.

Corpet's research group concluded heme is a promotor of carcinogenesis, but also noted important experimental protocol points that reflect on all of their studies. In an early paper from this group, it was noted that "*Results suggest that NOC from dietary bacon would not enhance colon carcinogenesis in rats"* based on their use of a control without initiation of carcinogenesis (Parnaud *et al*., 2000). In a later paper they indicated, "*We chose to initiate all rats with the carcinogen, since the study was designed to show dietary promotion, and because a 2-5% Hb diet does not initiate ACF in rats (F. Pierre and D.E. Corpet, unpublished results)"* (Pierre *et al.,* 2008). This lack of presenting non-initiated control data in most of these reports greatly weakens the inference of a heme-catalyzed nitrosation mechanism that may be associated with colon carcinogenesis.

The most recent paper published by this group, Bastide *et al.* (2015) concluded, "*In humans, red meat consumption increases fecal ATNC concentrations (11), as in our study with rats. Nevertheless, we could not detect any association between the ATNC level (Fig. 1D) and carcinogenesis (Fig. 1A). The highest level of ATNC was seen in the control group given nitrates/nitrites-supplemented water; this group had the fewest MDF. The lack of a relationship between ATNCs and the number of MDF does not support a strong role for ATNCs in the promotion of colon carcinogenesis by red meat*."

Sødring *et al*. (2015) used a genetic mouse model for familial adenomatous polyposis, and evaluated heme, nitrite and cured heme, *e.g.*, nitrosyl heme. Hemin addition to the diet resulted in elevated incidence of colonic lesions in the large intestine, but hemin also decreased lesions in the small intestine. Nitrite addition to the diet did not alter the incidence of colonic lesions but did suppress tumor growth in the small intestine. A similar lack of differential effect of nitrite has also been reported by other researchers who concluded:

> "*However, sodium nitrite in drinking water increased the level of ATNC, but level and nature of ATNC do not seem to increase carcinogenesis*." (*Chenni et al*., 2013)

Given these more recent findings demonstrating no effect of nitrite in heme-related animal experiments and the lack of an effect on colorectal tumor incidence in lifetime feeding bioassays of high levels of nitrite in both rats and mice (National Toxicology Program, 2001), there is a strong indication that there must be alternative mechanisms not involving heme-catalyzed N-nitrosation in inducing colorectal carcinogenesis.

Research on Cytotoxic Effects

A putative cytotoxic heme factor (CHF) has been proposed by Ijssennagger and colleagues as another heme-related mechanistic hypothesis for human colorectal cancer (Ijssennagger *et al.,* 2015), although a specific compound has still not been isolated or identified, despite being reported as a lipophilic compound (Ijssennagger *et al.,* 2013). The proposed carcinogenic mechanism involves oxidative stress and mucin-degrading bacteria, and these researchers suggested measurement of trisulfides as a marker for colonic dysfunction. This and previous reports from these researchers present a large number of methodologic problems in addition to a potentially flawed fundamental premise, which does not consider endogenous heme from blood and internal bleeding, as discussed above, as an important confounding factor.

The test materials used in these studies (Ijssennagger *et al*., 2012a,b,c; Ijssennagger *et al*., 2013; Ijssennagger *et al.,* 2015) could have introduced a number of artifacts. The use of a purchased heme product for the dietary supplement is a particularly significant problem. Heme from suppliers such as Sigma Aldrich is considered to be only 90 percent pure with a brown (oxidized to a Fe^{+3} state) to black color (potentially degraded) (Sigma-Aldrich, 2015). Thus, the cytotoxic effects reported by this research group could well have been produced by impurities and not by heme itself, making this test material an inadequate proxy for heme in the diet of the experimental animals. Notably, purified hemin is only soluble at very high pH, *i.e*., 1.4 M NaOH (Sigma-Aldrich, 2015), and thus would be expected to exist in a solid form throughout digestion in the animal studies. Therefore, this test material would not be representative of heme derived from digestion of meat by humans. In addition, the significant depression in animal weights for the heme-fed mice (Ijssennagger *et al.,* 2012c, 2015) reinforces this possibility of an acute toxic effect from potential impurities in the heme-supplemented diet.

Additionally in these studies, fecal samples were frozen at the relatively warm temperature of -20°C and freeze dried, followed by storage for unspecified time periods under unknown conditions as part of the experimental protocol. It is well recognized that freezing and freeze drying can induce oxidative degradation of biological materials (Fennema and Powrie, 1964). The lack of any baseline measure of lipid oxidation of the test materials used also raises the possibility of artifact formation that could be confounding the observed effects. Despite these shortcomings related to the heme preparations used, the studies do establish the important role that the bacterial colonic microflora play in producing cytotoxic effects.

The Microbiome and Colorectal Cancer

Crohn's disease is an established risk factor for development of colitis and colorectal cancers. An interaction in the progression of these disease conditions to invasive carcinoma has also been demonstrated in animal models, suggesting that colonic inflammation is a mechanism that can promote tumorigenesis by altering microbial composition to include microorganisms with genotoxic capabilities (Uronis and Jobin, 2009; Uronis *et al.,* 2009). A recent review on microbiome-host interactions concerning inflammatory bowel disease (Wlodarska *et al.,* 2015), in which the authors pointed out that the microbiome includes viruses and stressed that bacterial-related chronic intestinal inflammation is a major risk factor for human colorectal cancer. The specific organisms responsible for these endpoints remain to be fully elucidated, but may include commensal *Escherichia coli* strains (Arthur *et al.,* 2012). Researchers have also noted the presence of *Fusobacterium* in human colonic tumors (Castellarin *et al*., 2012; Kostic *et al.,* 2012, 2013; Rubinstein *et al*., 2013), and this organism must be considered in the etiology of colorectal cancer.

Arumugam *et al*. (2011) conducted a metagenomics analysis of fecal metagenomes from four countries and classified different enterotypes of the human gut genome. The researchers speculated on the importance of this type of analysis for human disorders, including colorectal cancer. In addition, a recent study on the metagenomics of the gut microbiome has listed 14 bacterial species that are found to be enhanced in humans with colorectal cancer (Mandal *et al.,* 2015). This newer area of research emphasizes new paradigms regarding the interaction of host genetics, commensal bacteria and colorectal cancer need to be considered by the Working Group.

The Microbiome and Mechanisms Of N-Nitrosation In The Colon

Kim *et al.* (2013) have reviewed colorectal cancer from the perspective of a dysbiosis of the microbiome and current knowledge about interactions with a number of dietary components related to meat and processed meat. The conclusion section of this paper very adequately summarized the current complexities and uncertainties surrounding diet and colorectal cancer. The authors discuss this in terms of a balance between "*...so-called "harmful" and "beneficial" bacteria (that) may influence carcinogen bioactivation and, thus, cancer risk*." Research gaps identified by the authors include: (1) more characterization of the chemical composition of the lumen of the large intestine; (2) determining genotoxicity and metabolic effects of microbiome metabolites; and (3) metabolic capacity of colonocytes for detoxification.

The potential for microorganisms in the colon to generate N-nitroso compounds has long been recognized (Garber and Hollocher, 1982; Ji and Hollocher, 1989; Kim and Hollocher, 1984; Mitchell *et al*., 2005; Pant and Crane, 2006). Massey *et al.* (1988) demonstrated this in differential animal experiments with germ-free rats given a cultured rat fecal extract. A number of other researchers also showed that common bacteria found in the colon, such as strains of *E. coli,* had a mechanistic feature involving nitrate reductase (Calmels *et al.,* 1987, 1988, 1991, 1996; Garber and Hollocher, 1982; Sobko *et al.,* 2005, 2006). In 1996, Calmels *et al.* (1996) noted that non-denitrifying bacteria, *i.e. E. coli*, present in the colon were able to also promote *N*-nitrosation. This potential was also examined in germ-free rats receiving either rat or human colonic bacterial inoculations (Mallett *et al.,* 1987, 1989). These studies showed elevated nitrate reductase and nitroreductase activities in the human feces inoculated into rats. It has also been postulated that bacteria can oxidize nitric oxide to nitrate using a flavohemoglobin pathway to deal with oxidative stress (Forrester and Foster, 2012).

The hypothesis that dietary fiber was protective against the development of colorectal cancer at one time was considered to be due to the gut microflora producing a variety of protective metabolites including butyrate, which was suggested to be an energy substrate for the colonic mucosa and an anti-proliferative and differentiating agent (Bingham, 1990). However, this same research group later discontinued their focus on this mechanism in favor of pursuing the heme-catalyzed *N*-nitrosation hypothesis using extreme levels of red meat intake (Cross *et al.,* 2003). In these intervening years, the importance of the microbiome, while acknowledged by many research groups, has not been adequately incorporated into interpretations of their research findings.

This apparent limitation can be highlighted in a series of papers representing studies done with simulated digestion using fecal cultures from human volunteers. Van Hecke and colleagues have demonstrated in a simulated gastric, duodenal and colonic digestion system, which used fecal microbiota obtained from 3 human volunteers, that curing meat actually reduced lipid and protein oxidation (Van Hecke *et al.,* 2014a,b). Measurement of O^6-carboxymethylguanine DNA adducts after 72 hours of colonic digestion was highly dependent on the individual bacterial inoculum used, and there was no significant effect of curing. While the authors discussed an adduct-increasing effect of heme, this was only shown in the data for one of the three fecal inocula. Thus, there was high individual sample variation in this series of experiments. One report by these researchers concluded as follows:

> "*The presented results demonstrate that haem-Fe is not solely responsible for oxidation and nitrosation reactions throughout an in vitro digestion approach but its effect is promoted by a higher fat content in mea.t*" (Van Hecke *et al.,* 2014b)

Vanden Bussche *et al.* (2014) reported the largest set of experiments from these studies with data using fecal samples from 15 healthy individuals. In their examination of O^6-carboxy-methylguanine DNA adduct formation reported in Table 1, eight of the fifteen subjects showed absolutely no response to the presence of red or poultry meat in the *in vitro* digestive system employed. The researchers reported:

> "*Upon linear mixed effect modelling of means, a borderline significant difference ($p = 0.055$) was observed in O^6-CMG DNA adduct formation between the different digested meats at the end of the colonic digestion. When looking at each of the seven O^6-CMG positive faecal inocula individually (by means of paired t-test), the beef indicated a significantly higher genotoxic effect compared to the digested chicken ($p < 0.05$) for two out of seven inocula. The inoculum of one volunteer displayed a borderline significantly higher genotoxic effect for beef ($p < 0.1$; Table 1). For one other inoculum, chicken proved to generate more DNA adducts compared to beef ($p < 0.1$; Table 1). Noteworthy was that O^6-MeG was never detected in any of the digestive fluids of the 15 volunteers."*

The extreme variation of response in these human experiments suggests the microbiome can have a far more important effect than the presence or absence of heme in the various meat products tested. Nevertheless, these researchers emphasized heme as a promoter of DNA adduct formation in their discussion based on data presented in Table 3, where a high O^6-CMG DNA adduct-producing fecal inoculum was used and was supplemented with purified myoglobin, the most predominant muscle protein in red meat. However, this experiment was missing two key controls not tested in their simulated digestion protocol: a low O^6-CMG DNA fecal inoculum; and no inoculum. In addition, intact myoglobin would not be expected to be present in the colon, because digestion in the stomach and duodenum would denature the protein moiety before it reaches the colon.

In the concluding discussion of the experiments reported in this paper, the authors acknowledged their primary finding:

> "*While endogenous alkylation was observed during colonic fermentation, MDA formation peaked during the small intestinal digestion. Moreover, the formation of the alkylated DNA adduct O^6-CMG appeared to depend on the microbial composition, since the inter-individual variability of the faecal inocula influenced the DNA adduct formation considerably* <u>and autoclavation completely inhibited the process.</u> *(emphasis added) A contributing factor in the MDA production was most likely the inherent fat content of the meat [9], since MDA is the main by-product of the peroxidation of polyunsaturated fatty acids, which is known to be prone to iron-mediation [80]*." ………

And:

> "...*while for the alkylated DNA adduct O^6-CMG, the colon and its innate microbial biota were proven to be vital and the basis for the significant observed variability between the individual faecal inocula*."

Therefore, in the body of studies performed in this Belgian laboratory measuring effects on DNA adduct formation and other markers of colon cancer from poultry, red meat and cured meat, they do not show an enhancing effect of nitrite and they also acknowledge a very large and variable impact of human microbiota used in the experiments. Thus, there does not appear to be any clear relationship established for a heme-catalyzed nitrosation mechanism.

Most Recent Comprehensive Review Of Nitrate And Nitrite In The Diet

A comprehensive review of nitrate and nitrate in the diet authored by a distinguished panel of experts commissioned by the Senate Commission on Food Safety (SKLM) of the German Research Foundation (Habermeyer *et al.,* 2015). The SKLM organized a round table meeting on "Nitrate and nitrite in the diet, benefit/risk for human health" with experts from The Netherlands, Sweden, UK, USA and Germany in Bonn, Germany in November, 2012. This expert panel reviewed the evidence regarding the beneficial and detrimental health effects related to dietary nitrate/nitrite intake, identified gaps in knowledge and also highlighted research needs to perform a reliable benefit/risk assessment in terms of long-term human health consequences due to dietary nitrate/nitrite intake. The information developed by the panel was updated and presented in the form of this just published review of numerous health effects related to nitrate and nitrite, including beneficial cardiovascular effects and cancer endpoints.

These expert reviewers addressed endogenous *N*-nitrosation in detail and came to the following conclusions:

> "*In conclusion, the epidemiological evidence regarding the role of endogenous NOC formation for human cancer risk is inconsistent. Obviously, there is a need for more elaborate studies, which make use of appropriate biomarkers and also include those determined by applying "omics" technologies, in order to establish causality for the association.*
>
> *Future comprehensive studies need to take into consideration, among other parameters, nitrate/nitrite exposure and the extent of NOC formation in vivo, the nature and relevance of N-nitrosatable precursors and the resulting NOC, the influence of individual dietary and physiologic factors, but also the individual health status, especially with respect to those conditions favoring endogenous NOC formation including inflammatory and/or infectious diseases."* [page 118]

The Meat Institute is cognizant that IARC does not evaluate the health benefits of agents being reviewed and classified, this comprehensive, scholarly review does conclude with a call for specific research needs for determining both risks and benefits. Six of their nine research needs were related to the potential carcinogenic effects are discussed within this submission. In addition, the Meat Institute supports the expert reviewers' advice urging that particular attention be given to the following research needs:

- to identify specific transcriptomic responses as an indication of short/long-term human health effects;
- to establish biomarkers that reflect the endogenous formation of carcinogenic NOC;
- to explore the influence of health status, especially bacterial infections and inflammatory diseases, on biomarker response;
- to update the database on human dietary intake of nitrate/nitrite and especially NOC;
- to explore the endogenous nitrosation kinetics of an array of amino compounds, which reflect the range of chemicals humans are exposed to and can plausibly be expected to act as precursors for carcinogenic NOC and/or to give rise to toxicologically relevant amounts of genotoxic electrophiles in vivo; and
- to more firmly establish the relationship between overall NOC exposure, both from endogenous and exogenous sources, and the induction of cancer.

* * * * * * *

The related topics of the potential role of heme-catalyzed *N*-nitrosation in human colorectal cancer and the following key points must be considered by the Working Group.

- IARC needs to first systematically evaluate mechanisms related to heme from all dietary sources in their future monograph evaluation of dietary iron and to avoid considering the mechanistic role of heme found in red and processed meat in your current evaluation process.
- The Working Group must consider the complexities and uncertainties surrounding mechanisms and regulation of intestinal iron absorption on the possible role of heme iron in the etiology of colorectal cancer.
- The Working Group should consider heme generated endogenously due to normal colonic blood loss that occurs in healthy humans, and also to recognize that such blood loss is exaggerated in certain pathological and disease conditions in the colon.
- The Working Group should carefully consider the roles of both *S*-nitrosation and *N*-nitrosation within the complexities of the "Nitrate–Nitrite–Nitric Oxide Pathway" as well as the conditions necessary for formation of *N*-nitroso compounds *in vivo.*

- The Working Group should recognize that the putative heme-catalyzed *N*-nitrosation hypothesis is not supported by any measurement of specific nitrosamines or known carcinogenic compounds, and that human studies on this mechanism employing red meat intakes approaching one pound per day intakes are inappropriate and irrelevant for this determination.
- The Working Group should recognize that colonic microbial composition, the interaction of host genetics and commensal organisms all interact in the *pathogenesis* of colorectal cancer and all need to be considered separately from the possible role of red and processed meat.

The research conducted to date attempting to establish that dietary heme from red or processed meat is a key mechanistic factor in the development of colorectal cancer, and in particular for generation of endogenous N-nitroso compounds in the colon, reflects a conundrum. Available experimental data in animals and humans do not support an independent effect converging on heme-catalyzed *N*-nitrosation in the development of colorectal cancer. The lack of a mediating effect from nitrite addition and a literature base that indicates nitrite *per se* is not a carcinogen argues against a heme-catalyzed, *N*-nitrosamine-related mechanism. In all pertinent studies performed to date, a microbiome effect cannot be sufficiently disentangled from any experimental result on heme-catalyzed *N*-nitrosation, suggesting that heme is not a contributing factor. Therefore, there is inadequate and insufficient evidence to implicate heme-catalyzed *N*-nitrosation as a plausible carcinogenic mechanism in the human colon.

Thank you for your consideration of these points. For your convenience, I have also included the scientific evidence referenced. Should you have any questions, please do not hesitate to contact me at bbooren@meatinstitute.org.

Respectfully submitted,

[redacted]

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Attachments:

Zip file enclosures: Heme-Catalyzed Nitrosation Evidence Submission1
Heme-Catalyzed Nitrosation Evidence Submission2
Heme-Catalyzed Nitrosation Evidence Submission3.0
Heme-Catalyzed Nitrosation Evidence Submission3.1
Heme-Catalyzed Nitrosation Evidence Submission3.2
Heme-Catalyzed Nitrosation Evidence Submission4
Heme-Catalyzed Nitrosation Evidence Submission5
Heme-Catalyzed Nitrosation Evidence Submission6
Heme-Catalyzed Nitrosation Evidence Submission7
Heme-Catalyzed Nitrosation Evidence Submission8
Heme-Catalyzed Nitrosation Evidence Submission9
Heme-Catalyzed Nitrosation Evidence Submission10
Heme-Catalyzed Nitrosation Evidence Submission11
Heme-Catalyzed Nitrosation Evidence Submission12

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NORTH AMERICAN MEAT INSTITUTE

September 9, 2015

Dr. Veronique Bouvard, Responsible Officer
Dr. Kurt Straif, Head of the IARC Monographs Programme
IARC
Lyon, France

Re: Volume 114: Red Meat and Processed Meat – Call For Data – 2012 Request to IARC to Reevaluate Group 2A Classification Of Nitrite And Nitrate Under Conditions Causing Endogenous Nitrosation

Dear Drs. Bouvard and Straif:

In 2012, Dr. Christopher Wild, IARC director, received a request[1] from a group of experts to reconsider its current classification of "Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)[2]," which was based on IARC's 2006 Monograph Working Group decision. As shared with Dr. Wild, these experts published an updated review of the evidence considered by the IARC's 2006 Working Group as well as new evidence since the 2006 review.[3] Many of the issues raised in the request are relevant to the review for red and processed meats and must be considered in October. To my knowledge, IARC has not responded or refuted the request and I respectfully request the data be shared with the Expert Panel that is evaluating red and processed meats.

