Received by email on October 10, 2016

From: Claire Kruger, Spherix Consulting

NTP is requesting information on four substances that have been nominated for possible review for future editions of the RoC; three of these substances are also under consideration for evaluation of non-cancer health outcomes (Federal Register Vol 81; no. 175. Friday September 9, 2016). One of the nominations is consumption of red meat: cancer and non-cancer health hazard evaluations. NTP has requested information on scientific issues important for prioritizing and assessing adverse health outcomes. A critical scientific issue to be considered in the prioritization of a nomination for the RoC is the weight of the available evidence that can be used to provide a robust scientific summary key to reaching a recommendation. The evidence base for red meat is too limited and premature to provide the quantity or rigor of scientific data required to warrant an evaluation at this time.

On October 26, 2015, IARC published a summary of their findings regarding the assessment of the association of cancer with consumption red meat or processed meat (The Lancet Oncology 2015). IARC classified the consumption of red meat as probably carcinogenic to humans (Group 2A). The largest body of epidemiological data concerned colorectal cancer. Data on the association of red meat consumption with colorectal cancer were available from 14 cohort studies. They concluded that chance, bias, and confounding could not be ruled out for the data on red meat consumption, since no clear association was seen in several of the high quality studies and residual confounding from other diet and lifestyle risk is difficult to exclude. The Working Group concluded that there is limited evidence in human beings for the carcinogenicity of the consumption of red meat. Limited evidence means that a positive association has been observed between exposure to the agent and cancer but that other explanations for the observations (technically termed chance, bias, or confounding) could not be ruled out. There is inadequate evidence in experimental animals for the carcinogenicity of consumption of red meat. Nevertheless, the working group opined that there is strong mechanistic evidence for the role of factors such as heme iron from red meat and nitrosamine formation, genotoxicity and oxidative stress as hazards in the production of colorectal cancer. On the basis of what they cited as strong mechanistic evidence, the Working Group classified consumption of red meat as “probably carcinogenic to humans (Group 2A)”.

An examination of publicly available data that investigates the mechanistic evidence cited by IARC as a justification for the assignment to Group 2A does not support the interpretation of the Working Group. The current evidence that links a mechanism of action for heme in red meat to the production of colorectal cancer is weak. A review of publicly available

in vitro assays, animal models and clinical trials (reference list attached) shows that many of the studies contain methodologic flaws as well as over-reaching conclusions based on limited evidence in models that are not appropriate to utilize in risk assessment. A lack of appropriate studies documenting dose-response and thresholds for the mechanisms investigated precludes assignment of these mechanisms as relevant under conditions of more modest exposures from red meat eaten as part of a usual diet. Studies that are currently available for review cannot be used to extrapolate to a mechanism or a health effect that would be manifested at usual red meat intakes. In conclusion, the data base is not sufficient to support nomination of red meat to the RoC.

**References**

Angeli JP, Garcia CC, Sena F, Freitas FP, Miyamoto S, Medeiros MH, Di Mascio P. Lipid hydroperoxide-induced and hemoglobin-enhanced oxidative damage to colon cancer cells. Free Radic Biol Med. 2011 Jul 15;51(2):503-15. doi: 10.1016/j.freeradbiomed.2011.04.015. Epub 2011 Apr 14.

Baradat M, Jouanin I, Dalleau S, Taché S, Gieules M, Debrauwer L, Canlet C, Huc L, Dupuy J, Pierre FH, Guéraud F. 4-Hydroxy-2(E)-nonenal metabolism differs in Apc(+/+) cells and in Apc(Min/+) cells: it may explain colon cancer promotion by heme iron. Chem Res Toxicol. 2011 Nov 21;24(11):1984-93. doi: 10.1021/tx2003036. Epub 2011 Oct 19.

Bastide NM, Chenni F, Audebert M, Santarelli RL, Taché S, Naud N, Baradat M, Jouanin I, Surya R, Hobbs DA, Kuhnle GG, Raymond-Letron I, Gueraud F, Corpet DE, Pierre FH. A central role for heme iron in colon carcinogenesis associated with red meat intake. Cancer Res. 2015 Mar 1;75(5):870-9. doi: 10.1158/0008-5472.CAN-14-2554. Epub 2015 Jan 15.

Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. J Nutr. 2002 Nov;132(11 Suppl):3522S-3525S.

Butler AR, Rhodes P. Chemistry, analysis, and biological roles of S-nitrosothiols. Anal Biochem. 1997 Jun 15;249(1):1-9.

Chenni FZ, Taché S, Naud N, Guéraud F, Hobbs DA, Kunhle GG, Pierre FH, Corpet DE. Heme-induced biomarkers associated with red meat promotion of colon cancer are not modulated by the intake of nitrite. Nutr Cancer. 2013;65(2):227-33. doi: 10.1080/01635581.2013.749291.

Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res. 2003 May 15;63(10):2358-60.

Cross AJ, Greetham HL, Pollock JR, Rowland IR, Bingham SA. Variability in fecal water genotoxicity, determined using the Comet assay, is independent of endogenous N-nitroso compound formation attributed to red meat consumption. Environ Mol Mutagen. 2006 Apr;47(3):179-84.

de Vogel J, van-Eck WB, Sesink AL, Jonker-Termont DS, Kleibeuker J, van der Meer R. Dietary heme injures surface epithelium resulting in hyperproliferation, inhibition of apoptosis and crypt hyperplasia in rat colon. Carcinogenesis. 2008 Feb;29(2):398-403. doi: 10.1093/carcin/bgm278. Epub 2008 Jan 3.

Gad SE 2008. Animal Models in Toxicology, Second Edition, edited by Shayne C. Gad. October 30, 2006 by CRC Press, ISBN 9781420014204 - CAT# DKE2079.

Gilsing AM, Fransen F, de Kok TM, Goldbohm AR, Schouten LJ, de Bruïne AP, van Engeland M, van den Brandt PA, de Goeij AF, Weijenberg MP. Dietary heme iron and the risk of colorectal cancer with specific mutations in KRAS and APC. Carcinogenesis. 2013 Dec;34(12):2757-66. doi: 10.1093/carcin/bgt290. Epub 2013 Aug 27.

Glei M, Klenow S, Sauer J, Wegewitz U, Richter K, Pool-Zobel BL. Hemoglobin and hemin induce DNA damage in human colon tumor cells HT29 clone 19A and in primary human colonocytes. Mutat Res. 2006 Feb 22;594(1-2):162-71. Epub 2005 Oct 13.

Guéraud F, Taché S, Steghens JP, Milkovic L, Borovic-Sunjic S, Zarkovic N, Gaultier E, Naud N, Héliès-Toussaint C, Pierre F, Priymenko N. Dietary polyunsaturated fatty acids and heme iron induce oxidative stress biomarkers and a cancer promoting environment in the colon of rats. Free Radic Biol Med. 2015 Jun;83:192-200. doi: 10.1016/j.freeradbiomed.2015.02.023. Epub 2015 Mar 3.

Hogg N. Red meat and colon cancer: heme proteins and nitrite in the gut. A commentary on "diet-induced endogenous formation of nitroso compounds in the GI tract". Free Radic Biol Med. 2007 Oct 1;43(7):1037-9. Epub 2007 Jul 14.

Hughes R, Cross AJ, Pollock JR, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis. 2001 Jan;22(1):199-202.

Ijssennagger N, de Wit N, Müller M, van der Meer R. Dietary heme-mediated PPARα activation does not affect the heme-induced epithelial hyperproliferation and hyperplasia in mouse colon. PLoS One. 2012;7(8):e43260. doi: 10.1371/journal.pone.0043260. Epub 2012 Aug 14.

Ijssennagger N, Rijnierse A, de Wit NJ, Boekschoten MV, Dekker J, Schonewille A, Müller M, van der Meer R. Dietary heme induces acute oxidative stress, but delayed cytotoxicity and compensatory hyperproliferation in mouse colon. Carcinogenesis. 2013 Jul;34(7):1628-35. doi: 10.1093/carcin/bgt084. Epub 2013 Mar 1.

Ishikawa S, Tamaki S, Ohata M, Arihara K, Itoh M. Heme induces DNA damage and hyperproliferation of colonic epithelial cells via hydrogen peroxide produced by heme oxygenase: a possible mechanism of heme-induced colon cancer. Mol Nutr Food Res. 2010 Aug;54(8):1182-91. doi: 10.1002/mnfr.200900348.

Joosen AM, Kuhnle GG, Aspinall SM, Barrow TM, Lecommandeur E, Azqueta A, Collins AR, Bingham SA. Effect of processed and red meat on endogenous nitrosation and DNA damage. Carcinogenesis. 2009 Aug;30(8):1402-7. doi: 10.1093/carcin/bgp130. Epub 2009 Jun 4.

Joosen AM, Lecommandeur E, Kuhnle GG, Aspinall SM, Kap L, Rodwell SA. Effect of dietary meat and fish on endogenous nitrosation, inflammation and genotoxicity of faecal water. Mutagenesis. 2010 May;25(3):243-7. doi: 10.1093/mutage/gep070. Epub 2010 Jan 27.