In 2012, a group of nitrite physiology, toxicology, meat curing chemistry, and epidemiology scientists concluded that if the following information had been considered by IARC's 2006 Monograph Working Group, the Group 2A classification would <u>not</u> have been scientifically supportable:

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[1] 2012 Letter to Dr. Christopher Wild from Dr. Nathan Bryan.

[2] Humans, I. W. G. o. t. E. o. C. R. t. (2010). "IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins." IARC Monogr Eval Carcinog Risks Hum 94: v-vii, 1-412.

[3] Bryan, N., Alexander, D., Coughlin, J., Milkowski, A. and Boffetta, P. (2012). Ingested nitrate and nitrite and stomach cancer risk: an updated review. Food Chem Tox. 50:3646-3665.

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- the human nitrogen oxide metabolism was not addressed, specifically the importance of *S*-nitrosation;
- new epidemiological evidence shows no association between dietary intake of nitrite and stomach cancer, which was the only organ determined by the IARC Working Group to demonstrate an increased incidence of cancer; and
- the U.S. National Toxicology Program (report TR495) provided compelling evidence that sodium nitrite is not an animal carcinogen. Yet the Monograph Working Group did not weight these peer reviewed conclusions appropriately against other smaller and less rigorous studies.

Thank you for your consideration of these points. For your convenience, I have also included the scientific evidence referenced. Should you have any questions, please do not hesitate to contact me at bbooren@meatinstitute.org.

Respectfully submitted,

[redacted]

Betsy Booren, Ph.D.
Vice President, Scientific Affairs

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Institute of Molecular
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IN COOPERATION *with* MEMORIAL-HERMANN HEALTHCARE SYSTEM

Dr. Christopher P. Wild
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Dear Dr. Wild,
We are authors of a new peer-reviewed publication (Bryan et al., 2012) that has important relevance to IARC's 2006 Monograph Working Group decision to classify "Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)"* (IARC, 2010). Attached is the paper and we wish to offer you below a summary of our thoughts.

Questions about the carcinogenicity of sodium nitrite arose from the discovery decades ago that most N-nitrosamines are carcinogenic and that humans are exposed to trace levels of them from foods, tobacco, some consumer products and the environment, facts well recognized in existing IARC Monographs. This led to understandable concerns about the reactants (various amines, amino acids and nitrite), and the focus was on nitrite and nitrite because of the recognition that secondary amines were ubiquitous in foods and the human body and that the carcinogenic potential of N-nitrosamines might be controlled by elimination of the nitrite. Unrecognized at the time was the important role that nitrogen oxides play in human physiology. The discovery that nitric oxide is endogenously synthesized and the profound importance of nitric oxide, nitrite and nitrate in human homeostasis have led to the current understanding that there is a metabolic nitrogen oxide cycle. Thus, human exposure to the nitrite and nitrate (a nitrite precursor) should now be considered as a normal part of human physiology.
IARC's evaluation leading to a Group 2A classification of nitrite and nitrate under conditions causing endogenous nitrosation was based on the following determinations by the Monograph Working Group (International Agency for Research on Cancer, 2010)

1. ***"There is limited evidence in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with an increased incidence of stomach cancer."***
2. ***"There is sufficient evidence in experimental animals for the carcinogenicity of nitrite in combination with amines or amides."***
   There is limited evidence in experimental animals for the carcinogenicity of nitrite per se."

Overall evaluation

> ***"Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).***
> ***There is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite, which are interconvertible in vivo. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary amines and amides, to generate N-nitroso compounds. These nitrosating conditions are enhanced following ingestion of additional nitrate, nitrite or nitrosatable compounds.***
> ***Some of the N-nitroso compounds that could be formed in humans under these conditions are known carcinogens."***

One of us, Dr. James R. Coughlin, attended the Monograph Working Group meeting in June 2006 as an Observer nominated by the American Meat Institute Foundation, so he is very familiar with the interactions that occurred during the 10-day monograph meeting. As you will see in our review, we strongly believe that IARC's rationale for a Group 2A classification is not scientifically supportable for the following reasons:

1. Nitrogen oxide physiology

   The profound beneficial effects of nitric oxide on human physiology are in many cases modulated via mechanisms involving S-nitrosation. The IARC Working Group did not review or evaluate any of this important S-nitrosation biochemistry. Thus, IARC's classification of "endogenous nitrosation" is actually very narrow and scientifically inaccurate, since it only focused on N-nitrosation. The human evidence for the carcinogenicity of nitrite in food is too weak to be classified as "limited evidence"

2. The Working Group focused on studies involving human stomach cancer because the evidence for other cancer sites was inadequate to make a conclusion. Exposure to ingested nitrite from food is minor compared to endogenous nitrite and nitrate recycling via the enterosalivary route, which most epidemiological studies have failed to address. This exposure is over 10 fold that from nitrite present in foods and if included would likely result in null associations. Additionally, a major prospective cohort study published in 2011 shows no association of nitrite with stomach cancer (Cross et al., 2011), which would necessitate a downgrading of the Working Group's conclusion to "inadequate evidence."

3. Animal toxicology studies show only "inadequate evidence" of carcinogenicity of nitrite per se

   We believe the Working Group erred on this classification on two fronts. First, the literature is overwhelming in that carcinogenic effects in animals require experimental exposure to extremely high levels of both nitrite **and** a specific nitrosatable amine for an effect to be observed. There is strong evidence that there is no carcinogenicity observed in free living animals even with almost acutely toxic levels of nitrite exposure in combination with animal rations.

   Second, we believe that the Working Group was inappropriately led to reinterpret the U.S. NTP 2001 bioassay study of sodium nitrite by Dr. Po C. Chan, who was the Chair of the Subgroup on Cancer in Experimental Animals. Dr. Chan had an undeclared conflict of interest serving in this role, since he was also the NTP "Study Scientist" for the Sodium Nitrite bioassay and argued at the May 2000 public peer review meeting for "some evidence" for female mouse forestomach tumors (National Toxicology Program, 2001). However, the NTP Peer Review Committee unanimously disagreed with Dr. Chan's interpretation then and downgraded the female mouse forestomach finding to "equivocal evidence" (see page 13). The Final NTP Technical Report concluded that there were no "neoplastic effects" observed for sodium nitrite at any dose. To essentially ignore this NTP Peer Review of the definitive assessment of the lack of carcinogenicity of sodium nitrite in rats and mice and to apply Dr. Chan's personal viewpoint during the Monograph deliberations is an obvious conflict of interest.

We believe that there is inadequate evidence for carcinogenicity in humans and also inadequate evidence in experimental animals for the carcinogenicity of nitrite *per se*. Therefore, according to IARC carcinogenicity criteria, the overall classification for ingested nitrite and nitrate would then be determined to be Group 3 "**The agent is *not classifiable as to its carcinogenicity to humans.*"**

Given these important questions we would request that IARC reconsider its current classification of nitrate and nitrite as Group 2A human carcinogens.

Thank you for consideration of our thoughts and please feel to contact any of us with questions.

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cc: Dr. Kurt Straif, IARC Monographs Section Head

References:

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Sincerely,

[redacted]

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NAMI Foundation
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September 9, 2015

Dr. Veronique Bouvard, Responsible Officer
Dr. Kurt Straif, Head of the IARC Monographs Programme
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Lyon, France

Re: Volume 114: Red Meat and Processed Meat – Call For Data – Considerations Regarding The Potential Human Exposure To Heterocyclic Amines From Cooked Meat Products

Dear Drs. Bouvard and Straif:

The North American Meat Institute Foundation (NAMIF or Foundation) is a non-profit research, education and information foundation established by the North American Meat Institute. NAMIF seeks to identify technologies and practices that enable meat and poultry companies to produce safer and more nutritious meat and poultry products. The Foundation publicly disseminates research findings, best practices, and other educational materials about a broad range of food safety, worker safety, animal welfare, nutrition, and consumer information projects. The Foundation appreciates the opportunity to provide scientific evidence for consideration by the International Agency for Research on Cancer (IARC) Expert Panel during its upcoming review of red and processed meats.

In 2007, the Foundation co-funded a series of research projects to understand better the human exposure to heterocyclic amines (HCA) from cooked meat products. Additional information for your consideration is provided below.

Heterocyclic Amine Content in Cooked Meat Products

Scientific evidence published indicate inconsistent results in determining HCA levels in cooked meat products. Much of this evidence was generated prior to significant technological advancements in measuring HCA compounds. Considerable data gaps still exist on accurate and precise HCA levels in meat products. Most of the scientific evidence does not include information based on the cooked internal temperature used by most consumers. HCAs are formed when amino acids, reducing sugars and creatine/creatinine products are cooked at temperatures higher than 300°F/149°C. Most Americans consume meat and poultry products cooked to internal temperatures recommended by the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) and those recommended temperatures do not exceed 165°F/73.9°C.[1]

[1] http://www.fsis.usda.gov/wps/wcm/connect/625d9435-4f14-46fe-b207-5d6688cb4db5/Safe_Miminum_Internal_Temperature_Chart.pdf?MOD=AJPERES. Accessed September 10, 2015.

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To fill these data gaps, the Foundation co-funded researchers from Kansas State University[2] to examine the occurrence and levels of HCA in various meat products, including those with antioxidant containing marinades and enhancements with various ingredients, using various cooking methods and temperatures preferred by the U.S. consumer. The researchers evaluated the HCA levels found in the outer layer of the meat sample (2 mm) because HCAs are primarily found of the surface when exposed to temperatures above 300°F/149°C. Researchers found that HCA content in cooked meat products was dependent on the type of meat, cooking method, and cooking time and temperature.

HCAs are formed when products are cooked at temperatures higher than 300°F/149°C. In the U.S., all commercially prepared ready-to-eat (RTE) meat and poultry products (including deli meats, sausages, frankfurters, hotdogs, pre-cooked bacon, *etc*.) are cooked to much lower temperatures, *e.g.*, less than 180°F/83°C and cooking ovens are less than 212°F/100°C. In addition, Vangnai *et al.* (2014) demonstrated marinated products reduce the formation of HCAs during high heat cooking, especially when the marinade or enhancement solution included antioxidants, in addition to salt and phosphate.[3] All of these ingredients are commonly found in RTE meat products as well as marinated or enhanced meat products in the U.S. Therefore, the HCA level is influenced by cooking conditions and ingredients. Consuming processed meats, which are primarily RTE meats, contributes very little to dietary HCA exposure

<u>Assessment of the Potential Human Exposure to Heterocyclic Amines from Cooked Meat Products</u>

The work of Puangsombat *et al.* (2012) was utilized to estimate human HCA exposure in an additional co-funded Foundation project. This study reviewed the significant categories of fresh and processed meat products that were candidates for HCA formation, and developed a matrix of levels of HCA among these categories (based on data in the published literature at that time), and conducted an exposure assessment based on known dietary consumption patterns.[4]

---

[2] Puangsombat, K., *et al*. (2012). "Occurrence of heterocyclic amines in cooked meat products." Meat Sci 90(3): 739-746.
[3] Vangnai, K., *et al*. (2014). "Effect of enhancement on the formation of heterocyclic amines in cooked pork loins: Preliminary studies." Meat Sci 98(2): 88-93.
[4] http://namif.org/research/assessment-potential-human-exposure-heterocyclic-amines-cooked-meat-products. Accessed September 10, 2015.

The researchers found significant uncertainties associated with the dietary exposure estimates, particularly those associated with the existing data gaps in HCA levels in foods. For the 83 meat cuts cooked to varying endpoint temperatures, existing data gaps for PhIP, MeIQx, DiMeIQx and B[a]P were indicated. Overall, the existing data gaps and how those gaps were extrapolated to complete the matrix of levels of HCA presented significant uncertainty in the exposure estimates. These results should be carefully interpreted because many data points were extrapolated to create the human exposure assessment framework. Once those data gaps are filled, the framework should be reevaluated to ensure adequate and accurate HCA exposure assessment data. It is premature to complete a hazard assessment without the full body of evidence.

* * * * *

Thank you for your consideration of these points and I have also included the scientific evidence reference. Should you have any questions, please contact me at bbooren@meatinstitute.org.

Respectfully submitted,

[redacted]

Betsy Booren, Ph.D.
President

United States Department of Agriculture
Food Safety and Inspection Service

Food Safety Information

Safe Minimum Internal Temperature Chart

Safe steps in food handling, cooking, and storage are essential in preventing foodborne illness. You can't see, smell, or taste harmful bacteria that may cause illness. In every step of food preparation, follow the four guidelines to keep food safe:

- **Clean**—Wash hands and surfaces often.
- **Separate**—Separate raw meat from other foods.
- **Cook**—Cook to the right temperature.
- **Chill**—Refrigerate food promptly.

Cook all food to these minimum internal temperatures as measured with a food thermometer before removing food from the heat source. For reasons of personal preference, consumers may choose to cook food to higher temperatures.

Product	Minimum Internal Temperature & Rest Time
Beef, Pork, Veal & Lamb Steaks, chops, roasts	145 °F (62.8 °C) and allow to rest for at least 3 minutes
Ground meats	160 °F (71.1 °C)
Ham, fresh or smoked (uncooked)	145 °F (62.8 °C) and allow to rest for at least 3 min.
Fully Cooked Ham (to reheat)	Reheat cooked hams packaged in USDA-inspected plants to 140 °F (60 °C); all others to 165 °F (73.9 °C).

Product	Minimum Internal Temperature
All Poultry (breasts, whole bird, legs, thighs, and wings, ground poultry, and stuffing)	165 °F (73.9 °C)
Eggs	160 °F (71.1 °C)
Fish & Shellfish	145 °F (62.8 °C)
Leftovers	165 °F (73.9 °C)
Casseroles	165 °F (73.9 °C)

Food Safety Questions?

Call the USDA Meat & Poultry Hotline

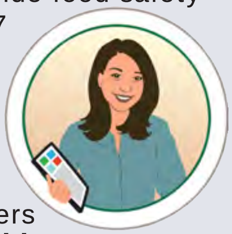
If you have a question about meat, poultry, or egg products, call the USDA Meat and Poultry Hotline toll free at **1-888-MPHotline (1-888-674-6854)**. The Hotline is open year-round

Monday through Friday from 10 a.m. to 4 p.m. ET (English or Spanish). Recorded food safety messages are available 24 hours a day. Check out the FSIS Web site at **www.fsis.usda.gov**.

Email questions to **MPHotline.fsis@usda.gov**.

AskKaren.gov

FSIS' automated response system can provide food safety information 24/7 and a live chat during Hotline hours.



Mobile phone users can access **m.askkaren.gov.**

PreguntaleaKaren.gov

The Food Safety and Inspection Service (FSIS) is the public health agency in the U.S. Department of Agriculture responsible for ensuring that the na tion's commercial supply of meat, poultry, and egg products is safe, whole some, and correctly labeled and packaged.

June 2012

Center for Chemical Regulation & Food Safety

Assessment of the Potential Human Exposure to Heterocyclic Amines from Cooked Meat Products

Assessment of the Potential Human Exposure to Heterocyclic Amines from Cooked Meat Products

Prepared for:
Susan Backus
Director of Research
American Meat Institute Foundation
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Prepared by:

Exponent
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August 15, 2009

Contents

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List of Acronyms

AC	1-amino-9-H-pyrido (2,3,b) Indole
AMI	American Meat Institute
AMIF	American Meat Institute Foundation
B(a)P	Benzo(a)pyrene
CDC	Centers for Disease Control
CFSII	Continuing Survey of Food Intakes by Individuals
CHARRED	Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease
DiMeIQx	2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxiline
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
FSIS	Food Safety and Inspection Service
HCA	Heterocyclic Amines
IQ	2-amino-3,4 dimethylimidazo (4,5,f) quinoline
MeIQ	2-amino-3-methylimidazo (4,5,f) quinoline
MeIQx	2-amino-3,8 dimethylimidazo (4,5,f) quinoxiline
NAS NRC	National Academy of Sciences National Research Council
NHANES	National Health and Nutrition Examination Survey
PAH	Polycyclic aromatic hydrocarbon
PhIP	2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine
USDA	United States Department of Agriculture

1 EXECUTIVE SUMMARY

The main objectives of this study are to review of the major categories of fresh and processed meat products that are candidates for heterocyclic amine (HCA) formation and develop a matrix of levels of HCA among the major consumed meat categories (based on data in the published literature); and to conduct an exposure assessment based on known dietary consumption patterns. The project was comprised of three parts, including: 1) literature review and data compilation, 2) a consumer behavior/preference survey, and 3) a dietary exposure assessment. In phase 1, data on HCA formation based on different methods of cooking/processing were reviewed and compiled. In phase 2, an internet survey was conducted to ascertain the prevalence of various meat cooking methods that are preferred among U.S. meat consumers. In phase 3 of the study, data from phases 1 and 2 were combined with food consumption data from the National Health and Nutrition Examination Survey 2003-2006 (NHANES 03-06), to derive estimates of exposure to HAs from meat consumption.

Based on the available published data, Exponent created an Excel database of HCA and B[a]P levels for 83 types of meat cuts by cooking method and degree of doneness that were included in the consumer behavior/preference survey. Based on NHANES 2003-2006 consumption data and the consumer's behavior/preference internet survey, food intake estimates for the 83 meat cuts by methods of cooking and degree of doneness were tabulated and summarized. In this report, we summarize the data and methods that were applied to develop dietary exposure estimates for PhIP, MeIQx, DiMeIQx, and B[a]P.

Uncertainties associated with the dietary exposure estimates, particularly those associated with the existing data gaps in HCA levels in foods are also described. For the 83 meat cut/degree of doneness, the existing data gaps for PhIP, MeIQx, DiMeIQx and B[a]P are indicated.
Overall, the existing data gaps and the extrapolation/surrogating from the available HCA level data present significant uncertainty in the exposure estimates and thus these results should be carefully interpreted. If it is possible in the future to fill the HCA data gaps (described in this report), then it would be recommended to re-estimate HCA exposure based on these improved data.

2 PROJECT OVERVIEW

The objectives of this project are to:

- Review of the major categories of fresh and processed meat products that are candidates for heterocyclic amine (HCA) formation and develop a matrix of levels of HCA among the major meat categories that are consumed.
- Conduct an exposure assessment based on the likelihood of HCA formation during normal processing and handling, and the likelihood and degree of human exposure based upon known dietary consumption patterns of major meat categories.

The project was comprised of three parts, including: 1) literature review and data compilation, 2) a consumer behavior survey, and 3) a dietary exposure assessment. In phase 1, data on HCA formation based on different methods of cooking/processing are reviewed and compiled. In phase 2, an Internet survey was conducted by IPSOS Observer to ascertain the prevalence of various meat cooking methods that are preferred among U.S. meat consumers. In phase 3 of the study, data from phases 1 and 2 were combined with food consumption data from the National Health and Nutrition Examination Survey 2003-2006 (NHANES 03-06), to derive estimates of exposure to HAs from meat consumption.

The goals were to assess dietary exposure to 3 major HCAs in meat/fish: PhIP, MeIQx, DiMeIQx, and B[a]P. In this report, we summarized the data and methods that were applied to develop dietary exposure estimates. Food intake estimates for the various meat cuts, methods of cooking and degree of doneness based on NHANES consumption data and the IPSOS consumer's behavior/preference survey were tabulated and summarized. For these meat cut/degree of doneness, the available concentration data for PHiP, MeIQx, DiMeIQx and B[a]P are also summarized in this report. Uncertainties associated with the HCA and B[a]P exposure estimates particularly those associated with the existing data gap in HCA levels in foods are also described.

3 DATA SOURCES

3.1 Consumption Data

The major publicly available consumption surveys (e.g., USDA's Continuing Survey of Food Intakes by Individuals (CSFII), and CDC's NHANES) that are typically used to assess potential dietary exposures to food additives or contaminants include very limited information about factors likely to affect HCA formation. Such factors include: the cut of meat and the degree of doneness (correlates of temperature and duration) associated with the cooking method used to prepare the various meat cuts. Based on an analysis of the types of foods included in the NHANES 2003-2006 food consumption survey, intake assessment based solely on the U.S. national consumption survey would be limited to following combination of meat/fish types and cooking methods:

Broad Meat Groups	Meat Types Reported in NHANES	Cooking Method Reported in NHANES
Beef	Steak, Brisket, Roast/Pot roast Ribs, Stew meat, Jerky, Liver, Other	Boiled, cooked, dried, fried, grilled/barbecued, roasted
Bacon	Pork, Turkey, Beef	Smoked
Burger	Ground beef	Cooked
Fish	Various fish species	Baked, baked or broiled, boiled, cooked, dried, dried, raw, smoked
Pork	Ground, patty, ham, liver, chop, jerky, roast, steak, ribs, tenderloins	Baked, baked or broiled, boiled, cooked, fried, Grilled/barbecued, smoked
Hotdog	Hotdogs	(blank)
Lunchmeat		Canned, cooked
Other meats	Ribs, ground or patty, chop, , roast, cutlet or steak, jerky	Baked, boiled, broiled, cooked, fried Roasted
Poultry	Breast w/ skin, breast, w/o skin, drumstick, w/ skin, drumstick, w/o skin, fillet, ground, leg (drumstick and thigh), w/ skin	baked or broiled, baked or fried, boiled, broiled, cooked, fried, roasted

	leg (drumstick and thigh), w/o skin, nuggets, patty, wing w/ skin, wing w/o skin, dark meat w/ skin, dark meat w/o skin	
Sausage	Beef, bratwurst, chicken and beef, Italian, polish, pork, pork and beef, link pork, link pork and beef, turkey, turkey and pork, Vienna, turkey, pork, beef	Canned, cooked, smoked

This study utilized the latest food consumption dataset to estimate meat/fish intake. The NHANES 2003-2006 (NCHS, 2008) is a complex multistage probability sample designed to be representative of the civilian U.S. population. The survey collects two days of food intake data, in addition to nutrition, demographic, and health information. The NHANES survey over-samples minorities, low-income groups, adolescents (12-19 years), and adults 60 years of age and older, and statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection. Participants included 10,122 subjects in 2003-2004 and 10,348 subjects in 2005-2006. Only individuals with complete and reliable 2-day dietary records were included in the analysis (N=16,783).

Given the limited descriptors for the method of cooking and degree of doneness for foods with reported consumption in NHANES, Exponent also conducted an internet-based survey to obtain data on consumers' preference for method of cooking and degree of doneness of the meat/fish that they consume. The development, implementation and application of the consumer's survey are described in the next section.

3.2 Consumers' Consumption Preference Survey (IPSOS Survey)

Exponent contracted with a market research survey company, IPSOS Observer, to conduct a specially designed survey to collect supplemental information on meat cut and cooking method preferences and data on degree of doneness preference. (Refer to Appendix A for more details)

3.2.1 Survey Instrument

Meat cooking behavior and meat consumption frequency have been ascertained using a validated FFQ developed by Sinha et al (2005), however, most of the data were gathered for specific cohort of individuals in an epidemiologic study (e.g. Martinez et al, 2007) and are not representative of meat cooking behavior among all US consumers. Exponent, along with its contractor IPSOS Observer, developed a survey questionnaire with close-ended questions to collect information on preferred cooking methods for home and restaurant prepared meat/fish products to supplement the food and method of cooking information extractable from the NHANES food consumption survey with degree of doneness preference and usual intake information. As such the purpose of the current survey is to ascertain the consumer preference and usual consumption as it relates to the degree of doneness of various cooked meat/fish products.

The cooking temperature and time have been shown to be correlated with HA formation. However, according to a 2006 FDA/FSIS survey[1], 67 % of the population owns a food thermometer, and while 54% always or often use a thermometer when cooking roasts or large cuts of meat, only 26% do so when cooking chicken parts, and 13% when cooking hamburgers. Sinha et al. (2005) report that the cooking technique and doneness level “serve as a reasonable proxy” for cooking temperature and time, the two most important elements in HA formation. Thus, for practical reasons the survey questionnaire relies on visual aids and description of degrees of doneness rather than on temperature measurements.

More details about the survey instrument and results are summarized in **Appendix A** of this report.

3.3 HCA Levels in Foods

A comprehensive review of the published literature was conducted to compile the readily available information on HCA levels in meat and fish products and associated method of cooking

[1] http://www.cfsan.fda.gov/~comm/crnutri7.html

and degree of doneness. There are two publicly available databases on HCA levels in meat and fish products:

NCI (National Cancer Institute) CHARRED (Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease) database:[2]

An excel spreadsheet of HCAs and benzo(a)pyrene (BaP) levels data is available from NCI. The data for MeIQx (ng/g meat), PhIP (ng/g meat), DiMeIQx (ng/g meat), and B[*a*]P (ng/g meat) are available for the following meat, cooking method and degree of doneness

- Meat types:
  - Bacon
  - Bacon fat
  - Chicken
  - Chicken (both)
  - Chicken (skin)
  - Chicken (skinless)
  - Chicken
  - Gravy
  - Ham slice
  - Hamburger
  - Pork chop
  - Sausage
  - Sausage links
  - Sausage patties
  - Steak

- Cooking method:

- Baked	Cooked in an oven with very little or no added liquid
- Boiled	Cooked in large amount of boiling liquid.
- Deep-fat fried	Cooked by immersing completely in hot fat
- Grilled/Barbecued	Cooked over a charcoal or gas grill.
- Microwaved	Cooked completely in a microwave oven.
- Oven-broiled	Cooked by direct exposure to the heat source in the oven.
- Pan-fried	Cooked in a small amount of hot fat in an open shallow pan.
- Stewed	Cooked by simmering in liquid in

- Degree of doneness: "just", "rare", "medium", "well", "very well".

Jakszyn et al (2004) Food Database of Nitrosamines, Heterocyclic amines, and Polycyclic Aromatic Hydrocarbons

This database is a compilation of available published data on food concentration on nitrosamines, HCAs and PAHs (polycyclic aromatic hydrocarbons) developed by the Catalan Institute of Oncology. The database contains information on HCA levels for 297 food items and PAH levels for 313 food items. The database is based on 139 references (1982 and 2003) from 23 different

[2] http://www.charred.cancer.gov

countries (including the US). HCAs included in the database are MeIQx, DiMeIQx, MeIQ, PhIP, AC, and IQ. Data on HCA and PAH levels are available for the following combinations of meat and fish products, cooking methods and degree of doneness:

Meat and Fish Products:
- Bacon (bacon, bacon fat, or pan residues)
- Beef (beef, hamburger, patty, minced, extract, minute steak, steak, stock cube)
- Chicken (breast, breast w skin, breast no skin, fast food sandwich, gravy, nuggets, thigh/leg, wings, white meat, liver
- Duck
- Turkey breast
- Pork (ham, steak, chop, belly, rib, cubes, rinds)
- Hotdog
- Lamb (chops
- Meat loaf
- Sausage
- Fish (fast food, herring, mackerel, pike-perch, salmon, swordfish, trout, tuna, whitefish, lobster, mussel, shrimp)

Cooking Methods:
- BA/GR: barbequed
- BK/ROA: baked
- BO: boiled
- BR: broiled
- FR: fried
- MW: microwaved
- NE: cooked
- RA: raw
- SM: smoked
- STW: stewed

Degree of Doneness:
- Uncooked
- Rare
- Medium
- Well-done
- Very well done
- Extra well done
- Not available

In addition to these two datasets, there are other published papers that provide more limited compilation of published studies, such as a publication by Keating and Bogen (2001). Although this compilation is not as extensive as that in the CHARRED and Jakszyn 2004 databases, Keating and Bogen (2004) updated this database in 2004 and provided HA concentration data for beef and chicken based on method of cooking, cooking temperature, duration and internal temperature. Toribio et al (2007) also measured concentrations of 15 HAs in different samples of griddled beef steak.

Based on the available data, Exponent created an Excel database of HCA levels for the meat cuts and cooking method and degree of doneness combinations that were included in the IPSOS survey. The database is summarized in **Appendix B, Tables B-1 to B-7**.

4 METHODS OF ESTIMATING DIETARY EXPOSURE

4.1 Estimating Food Intake

Detailed food consumption data from the NHANES 2003-2006 were used in conjunction with the IPSOS survey data to estimate food intake.

Each NHANES subject provided 2 days of food consumption information. Intakes for various meat/fish cuts by cooking method and degree of doneness were estimated using Exponent's Foods and Residue Evaluation Program (FARE™ 8.42) software. We identified each individual who reported consuming a food on either of the survey days, and we used that individual's responses for both survey days. Zero consumption days are included in calculating that individual's average daily intake. Provided the ingredient/contaminant of interest is not an acute toxicant, it is appropriate to average exposures over a longer period than one day. Therefore, Exponent® used each respondent's food consumption averaged over the two days of the NHANES 2003-2006 surveys. For example, if someone reported consuming 100 grams of steak on day 1 and 150 grams of steak on day 2, his/her 2-day average steak consumption would be 125 grams ([100+150]/2).

Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population and the selected age-gender subgroups.

A 2-day average typically overestimates long-term (chronic) daily intake; however, only two nonconsecutive days' worth of food consumption data are available in the most recent NHANES 2003-2006 survey. Although the 1989-91 CSFII included food consumption diaries on three nonconsecutive days, Exponent believes that rapidly evolving trends in diet and the pace of introduction of new foods call into question the representativeness of the older data for today's consumers. Therefore, Exponent used the best publicly available dietary intake data for this analysis. Further, since chronic health effect (cancer) is the toxicity endpoint of interest in the case of HCA, the appropriate exposure metric is mean *per capita*. In the estimation of the mean *per capita,* NHANES respondents who did not report consumption of a food are assumed as having zero intakes (assumed to be true non-eaters) and the reported consumption from the "eaters" of that food are averaged over all eaters and non-eaters. As such, it is unlikely that intakes are overestimated when expressed as mean *per capita.*

4.1.1 Bridging NHANES Food Consumption with IPSOS Survey Data

As previously described, NHANES food descriptors are limited in terms of method of cooking and the information on degree of doneness is not indicated. To develop intake for meat/fish by cuts, method of cooking and degree of doneness, Exponent developed a Monte Carlo sampling model to integrate the prevalence of type of meat cut consumed, cooking method, and degree of doneness preference among US consumers from the IPSOS survey with the NHANES 2003-2006 food consumption data. The following general approach was followed:

- When meat cut and cooking method were specified for a food of interest in the NHANES survey data, we applied the IPSOS survey's data on consumers' preference on the degree of doneness
- When meat cut was specified but cooking method was not specified for a food of interest in the NHANES survey data, we applied the IPSOS survey's data on consumers' preference on method of cooking and degree of doneness.
- When a meat/fish food was not further specified in terms of cut and cooking method in the NHANES survey data, we applied the IPSOS survey's data on consumption frequency for types of cuts, cooking method and degree of doneness.

Overall, NHANES food codes for meat/fish were mapped into 83 food groups based on type of meat, cut, and cooking method. Each of these was then classified into categories based on degree of doneness.

4.2 Estimating Exposure to HCA

The HCA intakes for each survey participant were derived by multiplying the amount of food consumed by concentration of the HCA in that food and then summing that information over all the foods reported consumed by that individual. Per capita estimates were derived by averaging the estimated HCA intakes over the population. Relative to the food intake database, the PhIP, MeIQx, DIMeIQx and B[a]P data were much less robust. Of the 83 foods classified based on meat cut and cooking method, concentration data were not available for 34 foods (41%). They include:

Beef Products
- Oven Baked Beef Steak
- Grilled/BBQ Beef Ribs
- Oven Broiled Beef Ribs
- Fried Beef Ribs
- Oven Baked Beef Ribs
- Microwave Baked Beef Ribs
- Grilled/BBQ Beef Brisket
- Oven Broiled Beef Brisket
- Fried Beef Brisket
- Oven Baked Beef Brisket
- Microwave Baked Beef Brisket
- Microwave Baked Beef Roast
- Oven Baked Hamburgers/Beef patties

Pork Products
- Oven Broiled Pork Ribs
- Fried Pork Ribs
- Microwave Baked Pork Ribs
- Oven Broiled Pork Tenderloins
- Fried Pork Tenderloins
- Oven Baked Pork Tenderloins
- Microwave Baked Pork Tenderloins
- Oven Broiled Pork Roast
- Fried Pork Roast
- Oven Baked Pork Roast
- Microwave Baked Pork Roast
- Oven Baked Ham Slices
- Microwave Baked Ham Slices

Other Meats (bacon, hotdog, and sausages)
- Oven Baked Bacon
- Grilled/BBQ Bacon
- Microwaved Baked Hot Dogs

Chicken Products
- Rotisserie Chicken

Fish Products
- Fried fish
- Oven broiled fish
- Oven baked fish
- Microwave baked fish

The B[a]P data are non-existent for all fish types. In order to carry out the exposure assessment, the following data treatment approach was applied to the foods with missing HCA information:

- When concentration data are not available for a specific meat cut or cooking method, values for a similar meat/cooking method were used as surrogate. For example, PhIP data were not available for oven baked beef steak, but data were available for oven broiled beef steaks and grilled/BBQ beef steaks. Since oven baking is more similar to oven broiling than grilling/BBQing, oven baked beef steak was assumed to have the same PhIP levels as oven broiled beef steak.
- When concentration data are not available for a degree of doneness, interpolation from levels reported for the lesser and higher degree of doneness was carried out. For instance if data were not available for the "Well done" category, but were available for the

"Medium well" and "Very well done" categories, linear interpolation between the two categories was used to estimate levels for the "Well done" category.

Overall, the existing data gaps in the HCA levels in meat/fish and the extrapolation/surrogating from the available data as described above present significant uncertainty in the exposure assessment. Dietary exposure estimates based on the extant HCA and B[a]P data should be carefully interpreted.

5 RESULTS AND DISCUSSION

5.1 IPSOS Survey

Consumers' preferences for degree of doneness by meat types are summarized in table 1. Based on the survey results, most consumers prefer their beef (cuts) just done (28.38%), medium well (20.10%), and well done (24.44%). The majority of consumers also prefer their hamburgers, chicken, pork, bacon and fish to be well done (32.73%, 56.29%, 46.59%, 50.07%, and 41.08%, respectively) and their hotdogs to be medium well (40.23%).

Table 1. Frequency of Consumer's Preference for Degree of Doneness

	Degree of Doneness (Frequency %)					
Meat/Fish Types	**Rare**	**Medium rare**	**Just done**	**Medium well**	**Well done**	**Very well done**
Beef	4.15%	16.86%	28.38%	20.10%	24.44%	6.08%
Hamburgers	2.79%	10.20%	24.27%	24.08%	32.73%	5.94%
Chicken	-	-	9.17%	23.40%	56.29%	11.14%
Pork	-	-	16.32%	29.26%	46.59%	7.82%
Bacon	-	-	10.41%	21.33%	50.07%	18.19%
Hot dogs	-	-	13.18%	40.23%	38.63%	7.96%
Fish	0.67%	2.49%	22.38%	28.13%	41.08%	5.25%

5.2 Meat Intake by Method of Cooking and Degree of Doneness

Since meat dishes can be either: (i) mostly meat (e.g., fried chicken) or (ii) mixed dishes (e.g., chicken fried rice), meat consumption from foods that are mostly meat captures a portion of total meat consumption. Table 2 provides a summary of per capita meat intakes when mixed dishes are included and when they are not. With the exception of chicken, consumption of non-mixed dishes, i.e., consumption of the mostly meat dishes, captured >75% of the overall meat/fish intake among US consumers. The current study focuses on non-mixed dishes and the intake results presented here are based on non-mixed (excluding mixed) dishes.

Based on NHANES 2003-2006 data, the mean *per capita* intake in g/day for the foods that were captured in this study was the highest for chicken (26.23 g/day) and the lowest for pork (7.57 g/day). Beef intake was second highest (mean *per capita* 19.76 g/day). Fish intake was slightly above pork consumption (mean *per capita* 8.81 g/day). Bacon and hotdogs combined mean *per capita* intake was 11.14 g/day

Table 2. U.S. Population Estimated Consumption of Meat (Mean *per Capita*, g/day, NHANES 2003-2006

Meat Type	Excluding Mixed Dishes	Including Mixed Dishes	% of Total
Beef	19.76	26.1	76%
Pork	7.57	9.8	77%
Chicken	26.23	35.8	73%
Bacon and hotdogs*	11.14	14.7	76%
Fish	8.81	9.03	95%

*include sausages

Consumption of meat and fish based on cut type are summarized in Table 3. In beef group, steak and hamburger have the highest consumption (mean per capita 8.52 g/day and 8.38 g/day, respectively). In the chicken meat group, skinless chicken breast has the highest intake (mean per capita 9.57 g/day), and in the pork meat group, pork chops have the highest intake (mean per capita 2.89 g/day).

Table 3. Estimated Meat Consumption by Cut (Mean per Capita, g/day); US Population, NHANES 2003-2006

Meat Type	Cut Type	g/day	g/kg-bw/day
Beef	Brisket	0.14	0.002
	Ribs	0.42	0.007
	Roast	1.86	0.027
	Steak	8.52	0.123
	NFS	0.45	0.007
Beef (Hamburger)	NA	8.38	0.131
Pork	Ham	1.87	0.028
	Pork chops	2.89	0.045
	Ribs	1.35	0.018
	Roast	0.81	0.011
	Tenderloins	0.24	0.004
	NFS	0.41	0.007
Chicken	Breast skin	2.22	0.031
	Breast skinless	9.57	0.162
	Other pieces skin	5.76	0.093
	Other pieces skinless	4.46	0.109
	NFS	4.22	0.063
Bacon	NA	1.39	0.022
Hot dog	NA	9.75	0.176
Fish	NA	8.54	0.129

Consumption of meat and fish based on degree of doneness are summarized in Table 4. Medium rare, just done and medium well done beef and hamburger have the highest consumption. Consumption for all other meat and fish are highest in the medium and well done categories.