Kuhnle GG, Story GW, Reda T, Mani AR, Moore KP, Lunn JC, Bingham SA. Diet-induced endogenous formation of nitroso compounds in the GI tract. Free Radic Biol Med. 2007 Oct 1;43(7):1040-7. Epub 2007 Mar 13.

Lakshmi VM, Clapper ML, Chang WC, Zenser TV. Hemin potentiates nitric oxide-mediated nitrosation of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) to 2-nitrosoamino-3-methylimidazo[4,5-f]quinoline. Chem Res Toxicol. 2005 Mar;18(3):528-35.

Lancet Oncology 2015. Carcinogenicity of consumption of red and processed meat. The Lancet Oncology, Volume 16, No. 16, p1599–1600, December 2015.

Lewin MH, Bailey N, Bandaletova T, Bowman R, Cross AJ, Pollock J, Shuker DE, Bingham SA. Red meat enhances the colonic formation of the DNA adduct O6-carboxymethyl guanine: implications for colorectal cancer risk. Cancer Res. 2006 Feb 1;66(3):1859-65.

Lunn JC, Kuhnle G, Mai V, Frankenfeld C, Shuker DE, Glen RC, Goodman JM, Pollock JR, Bingham SA. The effect of haem in red and processed meat on the endogenous formation of N-

nitroso compounds in the upper gastrointestinal tract. Carcinogenesis. 2007 Mar;28(3):685-90. Epub 2006 Oct 19.

Martin OC, Lin C, Naud N, Tache S, Raymond-Letron I, Corpet DE, Pierre FH. Antibiotic suppression of intestinal microbiota reduces heme-induced lipoperoxidation associated with colon carcinogenesis in rats. Nutr Cancer. 2015;67(1):119-25. doi: 10.1080/01635581.2015.976317. Epub 2014 Dec 16.

Pierre F, Freeman A, Taché S, Van der Meer R, Corpet DE. Beef meat and blood sausage promote the formation of azoxymethane-induced mucin-depleted foci and aberrant crypt foci in rat colons. J Nutr. 2004 Oct;134(10):2711-6.

Pierre F, Peiro G, Taché S, Cross AJ, Bingham SA, Gasc N, Gottardi G, Corpet DE, Guéraud F. New marker of colon cancer risk associated with heme intake: 1,4-dihydroxynonane mercapturic acid. Cancer Epidemiol Biomarkers Prev. 2006 Nov;15(11):2274-9.

Pierre F, Tache S, Guéraud F, Rerole AL, Jourdan ML, Petit C. Apc mutation induces resistance of colonic cells to lipoperoxide-triggered apoptosis induced by faecal water from haem-fed rats. Carcinogenesis. 2007 Feb;28(2):321-7. Epub 2006 Aug 2.

Sawa T, Akaike T, Kida K, Fukushima Y, Takagi K, Maeda H. Lipid peroxyl radicals from oxidized oils and heme-iron: implication of a high-fat diet in colon carcinogenesis. Cancer Epidemiol Biomarkers Prev. 1998 Nov;7(11):1007-12.

Schwartz S, and Ellefson M. Quantitative Fecal Recovery of Ingested Hemoglobin-Heme in Blood: Comparisons by HemoQuant Assay With Ingested Meat and Fish. 1985; 89:19-26.

Sesink AL, Termont DS, Kleibeuker JH, Van der Meer R. Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. Cancer Res. 1999 Nov 15;59(22):5704-9.

Surya R, Héliès-Toussaint C, Martin OC, Gauthier T, Guéraud F, Taché S, Naud N, Jouanin I, Chantelauze C, Durand D, Joly C, Pujos-Guillot E, Pierre FH, Huc L. Red meat and colorectal cancer: Nrf2-dependent antioxidant response contributes to the resistance of preneoplastic colon cells to fecal water of hemoglobin- and beef-fed rats. Carcinogenesis. 2016 Jun;37(6):635-45. doi: 10.1093/carcin/bgw035. Epub 2016 Mar 18.

Van Hecke T, Vanden Bussche J, Vanhaecke L, Vossen E, Van Camp J, De Smet S. Nitrite curing of chicken, pork, and beef inhibits oxidation but does not affect N-nitroso compound

(NOC)-specific DNA adduct formation during in vitro digestion. J Agric Food Chem. 2014 Feb 26;62(8):1980-8. doi: 10.1021/jf4057583. Epub 2014 Feb 11.

Winter J, Young GP, Hu Y, Gratz SW, Conlon MA, Le Leu RK. Accumulation of promutagenic DNA adducts in the mouse distal colon after consumption of heme does not induce colonic neoplasms in the western diet model of spontaneous colorectal cancer. Mol Nutr Food Res. 2014 Mar;58(3):550-8. doi: 10.1002/mnfr.201300430. Epub 2013 Oct 1.