Table 4. Estimated Meat Consumption by Degree of Doneness (Mean per Capita, g/day); US Population, NHANES 2003-2006

	Doneness Degree	g/day	g/kg-bw/day
Beef -	Rare	0.737	0.011
	Medium rare	2.937	0.043
	Just done	2.912	0.042
	Medium well	2.264	0.033
	Well done	2.065	0.030
	Very well done	0.469	0.007
Hamburgers -	Rare	0.226	0.004
	Medium rare	0.894	0.014
	Just done	2.112	0.033
	Medium well	2.060	0.032
	Well done	2.634	0.041
	Very well done	0.452	0.007
Chicken -	Rare	0.000	0.000
	Medium rare	0.000	0.000
	Just done	2.301	0.040
	Medium well	6.178	0.109
	Well done	14.818	0.258
	Very well done	2.931	0.051
Pork -	Rare	0.000	0.000
	Medium rare	0.000	0.000
	Just done	1.247	0.019
	Medium well	2.254	0.034
	Well done	3.576	0.053
	Very well done	0.495	0.007
Bacon -	Rare	0.000	0.000
	Medium rare	0.000	0.000
	Just done	0.123	0.002
	Medium well	0.275	0.004
	Well done	0.718	0.011
	Very well done	0.276	0.004
Hot dogs -	Rare	0.000	0.000
	Medium rare	0.000	0.000
	Just done	1.256	0.023
	Medium well	3.953	0.071
	Well done	3.848	0.069
	Very well done	0.695	0.013
Fish -	Rare	0.040	0.001
	Medium rare	0.162	0.002
	Just done	1.838	0.028
	Medium well	2.429	0.037
	Well done	3.600	0.054
	Very well done	0.470	0.007

5.3 Dietary Exposure to HCA

Tables 5-A and 5-B summarize the intake of HCA and B[a]P from meat and fish by the U.S. population. Data are expressed in µg/day (Table 5-A) and µg/kg-bw/day (Table 5-B) on the mean *per capita* basis. Overall, on a µg/day basis, chicken contribution to the total dietary exposure to PhIP and DiMeIQx and B[a]P were the highest, at 81% (0.82 µg/day) and 74% (0.0078 µg/day) respectively; fish contributes the most to total MeIQx dietary exposure (35% or 0.023 µg/day); and beef contributes the most to total B[a]P exposure (52% or 0.014 µg/day). On a µg/kg-bw/day basis, a similar pattern was observed.

Table 5-A. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Meat, Chicken, and Fish (µg/day, Mean *per Capita*, NHANES 2003-2006)

Meat Types	PhIP	% of total	MeIQx	% of total	DiMeIQx	% of total	B[a]P	% of total
Beef	6.7E-02	7%	1.2E-02	19%	1.2E-03	12%	1.4E-02	52%
Hamburgers	6.5E-03	1%	8.0E-03	12%	4.3E-04	4%	1.3E-03	5%
Chicken	8.2E-01	81%	1.6E-02	25%	7.8E-03	74%	1.2E-02	43%
Pork	3.0E-03	0%	3.2E-03	5%	3.0E-04	3%	4.6E-05	0%
Bacon Slides	7.7E-03	1%	2.3E-03	3%	1.7E-04	2%	1.5E-05	0%
Hot Dogs	8.7E-04	0%	1.0E-03	2%	0.0E+00	0%	4.3E-05	0%
Fish	1.0E-01	10%	2.3E-02	35%	6.1E-04	6%	0.0E+00	0%
TOTAL	1.0E+00	100%	6.6E-02	100%	1.1E-02	100%	2.7E-02	100%

Table 5-B. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Meat, Chicken, and Fish (µg/kg-BW/day, Mean *per Capita*, NHANES 2003-2006)

Meat Types	PhIP	% of total	MeIQx	% of total	DiMeIQx	% of total	B[a]P	% of total
Beef	9.7E-04	6%	1.8E-04	17%	1.8E-05	10%	2.0E-04	49%
Hamburgers	1.0E-04	1%	1.2E-04	12%	6.7E-06	4%	2.0E-05	5%
Chicken	1.4E-02	83%	2.7E-04	27%	1.3E-04	76%	1.9E-04	45%
Pork	4.2E-05	0%	4.9E-05	5%	4.7E-06	3%	6.8E-07	0%
Bacon Slices	1.2E-04	1%	3.6E-05	3%	2.7E-06	2%	2.4E-07	0%
Hot Dogs	1.6E-05	0%	1.8E-05	2%	0.0E+00	0%	7.8E-07	0%
Fish	1.6E-03	10%	3.5E-04	34%	9.2E-06	5%	0.0E+00	0%
TOTAL	1.6E-02	100%	1.0E-03	100%	1.7E-04	100%	4.1E-04	100%

Tables 6-A and 6-B summarize the dietary exposure to HCA and B[a]P from chicken consumption alone by the U.S. population in µg/day and µg/kg-bw/day. Table 6-A provides exposure estimates by degree of doneness while Table 6-B provides exposure estimates by cooking method. Well done chicken consumption provide the highest exposure to HCA and B[a]P (PhIP = 0.46 µg/day, MeIQx = 0.0088 µg/day, DiMeIQx = 0.0049 µg/day, and B[a]P = 0.0075 µg/day) (Table 6-A). Consumption of grilled/BBQ chicken resulted in the highest exposure to PhIP (0.54 µg/day), MeIQx (0.0083 µg/day) and B[a]P (0.01 µg/day), while consumption of fried chicken resulted in the highest exposure to DiMeIQx (0.005 µg/day) (Table 6-B).

Table 6-A. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Chicken by Degree of Doneness (µg/day, Mean *per Capita*, NHANES 2003-2006)

Doneness	µg/day				µg/kg-BW/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Rare	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Medium rare	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Just done	9.4E-03	1.1E-04	1.4E-05	2.5E-04	1.6E-04	1.9E-06	2.2E-07	4.0E-06
Medium well	1.2E-01	2.0E-03	1.1E-03	2.2E-03	1.9E-03	3.4E-05	1.8E-05	3.5E-05
Well done	4.6E-01	8.8E-03	4.9E-03	7.5E-03	7.6E-03	1.5E-04	8.2E-05	1.2E-04
Very well done	2.3E-01	5.3E-03	1.8E-03	1.7E-03	3.9E-03	8.8E-05	3.0E-05	2.7E-05
Sub-Total	8.2E-01	1.6E-02	7.8E-03	1.2E-02	1.4E-02	2.7E-04	1.3E-04	1.9E-04

Table 6-B. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Chicken by Cooking Method(µg/day, Mean *per Capita*, NHANES 2003-2006)

Cooking Method	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Fried	1.5E-01	6.9E-03	5.0E-03	7.2E-04	2.4E-03	1.2E-04	8.3E-05	1.2E-05
Grilled/ BBQ	5.4E-01	8.3E-03	2.8E-03	1.0E-02	8.9E-03	1.4E-04	4.7E-05	1.6E-04
Microwaved Baked	0.0E+00	8.2E-05	0.0E+00	1.0E-05	0.0E+00	1.5E-06	0.0E+00	1.8E-07
Oven Baked	0.0E+00	4.4E-04	0.0E+00	5.4E-05	0.0E+00	7.6E-06	0.0E+00	9.5E-07
Oven Broiled	1.3E-01	5.2E-04	3.1E-05	4.4E-04	2.1E-03	8.9E-06	5.7E-07	8.0E-06
Rotisserie Chicken	6.3E-03	1.6E-05	2.8E-06	2.3E-05	9.7E-05	2.5E-07	4.3E-08	3.5E-07
Sub-Total	8.2E-01	1.6E-02	7.8E-03	1.2E-02	1.4E-02	2.7E-04	1.3E-04	1.9E-04

Tables 7-A, 7-B, and 7-C summarize the dietary exposure to HCA and B[a]P from beef and hamburger consumption by the U.S. population in µg/day and µg/kg-bw/day. Table 7-A provides exposure estimates by beef types while Table 7-B provides exposure estimates by degree of doneness and Table 7-C provides exposure estimates by cooking method. Consistent with steak having high intake, the exposure estimates for HCA and B[a]P were the highest for steak (PhIP = 0.06 µg/day, MeIQx = 0.011 µg/day, DiMeIQx = 0.00098 µg/day, and B[a]P = 0.012 µg/day) (Table 7-A). Most of the exposure to HCA and B[a]P were from consumption of just done, medium well, and well done meat and hamburgers (Table 7-B). Consumption of grilled/BBQ beef and hamburgers resulted in the highest exposure to PhIP (0.049 µg/day) and B[a]P (0.015 µg/day), while consumption of fried beef and hamburgers resulted in the highest exposure to MeIQx (0.01 µg/day) and DiMeIQx (0.00071µg/day) (Table 7-C).

Table 7-A. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Beef and Hamburgers (µg/day, Mean *per Capita*, NHANES 2003-2006)

Beef Types	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Steak	6.0E-02	1.1E-02	9.8E-04	1.2E-02	8.7E-04	1.6E-04	1.4E-05	1.8E-04
Beef Brisket	1.4E-03	2.3E-04	2.0E-05	3.0E-04	2.1E-05	3.4E-06	2.9E-07	4.4E-06
Beef Ribs	4.7E-03	7.7E-04	6.1E-05	1.1E-03	7.6E-05	1.3E-05	9.9E-07	1.8E-05
Beef Roast	1.8E-04	1.8E-04	1.8E-04	1.6E-05	2.6E-06	2.6E-06	2.6E-06	2.3E-07
Hamburgers	6.5E-03	8.0E-03	4.3E-04	1.3E-03	1.0E-04	1.2E-04	6.7E-06	2.0E-05
Sub-Total	7.3E-02	2.0E-02	1.7E-03	1.5E-02	1.1E-03	3.0E-04	2.5E-05	2.2E-04

Table 7-B. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Beef and Hamburgers by Degree of Doneness(µg/day, Mean *per Capita*, NHANES 2003-2006)

Degree of Doneness	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Rare	2.4E-03	2.2E-04	6.3E-06	0.0E+00	3.4E-05	3.3E-06	9.2E-08	0.0E+00
Medium rare	1.4E-02	1.8E-03	1.2E-04	2.8E-03	2.0E-04	2.6E-05	1.8E-06	4.1E-05
Just done	1.6E-02	2.8E-03	3.0E-04	4.5E-03	2.3E-04	4.2E-05	4.4E-06	6.6E-05
Medium well	1.5E-02	4.7E-03	3.5E-04	3.7E-03	2.2E-04	7.0E-05	5.2E-06	5.4E-05
Well done	1.5E-02	7.5E-03	4.5E-04	3.2E-03	2.3E-04	1.1E-04	6.8E-06	4.8E-05
Very well done	1.1E-02	3.2E-03	4.3E-04	9.3E-04	1.6E-04	4.8E-05	6.4E-06	1.4E-05
Sub-Total	7.3E-02	2.0E-02	1.7E-03	1.5E-02	1.1E-03	3.0E-04	2.5E-05	2.2E-04

Table 7-C. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Beef and Hamburgers by Method of Cooking(µg/day, Mean *per Capita*, NHANES 2003-2006)

Cooking method	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Fried	1.2E-02	1.0E-02	7.1E-04	4.6E-05	1.7E-04	1.5E-04	1.0E-05	7.2E-07
Grilled/BBQ	4.9E-02	8.1E-03	6.5E-04	1.5E-02	7.2E-04	1.2E-04	9.8E-06	2.2E-04
Microwaved Baked	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Oven Baked	5.2E-03	1.2E-03	2.5E-04	3.3E-05	7.7E-05	1.8E-05	3.6E-06	4.9E-07
Oven Broiled	7.0E-03	8.5E-04	5.8E-05	2.2E-05	1.0E-04	1.3E-05	8.5E-07	3.4E-07
Sub-Total	7.3E-02	2.0E-02	1.7E-03	1.5E-02	1.1E-03	3.0E-04	2.5E-05	2.2E-04

Tables 8-A, 8-B, and 8-C summarize the dietary exposure to HCA and B[a]P from pork and bacon consumption by the U.S. population in µg/day and µg/kg-bw/day. Table 8-A provides exposure estimates by pork types, while Table 8-B provides exposure estimates by degree of doneness and Table 8-C provides exposure estimates by cooking method. Bacon consumption contributed the highest exposure to PhIP and MeIQx (0.0077 µg/day and 0.0023 µg/day, respectively) (Table 8-A). Pork chops intake lead to the highest exposure to DiMeIQx (0.00025 µg/day) and B[a]P (0.000022 µg/day). Well done pork consumption lead to the highest exposure estimates for all three HCA and B[a]P (PhIP = 0.0051 µg/day, MeIQx = 0.0028 µg/day, DiMeIQx = 0.00024 µg/day, and B[a]P = 0.000041 µg/day) (Table 8-B). Consumption of fried pork meats resulted in the highest exposure to MeIQx (0.003 µg/day), DiMeIQx (0.00023 µg/day) and B[a]P (0.000024 µg/day), while consumption of grilled/BBQ pork resulted in the highest exposure to PhIP (0.0037 µg/day) (Table 8-C).

Table 8-A. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Pork and Bacon(µg/day, Mean *per Capita*, NHANES 2003-2006)

Pork Type	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Bacon	7.7E-03	2.3E-03	1.7E-04	1.5E-05	1.2E-04	3.6E-05	2.7E-06	2.4E-07
Ham Slices	3.0E-04	4.0E-04	4.3E-06	5.7E-06	4.4E-06	6.0E-06	6.5E-08	8.6E-08
Pork Chop	7.0E-04	2.1E-03	2.5E-04	2.2E-05	1.1E-05	3.2E-05	3.9E-06	3.4E-07
Pork Ribs	1.8E-03	4.7E-04	3.0E-05	1.1E-05	2.4E-05	6.3E-06	4.0E-07	1.4E-07
Pork Roast	1.2E-04	1.8E-04	8.6E-06	6.2E-06	1.6E-06	2.5E-06	1.2E-07	8.6E-08
Pork Tenderloins	6.9E-05	1.2E-04	8.7E-06	2.0E-06	1.1E-06	2.0E-06	1.4E-07	3.3E-08
Sub-Total	1.1E-02	5.5E-03	4.8E-04	6.1E-05	1.6E-04	8.5E-05	7.4E-06	9.3E-07

Table 8-B. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Pork and Bacon by Degree of Doneness(µg/day, Mean *per Capita*, NHANES 2003-2006)

Doneness	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Rare	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Medium rare	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Just done	2.2E-04	1.2E-04	2.8E-06	5.8E-07	3.4E-06	3.8E-08	9.2E-09	1.9E-06
Medium well	1.5E-03	8.3E-04	6.8E-05	1.2E-05	2.3E-05	1.1E-06	1.8E-07	1.3E-05
Well done	5.1E-03	2.8E-03	2.4E-04	4.1E-05	7.7E-05	3.7E-06	6.2E-07	4.3E-05
Very well done	3.9E-03	1.8E-03	1.7E-04	7.9E-06	6.1E-05	2.6E-06	1.2E-07	2.8E-05
Sub-Total	1.1E-02	5.5E-03	4.8E-04	6.1E-05	1.6E-04	7.4E-06	9.3E-07	8.5E-05

Table 8-C. U.S. Population Estimated Intake of PhIP, MeIQx, DiMeIQx, and B[a]P from Consumption of Pork and Bacon by Method of Cooking(µg/day, Mean *per Capita*, NHANES 2003-2006)

Cooking Method	µg/day				µg/kg/day			
	PhIP	MeIQx	DiMeIQx	B[a]P	PhIP	MeIQx	DiMeIQx	B[a]P
Fried	1.3E-03	3.0E-03	2.3E-04	2.4E-05	2.1E-05	4.7E-05	3.7E-06	3.7E-07
Grilled/BBQ	3.7E-03	1.8E-03	1.4E-04	1.4E-05	5.7E-05	2.7E-05	2.2E-06	2.0E-07
Micro waved Baked	2.1E-04	1.8E-04	1.4E-05	3.3E-06	3.4E-06	2.9E-06	2.2E-07	5.2E-08
Microwaved Baked	0.0E+00	1.4E-05	0.0E+00	1.3E-06	0.0E+00	2.1E-07	0.0E+00	1.9E-08
Oven Baked	3.1E-03	3.0E-04	5.0E-05	1.3E-05	4.8E-05	4.6E-06	7.4E-07	1.9E-07
Oven Broiled	2.3E-03	2.1E-04	3.5E-05	6.5E-06	3.5E-05	3.2E-06	5.3E-07	9.6E-08
Sub-Total	1.1E-02	5.5E-03	4.8E-04	6.1E-05	1.6E-04	8.5E-05	7.4E-06	9.3E-07

5.4 Discussion

Representative and up-to-date food consumption rates can be readily obtained from national food consumption survey such as NHANES 2003-06. However, food descriptors are limited in terms of information about method of cooking and degree of doneness, which highly correlate with the formation of HCA and B[a]P in meat and fish. A survey was therefore conducted for Exponent by IPSOS Observer to collect information on the degree of doneness preference for 84 types of meats and cooking methods combinations among US adult consumers. The IPSOS survey collected information via a targeted internet survey of a representative sample of subjects ages 18 year or more. Further, data on levels of PhIP, MeIQx, DIMeIQx and B[a]P in meat and fish were obtained from the published literature. When such data were not available for a specific {meat cut X cooking method X degree of doneness} combination, they were imputed from the existing data.

In the current study we developed intake for meat/fish by cuts, method of cooking and degree of doneness by applying the Monte Carlo sampling model to integrate the prevalence of type of meat cut consumed, cooking method, and degree of doneness preference among US consumers from the IPSOS survey with the NHANES 2003-2006 food consumption data. The approach used to integrate the data from both surveys randomly allocated a degree of doneness preference to each meat eating occasion in the NHANES survey. The Monte Carlo model used did not assume any correlation in the doneness preference across meat cut types and cooking methods for a given individual. A graphical examination of the IPSOS data indicates that there may be some correlation in the doneness preference across cooking methods for some products, e.g., steaks (Figure 1) or beef ribs (Figure 2), but that this may not necessarily apply to all products, e.g., pork chops (Figure 3). Further, an examination of the data to determine if subjects tend to prefer similar doneness levels for given cooking methods (across multiple meats and cuts) indicates that this assumption may not be true (Figure 4). If correlations were accounted for the extreme percentile estimates (i.e. 5^{th}, 95^{th} percentiles) would be lower (at the 5^{th} percentile) or higher (at the 95^{th} percentile) than when correlations were not accounted for. However, these effects are not likely to be observed at the mean, which is the focus of this study analysis.

Relative to the robust food consumption and the consumer's preference data obtained from NHANES 2003-2006 and the IPSOS survey, respectively, the data on levels of PhIP, MeIQx, DIMeIQx and B[a]P in meat and fish were more limited. Of the 83 foods classified based on meat cut, cooking method and degree of doneness included in the current study, concentration data were not available for 34 foods (41%), most of which are beef and pork types. The B[a]P data were non-existent for all fish types. Further, since the concentration data were published compilation of existing studies, the quality of the data (and original studies) is unknown.

In assessing dietary exposure, values for the missing data were extrapolated based on the concentration data that are available for similar meat/fish types, cut and degree of doneness. Since the imputed data may not be representative of levels expected to occur in these meat cuts and degree of doneness the conclusions reached on the relative ranking of exposures for various products should be viewed caution. For instance, data from the IPSOS survey indicate that Grilled/BBQ steak are among the most frequently consumed beef foods of interest (percent consumers: 67.9%), and that the most preferred doneness level for Grilled/BBQ steak is "Medium rare" (33.9% of consumers of the food). However, no PhIP data were available for that combination of beef cut and degree of doneness, and the exposure assessment used a value interpolated between the levels reported for the "Rare" and "Just Done" categories. Since there is a large difference in levels reported for these two categories (2.53 ng/g vs. 12.0 ng/g), the impact of the linear assumption used in the derivation of the assumed level for the "Medium rare" category may be significant. Similar potential uncertainties exist for several meat cuts. In some instances, the published levels for the less done categories were higher than for the more done ones. For instance, in the case of oven broiled steaks, the levels reported in the published literature for the "Rare" and "Just done" categories were: 6.14 ng/g and 2.08 ng/g, respectively

Overall, the existing data gaps in the HCA levels in meat/fish and the extrapolation/surrogating from the available data as described in this report present significant uncertainty in the exposure estimates. Dietary exposure estimates for HCA and B[a]P based on the extant concentration data should be carefully interpreted.

FIGURE 1. CORRELATION IN DONENESS LEVEL PREFERENCE FOR BEEF STEAKS

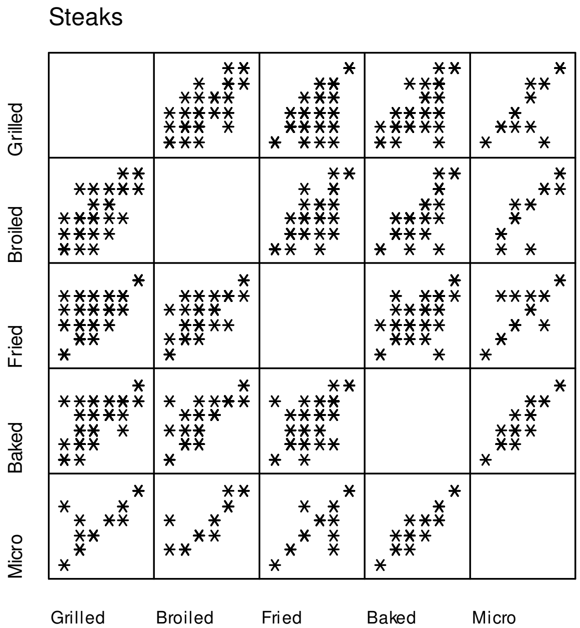


FIGURE 2. CORRELATION IN DONENESS LEVEL PREFERENCE FOR BEEF RIBS

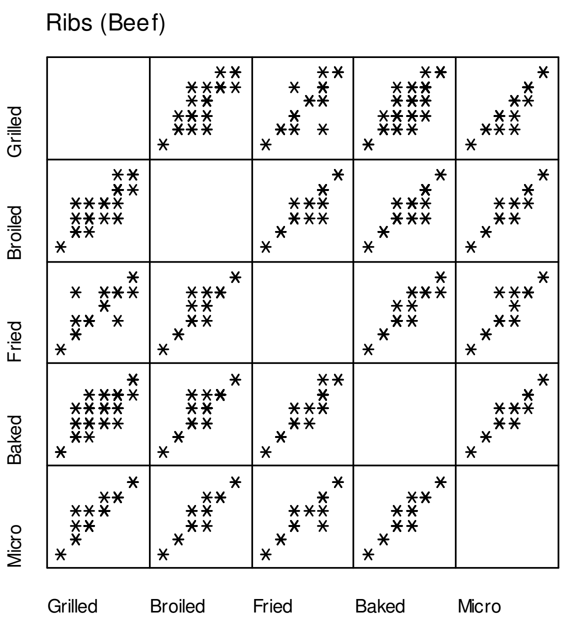


FIGURE 3. CORRELATION IN DONENESS LEVEL PREFERENCE FOR PORK CHOPS

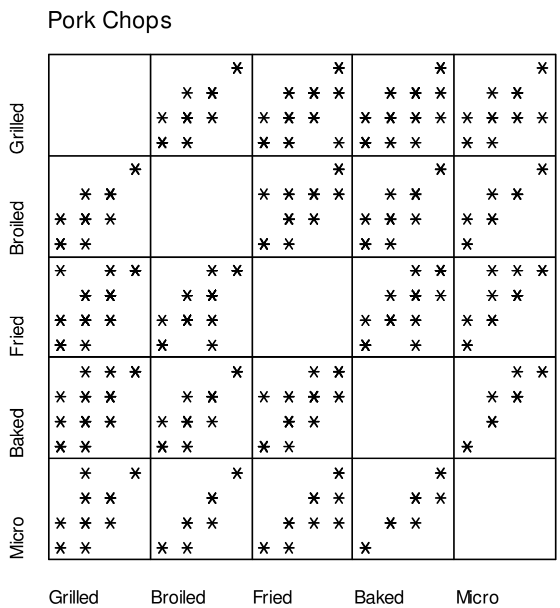
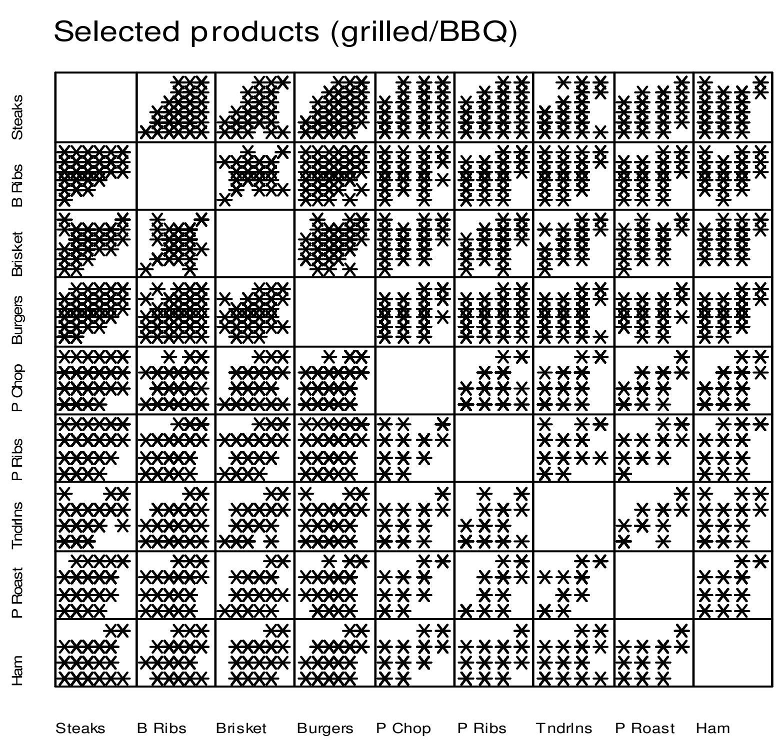


FIGURE 4. CORRELATION IN DONENESS LEVEL PREFERENCE FOR GRILLED PRODUCTS



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APPENDIX A. CONSUMER SURVEY

Exponent contracted with a market research survey company, IPSOS Observer, to conduct a specially designed survey to collect supplemental information on meat cut and cooking method preferences and data on degree of doneness preference. The IPSOS survey was a custom online survey conducted via the World Wide Web with data collected electronically on IPSOS secure servers. IPSOS Observer contacted a nationally representative sample of adults age 18 years and over to complete this survey. Respondents received an initial screen and only those who had reported consuming at least one of the following eight types of meat in the past 6 months: Beef, Ground Beef, Pork, Ham, Bacon, Hotdog, Chicken or Fish were included. A total of 1,086 subjects (498 male/588 female) completed the survey. Interviewing took place from November 19 – December 1, 2008

The questionnaire used to collect the information is provided below. The subjects were asked whether they had consumed any of the products of interest in the past six months. The web-based survey automatically guided respondents across follow-up questions regarding the cooking methods and doneness degree preferences. In addition, since different subjects may have different interpretation of the definition of the doneness levels, the questionnaire provided respondents with pictures illustrating the various doneness levels to ensure some level of consistency.

Figure A-1. Questionnaire used in the IPSOS survey: selected sections

EXPONENT MEAT PREPARATION SURVEY
Online Custom Survey
Ipsos Observer Job #: II0AVG100-1

Q.1
Please indicate the types of meat that you have eaten in the past 6 months.
(Select all that apply)

Beef
Ground Beef
Pork
Ham
Bacon
Hotdog
Chicken
Fish
None

[IF Q.1=NONE, THANK AND TERMINATE, OTHERWISE CONTINUE.]

SEGMENT BREAK--

[IF Q.1=BEEF CONTINUE. OTHERWISE SKIP TO Q.4.]
Q.2
Which of the following types of beef have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Steak
Oven Broiled Steak
Fried Steak
Oven Baked Steak
Microwave Baked Steak
Grilled/BBQ Ribs
Oven Broiled Ribs
Fried Ribs
Oven Baked Ribs
Microwave Baked Ribs
Grilled/BBQ Brisket
Oven Broiled Brisket
Fried Brisket
Oven Baked Brisket
Microwave Baked Brisket
Oven Baked Roast
Microwave Baked Roast
None of the above

SEGMENT BREAK--

[IF Q.2=NONE OF THE ABOVE SKIP TO Q.4. OTHERWISE CONTINUE.]
Q.3

Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Rare
Medium Rare
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q2]

SEGMENT BREAK---

[IF Q.1=GROUND BEEF CONTINUE. OTHERWISE SKIP TO Q.6.]
Q.4
Which of the following types of ground beef have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Hamburgers/Beef patties
Oven Broiled Hamburgers/Beef patties
Fried Hamburgers/Beef patties
Oven Baked Hamburgers/Beef patties
Microwave Baked Hamburgers/Beef patties
None of the above

SEGMENT BREAK---

[IF Q.4=NONE OF THE ABOVE SKIP TO Q.6. OTHERWISE CONTINUE.]
Q.5
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Rare
Medium Rare (Medium Rare is in between Rare and Just Done)
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q.4]

SEGMENT BREAK---

[IF Q.1=PORK CONTINUE. OTHERWISE SKIP TO Q.8.]
Q.6
Which of the following types of pork have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Pork Chop
Oven Broiled Pork Chop
Fried Pork Chop
Oven Baked Pork Chop
Microwave Baked Pork Chop
Grilled/BBQ Ribs
Oven Broiled Ribs
Fried Ribs
Oven Baked Ribs
Microwave Baked Ribs
Grilled/BBQ Tenderloins
Oven Broiled Tenderloins
Fried Tenderloins
Oven Baked Tenderloins
Microwave Baked Tenderloins
Grilled/BBQ Pork Roast
Oven Broiled Pork Roast
Fried Pork Roast
Oven Baked Pork Roast
Microwave Baked Pork Roast
None of the above

SEGMENT BREAK--

[IF Q.6=NONE OF THE ABOVE SKIP TO Q.8. OTHERWISE CONTINUE.]
Q.7
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q.6]

SEGMENT BREAK--

[IF Q.1=HAM CONTINUE. OTHERWISE SKIP TO Q.10.]
Q.8
Which of the following types of ham have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Ham Slices
Oven Broiled Ham Slices
Fried Ham Slices
Oven Baked Ham Slices
Microwave Baked Ham Slices
None of the above

SEGMENT BREAK--

[IF Q.8=NONE OF THE ABOVE SKIP TO Q.10. OTHERWISE CONTINUE.]
Q.9
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q.8]

SEGMENT BREAK--

[IF Q.1=BACON CONTINUE. OTHERWISE SKIP TO Q.12.]
Q.10
Which of the following types of bacon have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Bacon Slices
Oven Broiled Bacon Slices
Fried Bacon Slices
Oven Baked Bacon Slices
Microwave Baked Bacon Slices
None of the above

SEGMENT BREAK--

[IF Q.10=NONE OF THE ABOVE SKIP TO Q.12. OTHERWISE CONTINUE.]
Q.11
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q.10]

SEGMENT BREAK---

[IF Q.1=HOTDOG CONTINUE. OTHERWISE SKIP TO Q.14.]
Q.12
Which of the following types of hotdog have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Hotdog
Oven Broiled Hotdog
Fried Hotdog
Oven Baked Hotdog
Microwave Baked Hotdog
None of the above

SEGMENT BREAK---

[IF Q.12=NONE OF THE ABOVE SKIP TO Q.14. OTHERWISE CONTINUE.]
Q.13
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q.12]

SEGMENT BREAK---

[IF Q.1=CHICKEN CONTINUE. OTHERWISE SKIP TO Q.16.]
Q.14
Which of the following types of chicken have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Rotisserie Chicken
Grilled/BBQ Chicken Breasts, Skinless
Oven Broiled Chicken Breasts, Skinless
Fried Chicken Breasts, Skinless
Oven Baked Chicken Breasts, Skinless
Microwave Baked Chicken Breasts, Skinless
Grilled/BBQ Chicken Breasts, With Skin
Oven Broiled Chicken Breasts, With Skin
Fried Chicken Breasts, With Skin
Oven Baked Chicken Breasts, With Skin
Microwave Baked Chicken Breasts, With Skin

Grilled/BBQ Other Chicken Pieces, Skinless
Oven Broiled Other Chicken Pieces, Skinless
Fried Other Chicken Pieces, Skinless
Oven Baked Other Chicken Pieces, Skinless
Microwave Baked Other Chicken Pieces, Skinless
Grilled/BBQ Other Chicken Pieces, With Skin
Oven Broiled Other Chicken Pieces, With Skin
Fried Other Chicken Pieces, With Skin
Oven Baked Other Chicken Pieces, With Skin
Microwave Baked Other Chicken Pieces, With Skin
None of the above

SEGMENT BREAK--

[IF Q.14=NONE OF THE ABOVE SKIP TO Q.16. OTHERWISE CONTINUE.]
Q.15
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]
Just done (Medium)
Medium Well (Medium Well is in between Just Done and Well Done)
Well Done
Very Well Done

[STUB]
[PIPE-IN TYPES SELECTED IN Q14]

SEGMENT BREAK--

[IF Q.1=FISH CONTINUE. OTHERWISE SKIP TO Q.18.]
Q.16
Which of the following types of fish have you eaten either at home or away from home in the past 6 months?
(Select all that apply)

Grilled/BBQ Fish
Oven Broiled Fish
Fried Fish
Oven Baked Fish
Microwave Baked Fish
None of the above

SEGMENT BREAK--

[IF Q.16=NONE OF THE ABOVE SKIP TO Q.18. OTHERWISE CONTINUE.]
Q.17
Please indicate how you typically consume each of the following. If needed, you can click on each photo to enlarge it.
(Select One for each)

[BANNER WITH THUMBNAIL PICTURES. ALLOW RESPONDENTS TO ENLARGE PHOTO IF DESIRED]

Rare
Medium Rare
Just done (Medium)
Medium Well
Well Done
Very Well Done

SEGMENT BREAK---

Q.18
When you ate any of the following meat products, were they usually marinated before cooking?
(Select all that apply)

Yes
No
Don't know
Did Not Eat

Beef Steak
Beef Ribs
Beef Brisket
Pork Chop
Pork Rib
Pork Tenderloins
Chicken
Fish

Table A-1. Summary Results From the IPSOS Observer Survey: Percent Consumers and Degree of Doneness Preference

Meat type	Meat, Cut and Method of Cooking	Percent consumers	Preferred Degree of Doneness (%)					
			Rare	Medium Rare	Just done (Medium)	Medium Well	Well Done	Very Well Done
Beef		92.8						
	Grilled/BBQ Steak	67.9	8.9	33.9	25.4	17.3	11.4	3.1
	Oven Broiled Steak	30.7	8.7	30.4	27.2	18.8	11.0	3.9
	Fried Steak	31.7	5.3	25.3	25.3	20.0	20.6	3.4
	Oven Baked Steak	19.8	4.5	20.5	21.0	21.5	27.5	5.0
	Microwave Baked Steak	3.6	2.8	19.4	33.3	19.4	13.9	11.1
	Grilled/BBQ Beef Ribs	47.1	1.5	9.3	24.8	26.1	33.7	4.6
	Oven Broiled Beef Ribs	14.8	1.3	6.7	30.2	23.5	32.9	5.4
	Fried Beef Ribs	5.5	1.8	10.9	38.2	10.9	0.0	0.0
	Oven Baked Beef Ribs	25.7	1.5	5.0	23.6	27.8	37.5	4.6
	Microwave Baked Beef Ribs	3.7	5.4	10.8	21.6	27.0	27.0	8.1
	Grilled/BBQ Brisket	18.3	1.6	19.0	27.7	26.6	20.7	4.3
	Oven Broiled Brisket	9.9	4.0	19.0	38.0	15.0	17.0	7.0
	Fried Brisket	4.6	4.3	17.4	26.1	21.7	23.9	6.5
	Oven Baked Brisket	11.5	2.6	13.8	34.5	15.5	26.7	6.9
	Microwave Baked Brisket	3.8	5.3	13.2	34.2	13.2	26.3	7.9
	Oven Baked Roast	55.5	3.6	15.6	25.8	24.7	25.4	5.0
	Microwave Baked Roast	5.5	7.3	16.4	25.5	12.7	29.1	9.1
	None of the Above	6.1						
Ground Beef		92.5						
	Grilled/BBQ Hamburgers/Beef patties	77.8	2.8	13.2	26.5	25.1	27.6	4.9
	Oven Broiled Hamburgers/Beef patties	18.9	2.1	14.7	24.7	24.7	28.9	4.7
	Fried Hamburgers/Beef patties	60.0	2.5	7.3	24.7	24.4	35.5	5.6
	Oven Baked Hamburgers/Beef patties	13.4	3.7	5.9	21.5	23.7	37.8	7.4
	Microwave Baked Hamburgers/Beef patties	7.1	2.8	9.9	23.9	22.5	33.8	7.0

Meat type	Meat, Cut and Method of Cooking	Percent consumers	Preferred Degree of Doneness (%)					
			Rare	Medium Rare	Just done (Medium)	Medium Well	Well Done	Very Well Done
	None of the Above	3.2						
Pork		84.3						
	Grilled/BBQ Pork Chop	44.4	0.0	0.0	18.2	28.6	47.3	5.9
	Oven Broiled Pork Chop	25.5	0.0	0.0	13.7	31.8	49.4	5.2
	Fried Pork Chop	46.8	0.0	0.0	11.0	26.9	53.0	9.1
	Oven Baked Pork Chop	43.2	0.0	0.0	14.2	27.6	51.1	7.1
	Microwave Baked Pork Chop	3.8	0.0	0.0	17.1	28.6	42.9	11.4
	Grilled/BBQ Pork Ribs	41.4	0.0	0.0	12.9	28.8	51.5	6.9
	Oven Broiled Pork Ribs	13.3	0.0	0.0	11.5	32.0	48.4	8.2
	Fried Pork Ribs	5.1	0.0	0.0	10.6	25.5	55.3	8.5
	Oven Baked Pork Ribs	22.7	0.0	0.0	8.2	28.8	52.4	10.6
	Microwave Baked Pork Ribs	3.5	0.0	0.0	15.6	18.8	53.1	12.5
	Grilled/BBQ Tenderloins	23.7	0.0	0.0	24.9	34.6	36.4	4.1
	Oven Broiled Tenderloins	13.8	0.0	0.0	15.9	35.7	44.4	4.0
	Fried Tenderloins	14.3	0.0	0.0	11.5	26.7	55.0	6.9
	Oven Baked Tenderloins	22.3	0.0	0.0	14.2	31.4	46.1	8.3
	Microwave Baked Tenderloins	2.8	0.0	0.0	7.7	23.1	53.8	15.4
	Grilled/BBQ Pork Roast	14.1	0.0	0.0	20.2	31.0	40.3	8.5
	Oven Broiled Pork Roast	15.8	0.0	0.0	15.9	31.0	46.9	6.2
	Fried Pork Roast	5.1	0.0	0.0	12.8	27.7	44.7	14.9
	Oven Baked Pork Roast	43.6	0.0	0.0	14.3	23.6	53.6	8.5
	Microwave Baked Pork Roast	3.1	0.0	0.0	17.9	25.0	42.9	14.3
	None of the Above	2.5						
Ham		77.0						
	Grilled/BBQ Ham Slices	18.9	0.0	0.0	27.2	31.6	38.6	2.5
	Oven Broiled Ham Slices	18.7	0.0	0.0	24.4	37.8	34.6	3.2
	Fried Ham Slices	38.9	0.0	0.0	21.2	31.4	43.7	3.7
	Oven Baked Ham Slices	54.2	0.0	0.0	23.8	36.4	36.9	2.9

Meat type	Meat, Cut and Method of Cooking	Percent consumers	Preferred Degree of Doneness (%)					
			Rare	Medium Rare	Just done (Medium)	Medium Well	Well Done	Very Well Done
	Microwave Baked Ham Slices	8.7	0.0	0.0	23.3	27.4	42.5	6.8
	None of the Above	15.4						
Bacon		84.1						
	Grilled/BBQ Bacon Slices	12.2	0.0	0.0	10.8	20.7	49.5	18.9
	Oven Broiled Bacon Slices	11.7	0.0	0.0	13.1	27.1	45.8	14.0
	Fried Bacon Slices	83.4	0.0	0.0	7.9	18.9	52.7	20.5
	Oven Baked Bacon Slices	14.2	0.0	0.0	13.1	21.5	50.0	15.4
	Microwave Baked Bacon Slices	35.2	0.0	0.0	7.2	18.4	52.3	22.1
	None of the Above	1.8						
Hotdog		76.2						
	Grilled/BBQ Hotdog	64.0	0.0	0.0	7.5	37.5	46.8	8.1
	Oven Broiled Hotdog	17.0	0.0	0.0	11.3	44.7	37.6	6.4
	Fried Hotdog	25.2	0.0	0.0	7.7	32.1	50.7	9.6
	Oven Baked Hotdog	11.8	0.0	0.0	14.3	36.7	35.7	13.3
	Microwave Baked Hotdog	39.5	0.0	0.0	25.1	50.2	22.3	2.4
	None of the Above	10.6						
Chicken		97.6						
	Rotisserie Chicken	49.3	0.0	0.0	9.0	24.9	55.1	11.1
	Grilled/BBQ Chicken Breasts, Skinless	50.9	0.0	0.0	8.0	25.7	55.6	10.7
	Oven Broiled Chicken Breasts, Skinless	27.1	0.0	0.0	10.5	23.0	59.9	6.6
	Fried Chicken Breasts, Skinless	39.3	0.0	0.0	8.2	23.0	56.1	12.7
	Oven Baked Chicken Breasts, Skinless	50.3	0.0	0.0	9.8	22.9	57.4	9.9
	Microwave Baked Chicken Breasts, Skinless	8.4	0.0	0.0	9.0	21.3	58.4	11.2
	Grilled/BBQ Chicken Breasts, With Skin	26.2	0.0	0.0	8.3	23.4	56.5	11.9
	Oven Broiled Chicken Breasts, With Skin	14.2	0.0	0.0	7.9	20.5	63.6	7.9
	Fried Chicken Breasts, With Skin	33.6	0.0	0.0	6.2	21.1	59.8	12.9
	Oven Baked Chicken Breasts, With Skin	25.8	0.0	0.0	10.3	23.1	54.6	12.1
	Microwave Baked Chicken Breasts, With	3.9	0.0	0.0	12.2	24.4	53.7	9.8

Meat type	Meat, Cut and Method of Cooking	Percent consumers	Preferred Degree of Doneness (%)					
			Rare	Medium Rare	Just done (Medium)	Medium Well	Well Done	Very Well Done
	Skin							
	Grilled/BBQ Other Chicken Pieces, Skinless	19.2	0.0	0.0	8.8	27.0	53.4	10.8
	Oven Broiled Other Chicken Pieces, Skinless	11.5	0.0	0.0	9.8	25.4	51.6	13.1
	Fried Other Chicken Pieces, Skinless	20.4	0.0	0.0	8.8	22.7	57.9	10.6
	Oven Baked Other Chicken Pieces, Skinless	19.4	0.0	0.0	8.7	26.7	53.4	11.2
	Microwave Baked Other Chicken Pieces, Skinless	5.0	0.0	0.0	11.3	22.6	54.7	11.3
	Grilled/BBQ Other Chicken Pieces, With Skin	21.1	0.0	0.0	8.5	27.7	53.1	10.7
	Oven Broiled Other Chicken Pieces, With Skin	11.3	0.0	0.0	7.5	25.8	54.2	12.5
	Fried Other Chicken Pieces, With Skin	31.5	0.0	0.0	7.5	20.1	59.3	13.2
	Oven Baked Other Chicken Pieces, With Skin	22.5	0.0	0.0	9.6	20.9	56.5	13.0
	Microwave Baked Other Chicken Pieces, With Skin	4.4	0.0	0.0	12.8	19.1	57.4	10.6
	None of the Above	1.4						
Fish		79.1						
	Grilled/BBQ Fish	33.6	1.0	4.5	23.2	31.5	36.0	3.8
	Oven Broiled Fish	39.5	0.6	2.1	25.4	30.4	37.2	4.4
	Fried Fish	60.3	0.2	0.4	16.6	24.1	51.5	7.1
	Oven Baked Fish	54.7	0.2	1.5	22.8	30.6	39.4	5.5
	Microwave Baked Fish	8.7	1.3	4.0	24.0	24.0	41.3	5.3
	None of the Above	4.5						

Table A-2. Summary Results From the IPSOS Observer Survey: Percent Consuming Marinated Meat Products

Product	When you ate the following meat products, were they marinated?			
	Yes	No	Don't know	Did Not Eat Product
Beef Steak	45.0	41.3	5.4	8.3
Beef Ribs	39.7	29.2	7.0	24.1
Beef Brisket	26.5	29.2	8.2	36.1
Pork Chop	28.0	52.9	6.1	13.0
Pork Rib	30.9	35.7	8.1	25.2
Pork Tenderloins	30.6	38.4	7.0	24.0
Chicken	45.9	46.9	5.6	1.7
Fish	20.6	58.0	6.5	14.8

APPENDIX B. RESIDUE DATA AVAILABILITY

The following tables (B-1 to B-7) summarize the concentration data that are publicly available for the specific meat types, by cut, cooking method and degree of doneness or that were imputed from the available data. The sources for the data presented in this appendix are listed below. The {meat cut X cooking method X degree of doneness} combinations are the same as those included in the consumer internet-based survey and that were mapped to intakes (g/day) from the NHANES 2003-2006 and presented in this report. When the data for for PHiP, MeIQx, DiMeIQx and B[a]P for a given {meat cut X cooking method X degree of doneness} combination were not available in the published literature, the approach used to extrapolate the missing data is also noted in the comment field. This extrapolation represents a major source of uncertainty associated with the HCA intake estimates presented in this report.

Sources:

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National Cancer Institute (NCI). 2006. Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) version 1.7. Available via http://charred.cancer.gov. Accessed 17 October 2007.

Sinha, R, Rothman N, Salmon CP, Knize MG et al, 1998. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness in gravy made from meat drippings. Food Chemical Toxicology, 36: 279-287.

Table B-1. HCA Data Availability – Beef

Cut	Cooking Method	Doneness	PhIP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Steak	Grilled/ BBQ	Rare	2.53		0.21		0.00		0.00	
		Medium rare	7.27	Calculated - from doneness value above and below	0.66	Calculated - from doneness value above and below	0.03	Calculated - from doneness value above and below	2.08	Calculated - from doneness value above and below
		Just done	12.00		1.10		0.05		4.15	
		Medium well	13.50	Calculated - from doneness value above and below	1.64	Calculated - from doneness value above and below	0.05	Calculated - from doneness value above and below	4.45	Calculated - from doneness value above and below
		Well done	15.00		2.17		0.05		4.75	
		Very well done	33.30		5.78		1.90		4.86	
Steak	Oven Broiled	Rare	6.14		0.00		0.00		0.00	
		Medium rare	4.11	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
		Just done	2.08		0.00		0.00		0.01	
		Medium well	3.33	Calculated - from doneness value above and below	0.84	Calculated - from doneness value above and below	0.06	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
		Well done	4.57		1.67		0.11		0.01	
		Very well done	7.08		1.51		0.19		0.01	

Steak	Fried	Rare	1.89		1.25		0.00		0.00	
		Medium rare	2.21	Calculated - from doneness value above and below	1.60	Calculated - from doneness value above and below	0.11	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below
		Just done	2.53		1.94		0.21		0.00	
		Medium well	4.53	Calculated - from doneness value above and below	3.01	Calculated - from doneness value above and below	0.33	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
		Well done	6.53		4.07		0.45		0.01	
		Very well done	23.22		8.19		1.30		0.01	
Steak	Oven Baked	Rare	6.14	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak
		Medium rare	4.11		0.00		0.00		0.01	
		Just done	2.08		0.00		0.00		0.01	
		Medium well	3.33		0.84		0.06		0.01	
		Well done	4.57		1.67		0.11		0.01	
		Very well done	7.08		1.51		0.19		0.01	
Steak	Microwaved	Rare	0.00		0.00		0.00		0.00	
		Medium rare	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.00		0.00		0.00	

Beef Ribs	Grilled/ BBQ	Rare	2.53	Surrogate - from grilled/bbq steak	0.21	Surrogate - from grilled/bbq steak	0.00	Surrogate - from grilled/bbq steak	0.00	Surrogate - from grilled/bbq steak
		Medium rare	7.27		0.66		0.03		2.08	
		Just done	12.00		1.10		0.05		4.15	
		Medium well	13.50		1.64		0.05		4.45	
		Well done	15.00		2.17		0.05		4.75	
		Very well done	33.30		5.78		1.90		4.86	
Beef Ribs	Oven Broiled	Rare	6.14	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak	0.00	Surrogate - value taken from oven broiled steak	0.00	Surrogate - from oven broiled steak
		Medium rare	4.11		0.00		0.00		0.01	
		Just done	2.08		0.00		0.00		0.01	
		Medium well	3.33		0.84		0.06		0.01	
		Well done	4.57		1.67		0.11		0.01	
		Very well done	7.08		1.51		0.19		0.01	
Beef Ribs	Fried	Rare	1.89	Surrogate - from fried steak	1.25	Surrogate - from fried steak	0.00	Surrogate - value taken from fried steak	0.00	Surrogate - from fried steak
		Medium rare	2.21		1.60		0.11		0.00	
		Just done	2.53		1.94		0.21		0.00	
		Medium well	4.53		3.01		0.33		0.01	
		Well done	6.53		4.07		0.45		0.01	
		Very well done	23.22		8.19		1.30		0.01	
Beef Ribs	Oven Baked	Rare	6.14	Surrogate - from oven baked steak	0.00	Surrogate - from oven baked steak	0.00	Surrogate - from oven baked steak	0.00	Surrogate - from oven baked steak
		Medium rare	4.11		0.00		0.00		0.01	
		Just done	2.08		0.00		0.00		0.01	
		Medium well	3.33		0.84		0.06		0.01	
		Well done	4.57		1.67		0.11		0.01	
		Very well done	7.08		1.51		0.19		0.01	
Beef Ribs	Microw aved	Rare	0.00	Surrogate - from microwaved steak	0.00	Surrogate - from microwaved steak	0.00	Surrogate - from microwaved steak	0.00	Surrogate - from microwaved steak
		Medium rare	0.00		0.00		0.00		0.00	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00		0.00		0.00		0.00	
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.00		0.00		0.00	

Beef Brisket	Grilled/ BBQ	Rare	2.53	Surrogate - from grilled/bbq steak	0.21	Surrogate - from grilled/bbq steak	0.00	Surrogate - from grilled/bbq steak	0.00	Surrogate - from grilled/bbq steak
		Medium rare	7.27		0.66		0.03		2.08	
		Just done	12.00		1.10		0.05		4.15	
		Medium well	13.50		1.64		0.05		4.45	
		Well done	15.00		2.17		0.05		4.75	
		Very well done	33.30		5.78		1.90		4.86	
Beef Brisket	Oven Broiled	Rare	6.14	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak	0.00	Surrogate - from oven broiled steak
		Medium rare	4.11		0.00		0.00		0.01	
		Just done	2.08		0.00		0.00		0.01	
		Medium well	3.33		0.84		0.06		0.01	
		Well done	4.57		1.67		0.11		0.01	
		Very well done	7.08		1.51		0.19		0.01	
Beef Brisket	Fried	Rare	1.89	Surrogate - from fried steak	1.25	Surrogate - from fried steak	0.00	Surrogate - from fried steak	0.00	Surrogate - from fried steak
		Medium rare	2.21		1.60		0.11		0.00	
		Just done	2.53		1.94		0.21		0.00	
		Medium well	4.53		3.01		0.33		0.01	
		Well done	6.53		4.07		0.45		0.01	
		Very well done	23.22		8.19		1.30		0.01	
Beef Brisket	Oven Baked	Rare	6.14	Surrogate - from oven baked steak	0.00	Surrogate - from oven baked steak	0.00	Surrogate - from oven baked steak	0.00	Surrogate - from oven baked steak
		Medium rare	4.11		0.00		0.00		0.01	
		Just done	2.08		0.00		0.00		0.01	
		Medium well	3.33		0.84		0.06		0.01	
		Well done	4.57		1.67		0.11		0.01	
		Very well done	7.08		1.51		0.19		0.01	
Beef Brisket	Microw aved	Rare	0.00	Surrogate from microwaved steak	0.00	Surrogate from microwaved steak	0.00	Surrogate from microwaved steak	0.00	Surrogate from microwaved steak
		Medium rare	0.00		0.00		0.00		0.00	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00		0.00		0.00		0.00	
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.00		0.00		0.00	

Beef Roast	Oven Baked	Rare	0.10		0.10		0.10		0.00	Surrogate from oven baked steak
		Medium rare	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.01	
		Just done	0.10		0.10		0.10		0.01	
		Medium well	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.01	
		Well done	0.10		0.10		0.10		0.01	
		Very well done	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.01	
Beef Roast	Microw aved	Rare	0.00	Surrogate from microwaved steak	0.00	Surrogate from microwaved steak	0.00	Surrogate from microwaved steak	0.00	Surrogate from microwaved steak
		Medium rare	0.00		0.00		0.00		0.00	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00		0.00		0.00		0.00	
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.00		0.00		0.00	

Table B-2. HCA Data Availability – Hamburger

Cooking Method	Doneness	PhIP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Grilled/ BBQ	Rare	0.00		0.00		0.00		0.00	
	Medium rare	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.05	Calculated - from doneness value above and below
	Just done	0.00		0.00		0.00		0.09	
	Medium well	0.00	Assumed to be same as levels above and below	0.66	Calculated - from doneness value above and below	0.07	Calculated - from doneness value above and below	0.33	Calculated - from doneness value above and below
	Well done	0.00		1.31		0.13		0.56	
	Very well done	16.79		4.55		0.31		1.52	
Oven Broiled	Rare	0.00		0.00		0.00		0.00	
	Medium rare	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.01	Calculated - from doneness value above and below
	Just done	0.00		0.00		0.00		0.01	
	Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.01	Calculated - from doneness value above and below
	Well done	0.00		0.00		0.00		0.01	
	Very well done	0.00		1.61		0.00		0.01	
Fried	Rare	0.00		0.34		0.00		0.00	
	Medium rare	0.00	Assumed to be same as levels above and below	0.67	Calculated - from doneness value above and below	0.07	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
	Just done	0.00		1.00		0.14		0.01	
	Medium well	0.97	Calculated - from doneness value above and below	1.68	Calculated - from doneness value above and below	0.08	Calculated - from doneness value above and below	0.02	Calculated - from doneness value above and below
	Well done	1.94		2.35		0.01		0.02	
	Very well done	5.18		4.25		0.03		0.01	

Cooking Method	Doneness	PhIP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Oven Baked	Rare	0.00	Surrogate - value taken from oven broiled hamburger	0.00	Surrogate - value taken from oven broiled hamburger	0.00	Surrogate - value taken from oven broiled hamburger	0.00	Surrogate - value taken from oven broiled hamburger
	Medium rare	0.00		0.00		0.00		0.01	
	Just done	0.00		0.00		0.00		0.01	
	Medium well	0.00		0.00		0.00		0.01	
	Well done	0.00		0.00		0.00		0.01	
	Very well done	0.00		1.61		0.00		0.01	
Microwaved	Rare	0.00		0.00		0.00		0.00	
	Medium rare	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below
	Just done	0.00		0.00		0.00		0.00	
	Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below
	Well done	0.00		0.00		0.00		0.00	
	Very well done	0.00		0.00		0.00		0.00	

Table B-3. HCA Data Availability – Chicken

Cut	Cooking Method	Doneness	PhIP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Chicken, with skin	Rotisserie	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	2.61	Surrogate - from oven broiled breasts with skin	0.04	Surrogate - from oven broiled breasts with skin	0.00	Surrogate - from oven broiled breasts with skin	0.04	Surrogate - from oven broiled breasts with skin
		Medium well	5.34 66.8		0.03		0.00		0.06	
		Well done	8.06 131		0.02		0.00		0.08	
		Very well done	22.87		0.32		0.09		0.16	
Breasts, skinless	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	27.00		0.00		0.00		0.06	
		Medium well	83.50	Calculated - from doneness value above and below	1.00	Calculated - from doneness value above and below	0.50	Calculated - from doneness value above and below	0.23	Calculated - from doneness value above and below
		Well done	140.0		2.00		1.00		0.39	
		Very well done	480.0		9.00		1.00		0.40	

Breasts, skinless	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.24		0.00		0.00		0.06	
		Medium well	32.12	Calculated - from doneness value above and below	0.02	Calculated - from doneness value above and below	0.00	Assumed to be same as levels above and below	0.09	Calculated - from doneness value above and below
		Well done	64.00		0.04		0.00		0.12	
		Very well done	150.00		3.00		0.09		0.48	
Breasts, skinless	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.96		0.11		0.00		0.00	
		Medium well	18.98	Calculated - from doneness value above and below	1.06	Calculated - from doneness value above and below	1.00	Calculated - from doneness value above and below	0.05	Calculated - from doneness value above and below
		Well done	37.00		2.00		2.00		0.10	
		Very well done	70.00		3.00		4.00		0.10	
Breasts, skinless	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	

Breasts, skinless	Microwaved	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	
Breasts, with skin	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	5.12		0.07		0.00		1.00	
		Medium well	7.68 20.56	Calculated - from doneness value above and below	0.09	Calculated - from doneness value above and below	0.00	Assume to contain no levels	2.79	Calculated - from doneness value above and below
		Well done	10.24 36.0		0.11		0.00		4.57	
		Very well done	10.24		0.55		0.00		4.57	
Breasts, with skin	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	2.61		0.04		0.00		0.04	
		Medium well	5.335 66.805	Calculated - from doneness value above and below	0.03	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.06	Calculated - from doneness value above and below
		Well done	8.06 131		0.02		0.00		0.08	
		Very well done	22.87		0.32		0.09		0.16	

Breasts, with skin	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	2.43		0.25		0.06		0.06	
		Medium well	13.72	Calculated - from doneness value above and below	0.45	Calculated - from doneness value above and below	0.06	Calculated - from doneness value above and below	0.09	Calculated - from doneness value above and below
		Well done	25.00		0.65		0.06		0.12	
		Very well done	30.90		0.72		0.13		0.24	
Breasts, with skin	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	
Breasts, with skin	Microwaved	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	

Other Pieces, skinless	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	1.52		0.00		0.00		0.06	
		Medium well	5.03	Calculated - from doneness value above and below	0.12	Calculated - from doneness value above and below	0.09	Calculated - from doneness value above and below	0.23	Calculated - from doneness value above and below
		Well done	8.54		0.24		0.18		0.39	
		Very well done	8.54		0.76		0.22		0.40	
Other Pieces, skinless	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.24		0.00		0.00		0.06	
		Medium well	1.10	Calculated - from doneness value above and below	0.02	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below	0.09	Calculated - from doneness value above and below
		Well done	1.96		0.04		0.00		0.12	
		Very well done	14.78		0.34		0.09		0.48	
Other Pieces, skinless	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.96		0.11		0.00		0.00	
		Medium well	4.90	Calculated - from doneness value above and below	0.27	Calculated - from doneness value above and below	0.02	Calculated - from doneness value above and below	0.05	Calculated - from doneness value above and below
		Well done	8.84		0.42		0.04		0.10	
		Very well done	30.90		0.64		0.25		0.10	

Other Pieces, skinless	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	
Other Pieces, skinless	Microwaved Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	
Other Pieces, with skin	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	5.12		0.07		0.00		1.00	
		Medium well	7.68	Calculated - from doneness value above and below	0.09	Calculated - from doneness value above and below	0.00	Assume to contain no levels	2.79	Calculated - from doneness value above and below
		Well done	10.24		0.11		0.00		4.57	
		Very well done	10.24		0.55		0.00		4.57	

Other Pieces, with skin	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	2.61		0.04		0.00		0.04	
		Medium well	5.34	Calculated - from doneness value above and below	0.03	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.06	Calculated - from doneness value above and below
		Well done	8.06		0.02		0.00		0.08	
		Very well done	22.87		0.32		0.09		0.16	
Other Pieces, with skin	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	2.43		0.25		0.06		0.06	
		Medium well	5.64	Calculated - from doneness value above and below	0.45	Calculated - from doneness value above and below	0.06	Calculated - from doneness value above and below	0.09	Calculated - from doneness value above and below
		Well done	8.84		0.65		0.06		0.12	
		Very well done	30.90		0.72		0.13		0.24	
Other Pieces, with skin	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	

Other Pieces, with skin	Microwaved Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assume to contain no levels	0.04	Calculated - from doneness value above and below	0.00	Assume to contain no levels	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.08		0.00		0.01	
		Very well done	0.00		0.08		0.00		0.01	

Table B-4. HCA Data Availability – Pork

Cut	Cooking Method	Doneness	PhIP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Pork Chop	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.32		0.23		0.00		0.00	
		Medium well	0.62	Calculated - from doneness value above and below	0.79	Calculated - from doneness value above and below	0.10	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
		Well done	0.91		1.34		0.20		0.01	
		Very well done	1.50		3.83		0.28		0.01	
Pork Chop	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Chop	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.10		0.10		0.00		0.00	
		Medium well	0.10	Calculated - from doneness value above and below	0.72	Calculated - from doneness value above and below	0.10	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
		Well done	0.10		1.34		0.20		0.01	
		Very well done	0.10		3.83		0.28		0.01	

Pork Chop	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Chop	Microwaved	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.01	Calculated - from doneness value above and below
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Ribs	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.32		0.23		0.00		0.00	Surrogate - value taken from grilled/bbq pork chop
		Medium well	0.62	Calculated - from doneness value above and below	0.44	Calculated - from doneness value above and below	0.00	Assumed to be same as levels above and below	0.01	
		Well done	0.91		0.65		0.00		0.01	
		Very well done	1.50		1.07		0.00		0.01	

Pork Ribs	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.50	Surrogate - from oven baked ribs	0.05	Surrogate - from oven baked ribs	0.05	Surrogate - from oven baked ribs	0.00	Surrogate - from oven broiled pork chop
		Medium well	1.40		0.05		0.05		0.01	
		Well done	2.30		0.05		0.05		0.01	
		Very well done	3.79		0.05		0.05		0.01	
Pork Ribs	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.32	Surrogate - value taken from grilled/bbq ribs	0.23	Surrogate - value taken from grilled/bbq ribs	0.00	Surrogate - value taken from grilled/bbq ribs	0.00	Surrogate - value taken from fried prok chop
		Medium well	0.62		0.44		0.00		0.01	
		Well done	0.91		0.65		0.00		0.01	
		Very well done	1.50		1.07		0.00		0.01	
Pork Ribs	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.50		0.05		0.05		0.00	Surrogate - value taken from oven baked pork chop
		Medium well	1.40	Calculated - from doneness value above and below	0.05	Assumed to be same as levels above and below	0.05	Assumed to be same as levels above and below	0.01	
		Well done	2.30		0.05		0.05		0.01	
		Very well done	3.79	Calculated - from grilled/bbq tenderloins ratio of well done to very well done	0.05	Assumed to be same as level below	0.05	Assumed to be same as level below	0.01	
Pork Ribs	Microwaved	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from microwaved steak	0.00	Surrogate - value taken from microwaved baked pork chop	0.00	Surrogate - value taken from microwaved steak	0.00	Surrogate - value taken from microwaved baked pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	

Pork Tenderloins	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.32		0.23		0.00		0.00	Surrogate - from grilled/bbq pork chop
		Medium well	0.62	Calculated - from doneness value above and below	0.44	Calculated - from doneness value above and below	0.00	Assumed to be same as level below	0.01	
		Well done	0.91		0.65		0.00		0.01	
		Very well done	1.50		1.07		0.00		0.01	
Pork Tenderloins	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from oven broiled pork chop	0.00	Surrogate - value taken from oven broiled pork chop	0.00	Surrogate - value taken from oven broiled pork chop	0.00	Surrogate - value taken from oven broiled pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Tenderloins	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.10	Surrogate - value taken from fried pork chop	0.10	Surrogate - value taken from fried pork chop	0.00	Surrogate - value taken from fried pork chop	0.00	Surrogate - value taken from fried pork chop
		Medium well	0.10		0.72		0.10		0.01	
		Well done	0.10		1.34		0.20		0.01	
		Very well done	0.10		3.83		0.28		0.01	
Pork Tenderloins	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from oven baked pork chop	0.00	Surrogate - value taken from oven baked pork chop	0.00	Surrogate - value taken from oven baked pork chop	0.00	Surrogate - value taken from oven baked pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	

Pork Tenderloins	Microwaved	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from microwaved pork chop	0.00	Surrogate - value taken from microwaved pork chop	0.00	Surrogate - value taken from microwaved pork chop	0.00	Surrogate - value taken from microwaved pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Roast	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.32		0.23		0.00		0.00	Surrogate - value taken from grilled/bbq pork chop
		Medium well	0.62	Calculated - from doneness value above and below	0.44	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below	0.01	
		Well done	0.91		0.65		0.00		0.01	
		Very well done	1.50		1.07		0.00		0.01	
Pork Roast	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - from oven broiled pork chop	0.00	Surrogate - from oven broiled pork chop	0.00	Surrogate - from oven broiled pork chop	0.00	Surrogate - from oven broiled pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Roast	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.10	Surrogate - value taken from fried pork chop	0.10	Surrogate - value taken from fried pork chop	0.00	Surrogate - value taken from fried pork chop	0.00	Surrogate - value taken from fried pork chop
		Medium well	0.10		0.72		0.10		0.01	
		Well done	0.10		1.34		0.20		0.01	
		Very well done	0.10		3.83		0.28		0.01	

Pork Roast	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from oven baked pork chop	0.00	Surrogate - value taken from oven baked pork chop	0.00	Surrogate - value taken from oven baked pork chop	0.00	Surrogate - value taken from oven baked pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Pork Roast	Microw aved	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from microwaved pork chop	0.00	Surrogate - value taken from microwaved pork chop	0.00	Surrogate - value taken from microwaved pork chop	0.00	Surrogate - value taken from microwaved baked pork chop
		Medium well	0.00		0.00		0.00		0.01	
		Well done	0.00		0.00		0.00		0.01	
		Very well done	0.00		0.52		0.00		0.01	
Ham Slices	Grilled/ BBQ	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.32		0.23		0.00		0.00	Surrogate - value taken from fried ham slices
		Medium well	0.62	Calculated - from doneness value above and below	0.44	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below	0.01	
		Well done	0.91		0.65		0.00		0.01	
		Very well done	1.50		1.07		0.00		0.01	

Ham Slices	Oven Broiled	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	0.00	Calculated - from doneness value above and below	0.00	Assumed to be same as level below
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.35		0.00		0.01	
Ham Slices	Fried	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00		0.00		0.00		0.00	
		Medium well	0.15	Calculated - from doneness value above and below	0.30	Calculated - from doneness value above and below	0.00	Calculated - from doneness value above and below	0.01	Calculated - from doneness value above and below
		Well done	0.30		0.60		0.00		0.01	
		Very well done	0.00		1.80		0.20		0.01	
Ham Slices	Oven Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from oven broiled ham	0.00	Surrogate - value taken from oven broiled ham	0.00	Surrogate - value taken from oven broiled ham	0.00	Surrogate - value taken from oven broiled ham
		Medium well	0.00		0.00		0.00		0.00	
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.35		0.00		0.01	

Ham Slices	Microwaved Baked	Rare	-		-		-		-	
		Medium rare	-		-		-		-	
		Just done	0.00	Surrogate - value taken from oven broiled ham	0.00	Surrogate - value taken from oven broiled ham	0.00	Surrogate - value taken from oven broiled ham	0.00	Surrogate - value taken from oven broiled ham
		Medium well	0.00		0.00		0.00		0.00	
		Well done	0.00		0.00		0.00		0.00	
		Very well done	0.00		0.35		0.00		0.01	

Table B-5. HCA Data Availability – Bacon Slices

Cooking Method	Doneness	PhIP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Grilled/BBQ	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	1.39	Surrogate - value taken from oven broiled values	0.10	Surrogate - value taken from oven broiled values	0.00	Surrogate - value taken from oven broiled values	0.00	Surrogate - value taken from oven broiled values
	Medium well	10.01		0.85		0.13		0.00	
	Well done	18.62		1.60		0.26		0.00	
	Very well done	46.20		4.84		0.57		0.00	
Oven Broiled	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	1.39		0.10		0.00		0.00	
	Medium well	10.01	Calculated - from doneness value above and below	0.85	Calculated - from doneness value above and below	0.13	Calculated - from doneness value above and below	0.00	Assumed to be same as levels above and below
	Well done	18.62		1.60		0.26		0.00	
	Very well done	46.20		4.84		0.57		0.00	
Fried	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.10		0.35		0.00		0.01	
	Medium well	0.41	Calculated - from doneness value above and below	1.03	Calculated - from doneness value above and below	0.00	Assumed to be same as levels above and below	0.02	Calculated - from doneness value above and below
	Well done	0.71		1.71		0.00		0.02	
	Very well done	4.80		4.30		0.50		0.01	

Oven Baked	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	1.39	Surrogate - value taken from oven broiled values	0.10	Surrogate - value taken from oven broiled values	0.00	Surrogate - value taken from oven broiled values	0.00	Surrogate - value taken from oven broiled values
	Medium well	10.01		0.85		0.13		0.00	
	Well done	18.62		1.60		0.26		0.00	
	Very well done	46.20		4.84		0.57		0.00	
Micro waved	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.00		0.00		0.00		0.00	
	Medium well	0.00		0.20	Calculated - from doneness value above and below	0.00	Assumed to be same as levels above and below	0.01	Calculated - from doneness value above and below
	Well done	0.00		0.40		0.00		0.01	
	Very well done	3.10		1.50		0.20		0.02	

Table B-6. HCA Data Availability – Hot Dogs

Cooking Method	Doneness	PhiP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Grilled/BBQ	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.10		0.10		0.00	Assume to contain no levels	0.00	Surrogate - value taken from ham slice values
	Medium well	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.00	Assume to contain no levels	0.01	
	Well done	0.10		0.10		0.00		0.01	
	Very well done	0.10		0.10		0.00		0.01	
Oven Broiled	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.00	Assumed to be same as level below	0.10	Surrogate - value taken from oven baked values	0.00	Assume to contain no levels	0.00	Surrogate - value taken from ham slice values
	Medium well	0.00	Assumed to be same as levels above and below	0.10		0.00	Assume to contain no levels	0.00	
	Well done	0.00		0.19		0.00		0.00	
	Very well done	0.00		0.10	Surrogate - value taken from oven baked values	0.00		0.01	

Fried	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.10		0.10		0.00	Assume to contain no levels	0.00	Surrogate - value taken from ham slice values
	Medium well	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.00	Assume to contain no levels	0.01	
	Well done	0.10		0.10		0.00		0.01	
	Very well done	0.10		0.10		0.00		0.01	
Oven Baked	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.10		0.10		0.00	Surrogate - value taken from oven broiled hot dog values	0.00	Surrogate - value taken from ham slice values
	Medium well	0.10	Assumed to be same as levels above and below	0.10	Assumed to be same as levels above and below	0.00		0.00	
	Well done	0.10		0.10		0.00		0.00	
	Very well done	0.10		0.10		0.00		0.01	
Micro waved	Rare	-		-		-		-	
	Medium rare	-		-		-		-	
	Just done	0.10	Surrogate - value taken from oven baked values	0.10	Surrogate - value taken from oven baked values	0.00	Surrogate - value taken from oven baked hot dog values	0.00	Surrogate - value taken from ham slice values
	Medium well	0.10		0.10		0.00		0.00	
	Well done	0.10		0.10		0.00		0.00	
	Very well done	0.10		0.10		0.00		0.01	

Table B-7. HCA Data Availability – Fish

Cooking Method	Doneness	PhiP (ng/g)	Comment	MeIQx (ng/g)	Comment	DiMeIQx (ng/g)	Comment	B[a]P (ng/g)	Comment
Grilled/BBQ	Rare	0.00		0.00	Assume to contain no levels	0.00	Assume to contain no levels	NA	No data available
	Medium rare	2.00		0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	NA	No data available
	Just done	0.066 6.2		0.00		0.00		NA	No data available
	Medium well	2.688 37.6	Calculated - from doneness value above and below	0.00	Assumed to be same as levels above and below	0.00	Assumed to be same as levels above and below	NA	No data available
	Well done	5.31 69		0.00		0.00		NA	No data available
	Very well done	28.55 73		0.00		0.00		NA	No data available
Oven Broiled	Rare	0.00	Assume to contain no levels	0.00	Assume to contain no levels	0.00	Surrogate - value taken from grilled/bbq fish	NA	No data available
	Medium rare	1.70	Calculated - from doneness value above and below	1.40		0.00		NA	No data available
	Just done	23.00		5.00		0.00		NA	No data available
	Medium well	18.50	Calculated - from doneness value above and below	4.85	Calculated - from doneness value above and below	0.00		NA	No data available
	Well done	14.00		4.70		0.00		NA	No data available
	Very well done	17.00		3.70		0.00		NA	No data available

Fried	Rare	0.00	Assume to contain no levels	0.00	Assume to contain no levels	0.00	Assume to contain no levels	NA	No data available
	Medium rare	0.80	Calculated - from doneness value above and below	0.10	Calculated - from doneness value above and below	0.03	Calculated - from doneness value above and below	NA	No data available
	Just done	1.60		0.20		0.05		NA	No data available
	Medium well	3.80	Calculated - from doneness value above and below	1.85	Calculated - from doneness value above and below	0.18	Calculated - from doneness value above and below	NA	No data available
	Well done	6.00		3.50		0.30		NA	No data available
	Very well done	12.80		5.80		0.40		NA	No data available
Oven Baked	Rare	0.00	Assume to contain no levels	0.00	Assume to contain no levels	0.00	Surrogate - value taken from grilled/bbq fish	NA	No data available
	Medium rare	0.25	Calculated - from doneness value above and below	0.25	Calculated - from doneness value above and below	0.00		NA	No data available
	Just done	0.50		0.50		0.00		NA	No data available
	Medium well	9.25	Calculated - from doneness value above and below	2.55	Calculated - from doneness value above and below	0.00		NA	No data available
	Well done	18.00		4.60		0.00		NA	No data available
	Very well done	5.90		3.40		0.00		NA	No data available

Microwaved	Rare	0.00	Surrogate - value taken from oven baked values	0.00	Surrogate - value taken from oven baked values	0.00	Surrogate - value taken from grilled/bbq fish	NA	No data available
	Medium rare	0.25		0.25		0.00		NA	No data available
	Just done	0.50		0.50		0.00		NA	No data available
	Medium well	9.25		2.55		0.00		NA	No data available
	Well done	18.00		4.60		0.00		NA	No data available
	Very well done	5.90		3.40		0.00		NA	No data available

APPENDIX C. FOOD INTAKE BY SPECIFIC MEAT TYPE

Intakes based on cooking method and degree of doneness for the 7 meat types (beef cuts, hamburgers, chicken, pork, bacon, hotdogs, and fish) included in this study are summarized in this section, see Tables C-1 to C-7

Table C-1. Beef Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

Beef Cuts	Cooking Method	Doneness Degree	g/day	g/kg/day
Steak	Grilled/BBQ	Rare	0.343	0.0050
		Medium rare	1.305	0.0189
		Just done	0.978	0.0142
		Medium well	0.664	0.0096
		Well done	0.439	0.0063
		Very well done	0.118	0.0017
Steak	Oven Broiled	Rare	0.152	0.0022
		Medium rare	0.529	0.0077
		Just done	0.473	0.0068
		Medium well	0.326	0.0047
		Well done	0.191	0.0028
		Very well done	0.068	0.0010
Steak	Fried	Rare	0.095	0.0014
		Medium rare	0.455	0.0066
		Just done	0.455	0.0066
		Medium well	0.359	0.0052
		Well done	0.370	0.0054
		Very well done	0.062	0.0009
Steak	Oven Baked	Rare	0.050	0.0007
		Medium rare	0.230	0.0033
		Just done	0.236	0.0034
		Medium well	0.241	0.0035
		Well done	0.308	0.0045
		Very well done	0.056	0.0008
Steak	Microwaved Baked	Rare	0.006	0.0001
		Medium rare	0.040	0.0006
		Just done	0.068	0.0010
		Medium well	0.040	0.0006
		Well done	0.028	0.0004
		Very well done	0.023	0.0003

Beef Cuts	Cooking Method	Doneness Degree	g/day	g/kg/day
Beef Ribs	Grilled/BBQ	Rare	0.004	0.0001
		Medium rare	0.025	0.0004
		Just done	0.066	0.0011
		Medium well	0.069	0.0011
		Well done	0.089	0.0015
		Very well done	0.012	0.0002
Beef Ribs	Oven Broiled	Rare	0.001	0.0000
		Medium rare	0.006	0.0001
		Just done	0.025	0.0004
		Medium well	0.020	0.0003
		Well done	0.027	0.0004
		Very well done	0.004	0.0001
Beef Ribs	Fried	Rare	0.001	0.0000
		Medium rare	0.003	0.0001
		Just done	0.012	0.0002
		Medium well	0.003	0.0001
		Well done	0.010	0.0002
		Very well done	0.002	0.0000
Beef Ribs	Oven Baked	Rare	0.002	0.0000
		Medium rare	0.007	0.0001
		Just done	0.034	0.0006
		Medium well	0.040	0.0007
		Well done	0.054	0.0009
		Very well done	0.007	0.0001
Beef Ribs	Microwaved Baked	Rare	0.001	0.0000
		Medium rare	0.002	0.0000
		Just done	0.004	0.0001
		Medium well	0.006	0.0001
		Well done	0.006	0.0001
		Very well done	0.002	0.0000
Beef Brisket	Grilled/BBQ	Rare	0.001	0.0000
		Medium rare	0.015	0.0002
		Just done	0.021	0.0003
		Medium well	0.020	0.0003
		Well done	0.016	0.0002
		Very well done	0.003	0.0000
Beef Brisket	Oven Broiled	Rare	0.002	0.0000
		Medium rare	0.008	0.0001
		Just done	0.016	0.0002
		Medium well	0.006	0.0001
		Well done	0.007	0.0001
		Very well done	0.003	0.0000

Beef Cuts	Cooking Method	Doneness Degree	g/day	g/kg/day
Beef Brisket	Fried	Rare	0.001	0.0000
		Medium rare	0.003	0.0000
		Just done	0.005	0.0001
		Medium well	0.004	0.0001
		Well done	0.005	0.0001
		Very well done	0.001	0.0000
Beef Brisket	Oven Baked	Rare	0.001	0.0000
		Medium rare	0.007	0.0001
		Just done	0.017	0.0002
		Medium well	0.007	0.0001
		Well done	0.013	0.0002
		Very well done	0.003	0.0000
Beef Brisket	Microwaved Baked	Rare	0.001	0.0000
		Medium rare	0.002	0.0000
		Just done	0.005	0.0001
		Medium well	0.002	0.0000
		Well done	0.004	0.0001
		Very well done	0.001	0.0000
Beef Roast	Oven Baked	Rare	0.063	0.0009
		Medium rare	0.274	0.0040
		Just done	0.453	0.0066
		Medium well	0.434	0.0063
		Well done	0.447	0.0065
		Very well done	0.088	0.0013
Beef Roast	Microwaved Baked	Rare	0.013	0.0002
		Medium rare	0.029	0.0004
		Just done	0.044	0.0006
		Medium well	0.022	0.0003
		Well done	0.051	0.0007
		Very well done	0.016	0.0002
Total Beef intake			**11.384**	**0.166**

Table C-2. Hamburgers Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

Cooking Method	Doneness	g/day	g/kg-bw/day
Grilled/BBQ	Rare	0.103	0.0016
	Medium rare	0.484	0.0076
	Just done	0.974	0.0153
	Medium well	0.922	0.0145
	Well done	1.016	0.0159
	Very well done	0.179	0.0028
Oven Broiled	Rare	0.019	0.0003
	Medium rare	0.132	0.0021
	Just done	0.221	0.0035
	Medium well	0.221	0.0035
	Well done	0.259	0.0041
	Very well done	0.042	0.0007
Fried	Rare	0.071	0.0011
	Medium rare	0.207	0.0032
	Just done	0.701	0.0110
	Medium well	0.691	0.0108
	Well done	1.007	0.0158
	Very well done	0.160	0.0025
Oven Baked	Rare	0.023	0.0004
	Medium rare	0.038	0.0006
	Just done	0.136	0.0021
	Medium well	0.150	0.0024
	Well done	0.239	0.0038
	Very well done	0.047	0.0007
Microwaved	Rare	0.009	0.0001
	Medium rare	0.033	0.0005
	Just done	0.080	0.0013
	Medium well	0.076	0.0012
	Well done	0.113	0.0018
	Very well done	0.024	0.0004
Total Hamburger Intake		**8.377**	**0.131**

Table C-3. Chicken Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

CHICKEN CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
Rotisserie Chicken, with skin	Rotisserie Chicken	Rare	-	-
		Medium rare	-	-
		Just done	0.025	0.0004
		Medium well	0.070	0.0011
		Well done	0.155	0.0024
		Very well done	0.031	0.0005
Breasts, skinless	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.260	0.0043
		Medium well	0.839	0.0139
		Well done	1.812	0.0300
		Very well done	0.350	0.0058
Breasts, skinless	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.181	0.0030
		Medium well	0.399	0.0066
		Well done	1.041	0.0172
		Very well done	0.115	0.0019
Breasts, skinless	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.205	0.0034
		Medium well	0.580	0.0096
		Well done	1.413	0.0234
		Very well done	0.320	0.0053
Breasts, skinless	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.314	0.0052
		Medium well	0.738	0.0122
		Well done	1.850	0.0307
		Very well done	0.320	0.0053
Breasts, skinless	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.048	0.0008
		Medium well	0.115	0.0019
		Well done	0.314	0.0052
		Very well done	0.060	0.0010
Breasts, with skin	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.071	0.0010

CHICKEN CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
		Medium well	0.200	0.0028
		Well done	0.484	0.0068
		Very well done	0.102	0.0014
Breasts, with skin	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.037	0.0005
		Medium well	0.095	0.0013
		Well done	0.295	0.0042
		Very well done	0.037	0.0005
Breasts, with skin	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.068	0.0010
		Medium well	0.231	0.0033
		Well done	0.657	0.0093
		Very well done	0.142	0.0020
Breasts, with skin	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.086	0.0012
		Medium well	0.195	0.0027
		Well done	0.460	0.0065
		Very well done	0.102	0.0014
Breasts, with skin	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.016	0.0002
		Medium well	0.031	0.0004
		Well done	0.068	0.0010
		Very well done	0.012	0.0002
Other Pieces, skinless	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.109	0.0026
		Medium well	0.334	0.0079
		Well done	0.663	0.0157
		Very well done	0.134	0.0032
Other Pieces, skinless	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.073	0.0017
		Medium well	0.189	0.0045
		Well done	0.384	0.0091
		Very well done	0.097	0.0023
Other Pieces,	Fried	Rare	-	-

CHICKEN CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
skinless				
		Medium rare	-	-
		Just done	0.116	0.0028
		Medium well	0.299	0.0071
		Well done	0.762	0.0181
		Very well done	0.140	0.0033
Other Pieces, skinless	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.109	0.0026
		Medium well	0.335	0.0079
		Well done	0.669	0.0159
		Very well done	0.140	0.0033
Other Pieces, skinless	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.037	0.0009
		Medium well	0.073	0.0017
		Well done	0.177	0.0042
		Very well done	0.037	0.0009
Other Pieces, with skin	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.126	0.0020
		Medium well	0.412	0.0066
		Well done	0.791	0.0127
		Very well done	0.159	0.0026
Other Pieces, with skin	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.060	0.0010
		Medium well	0.206	0.0033
		Well done	0.432	0.0070
		Very well done	0.100	0.0016
Other Pieces, with skin	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.166	0.0027
		Medium well	0.446	0.0072
		Well done	1.317	0.0212
		Very well done	0.293	0.0047
Other Pieces, with skin	Oven Baked	Rare	-	-

CHICKEN CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
		Medium rare	-	-
		Just done	0.153	0.0025
		Medium well	0.332	0.0053
		Well done	0.896	0.0144
		Very well done	0.206	0.0033
Other Pieces, with skin	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.040	0.0006
		Medium well	0.059	0.0010
		Well done	0.178	0.0029
		Very well done	0.033	0.0005
Total Chicken Intake			**26.228**	**0.458**

Table C-4. Pork Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

PORK CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
Pork Chop	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.149	0.0023
		Medium well	0.233	0.0037
		Well done	0.386	0.0060
		Very well done	0.048	0.0008
Pork Chop	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.064	0.0010
		Medium well	0.149	0.0023
		Well done	0.231	0.0036
		Very well done	0.024	0.0004
Pork Chop	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.094	0.0015
		Medium well	0.231	0.0036
		Well done	0.456	0.0071
		Very well done	0.078	0.0012
Pork Chop	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.113	0.0018
		Medium well	0.219	0.0034
		Well done	0.406	0.0064
		Very well done	0.056	0.0009
Pork Chop	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.012	0.0002
		Medium well	0.020	0.0003
		Well done	0.030	0.0005
		Very well done	0.008	0.0001
Pork Ribs	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.088	0.0012
		Medium well	0.196	0.0026
		Well done	0.351	0.0047
		Very well done	0.047	0.0006
Pork Ribs	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.025	0.0003
		Medium well	0.070	0.0009

PORK CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
		Well done	0.106	0.0014
		Very well done	0.018	0.0002
Pork Ribs	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.009	0.0001
		Medium well	0.021	0.0003
		Well done	0.047	0.0006
		Very well done	0.007	0.0001
Pork Ribs	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.031	0.0004
		Medium well	0.108	0.0015
		Well done	0.196	0.0026
		Very well done	0.040	0.0005
Pork Ribs	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.009	0.0001
		Medium well	0.011	0.0001
		Well done	0.031	0.0004
		Very well done	0.007	0.0001
Pork Tenderloins	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.023	0.0004
		Medium well	0.032	0.0005
		Well done	0.034	0.0006
		Very well done	0.004	0.0001
Pork Tenderloins	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.009	0.0001
		Medium well	0.019	0.0003
		Well done	0.024	0.0004
		Very well done	0.002	0.0000
Pork Tenderloins	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.006	0.0001
		Medium well	0.015	0.0002
		Well done	0.031	0.0005
		Very well done	0.004	0.0001
Pork Tenderloins	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.012	0.0002
		Medium well	0.027	0.0005

PORK CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
		Well done	0.040	0.0007
		Very well done	0.007	0.0001
Pork Tenderloins	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.001	0.0000
		Medium well	0.003	0.0000
		Well done	0.006	0.0001
		Very well done	0.002	0.0000
Pork Roast	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.030	0.0004
		Medium well	0.047	0.0006
		Well done	0.061	0.0008
		Very well done	0.013	0.0002
Pork Roast	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.027	0.0004
		Medium well	0.052	0.0007
		Well done	0.079	0.0011
		Very well done	0.010	0.0001
Pork Roast	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.007	0.0001
		Medium well	0.015	0.0002
		Well done	0.024	0.0003
		Very well done	0.008	0.0001
Pork Roast	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.067	0.0009
		Medium well	0.110	0.0015
		Well done	0.250	0.0035
		Very well done	0.040	0.0005
Pork Roast	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.006	0.0001
		Medium well	0.008	0.0001
		Well done	0.014	0.0002
		Very well done	0.005	0.0001
Ham Slices	Grilled/BBQ	Rare	-	-
		Medium rare	-	-
		Just done	0.077	0.0012
		Medium well	0.090	0.0014

PORK CUT	Cooking Method	Doneness	g/day	g/kg-bw/day
		Well done	0.110	0.0017
		Very well done	0.007	0.0001
Ham Slices	Oven Broiled	Rare	-	-
		Medium rare	-	-
		Just done	0.069	0.0010
		Medium well	0.106	0.0016
		Well done	0.097	0.0015
		Very well done	0.009	0.0001
Ham Slices	Fried	Rare	-	-
		Medium rare	-	-
		Just done	0.124	0.0019
		Medium well	0.184	0.0028
		Well done	0.256	0.0039
		Very well done	0.022	0.0003
Ham Slices	Oven Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.164	0.0025
		Medium well	0.251	0.0038
		Well done	0.254	0.0038
		Very well done	0.020	0.0003
Ham Slices	Microwaved Baked	Rare	-	-
		Medium rare	-	-
		Just done	0.030	0.0005
		Medium well	0.036	0.0005
		Well done	0.056	0.0008
		Very well done	0.009	0.0001
Total Pork Intake			**7.572**	**0.113**

Table C-5. Bacon Slice Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

Cooking Method	Doneness	g/day	g/kg-bw/day
Grilled/BBQ	Rare	-	-
	Medium rare	-	-
	Just done	0.012	0.0002
	Medium well	0.022	0.0004
	Well done	0.054	0.0008
	Very well done	0.021	0.0003
Oven Broiled	Rare	-	-
	Medium rare	-	-
	Just done	0.014	0.0002
	Medium well	0.028	0.0004
	Well done	0.048	0.0008
	Very well done	0.015	0.0002
Fried	Rare	-	-
	Medium rare	-	-
	Just done	0.058	0.0009
	Medium well	0.140	0.0022
	Well done	0.390	0.0062
	Very well done	0.152	0.0024
Oven Baked	Rare	-	-
	Medium rare	-	-
	Just done	0.017	0.0003
	Medium well	0.027	0.0004
	Well done	0.063	0.0010
	Very well done	0.019	0.0003
Micro waved	Rare	-	-
	Medium rare	-	-
	Just done	0.022	0.0004
	Medium well	0.057	0.0009
	Well done	0.164	0.0026
	Very well done	0.069	0.0011
Total Bacon Slices Intake		**1.392**	**0.022**

Table C-6. Hot Dog Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

Cooking Method	Doneness	g/day	g/kg-bw/day
Grilled/BBQ	Rare	-	-
	Medium rare	-	-
	Just done	0.299	0.0054
	Medium well	1.488	0.0269
	Well done	1.854	0.0335
	Very well done	0.321	0.0058
Oven Broiled	Rare	-	-
	Medium rare	-	-
	Just done	0.119	0.0022
	Medium well	0.470	0.0085
	Well done	0.396	0.0071
	Very well done	0.067	0.0012
Fried	Rare	-	-
	Medium rare	-	-
	Just done	0.119	0.0022
	Medium well	0.500	0.0090
	Well done	0.791	0.0143
	Very well done	0.149	0.0027
Oven Baked	Rare	-	-
	Medium rare	-	-
	Just done	0.104	0.0019
	Medium well	0.268	0.0048
	Well done	0.261	0.0047
	Very well done	0.097	0.0017
Micro waved Baked	Rare	-	-
	Medium rare	-	-
	Just done	0.613	0.0111
	Medium well	1.226	0.0221
	Well done	0.546	0.0099
	Very well done	0.060	0.0011
Total Hog Dog Intake		**9.751**	**0.176**

Table C-7. Fish Consumption by Method of Cooking and Degree of Doneness (Mean *per Capita*, US Population, NHANES 03-06)

Cooking Method	Doneness	g/day	g/kg-bw/day
Grilled/BBQ	Rare	0.015	0.0002
	Medium rare	0.066	0.0010
	Just done	0.338	0.0051
	Medium well	0.459	0.0069
	Well done	0.525	0.0079
	Very well done	0.055	0.0008
Oven Broiled	Rare	0.010	0.0002
	Medium rare	0.035	0.0005
	Just done	0.435	0.0066
	Medium well	0.521	0.0079
	Well done	0.637	0.0096
	Very well done	0.076	0.0011
Fried	Rare	0.005	0.0001
	Medium rare	0.010	0.0002
	Just done	0.434	0.0066
	Medium well	0.631	0.0095
	Well done	1.349	0.0204
	Very well done	0.187	0.0028
Oven Baked	Rare	0.005	0.0001
	Medium rare	0.035	0.0005
	Just done	0.540	0.0082
	Medium well	0.727	0.0110
	Well done	0.934	0.0141
	Very well done	0.131	0.0020
Microwaved Baked	Rare	0.005	0.0001
	Medium rare	0.015	0.0002
	Just done	0.091	0.0014
	Medium well	0.091	0.0014
	Well done	0.156	0.0024
	Very well done	0.020	0.0003
Total Fish Intake		**8.539**	**0.129**

NAMI Foundation
NORTH AMERICAN MEAT INSTITUTE

September 9, 2015

Dr. Veronique Bouvard, Responsible Officer
Dr. Kurt Straif, Head of the IARC Monographs Programme
IARC
Lyon, France

Re: Volume 114: Red Meat And Processed Meat – Call For Data – Nitrite/Nitrate Concentrations In Cured Meat Products And Non-Meat Foods Available In U.S. Retail Stores

Dear Drs. Bouvard and Straif:

The North American Meat Institute Foundation (NAMIF or Foundation) is a non-profit research, education and information foundation. NAMIF seeks to identify technologies and practices that enable meat and poultry companies to produce safer and more nutritious meat and poultry products. The Foundation publicly disseminates research findings, best practices and other educational materials about a broad range of food safety, worker safety, animal welfare, nutrition, and consumer information projects. The Foundation appreciates the opportunity to provide scientific evidence for consideration by the International Agency for Research on Cancer (IARC) Expert Panel during its upcoming review of red and processed meats.

In 2007, the Foundation co-funded a national survey of nitrite/nitrate concentrations in cured meat products and non-meat foods available in U.S. retail stores.[1,2] This survey was a follow-up to one completed in 1997. Specifically, this study surveyed the most significant categories of cured meats and highly consumed, raw, nitrate rich vegetables available at retail in five geographic regions of the U.S. and analyzed each category for nitrite/nitrate content. Comparisons were made with historic databases to determine if changes had occurred in nitrite/nitrate levels since the 1997 survey.

[1] Nunez De Gonzalez, M. T., *et al*. (2012). "Survey of residual nitrite and nitrate in conventional and organic/natural/uncured/indirectly cured meats available at retail in the United States." J Agric Food Chem 60(15): 3981-3990.
[2] Nunez de Gonzalez, M. T., *et al*. (2015). "A Survey of Nitrate and Nitrite Concentrations in Conventional and Organic-Labeled Raw Vegetables at Retail." J Food Sci.

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Overall, the findings show that nitrite/nitrate contents of U.S. cured meat products have remained low since the last national survey in 1997. The U.S. Department of Agriculture's Food Safety and Inspection Service's regulations and manufacturer's processing procedures are consistently controlling the levels of nitrite/nitrate in cured meat products and continue to be effective for minimizing their contribution to the dietary nitrite/nitrate load.

The survey also found variations in the compositional content, specifically nitrate concentration, of organically and conventionally produced raw vegetables may need to be considered when compiling nutrient composition databases. If compositional differences are of sufficient magnitude, this might warrant an "organic" category in databases to be considered when modeling nutrient intake.

Nitrate/nitrite concentrations of drinking water in 25 U.S. cities were compiled to evaluate their potential contribution to nitrite/nitrate load. All reported drinking water sources were within the U.S. Environmental Protection Agency's allowable limits for nitrate and nitrite.

* * * * *

Thank you for your consideration of these points and for your convenience, I have also included the referenced scientific evidence. Should you have questions, please contact me at bbooren@meatinstitute.org.

Respectfully submitted,

[redacted]

Betsy Booren, Ph.D.
President

Additional References

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