Mechanistic Development of Cancers Associated with Processed Meat Products: A Review

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Abstract: Epidemiological data link processed meat products to various cancers, especially colorectal cancer; however, such evidence cannot prove causation. Clear mechanistic evidence of how these foods promote carcinogenesis strengthens the case for causation. Because the complexity and heterogeneity of processed meats as a food category complicate both epidemiological and mechanistic assessments, the study of carcinogenic mechanisms associated with specific components of such foods is often undertaken. These include components that are intrinsic to meats, those that contaminate meat, and those ingredients that are added to or form in meats during processing. Consumption of processed meats also leads to endogenous production of agents, epigenetic changes, and alterations in the microbiota of the digestive tract; therefore, the potential contributions of these endogenous responses to carcinogenesis are also discussed. This review highlights data that illuminate potential mechanisms by which agents associated with processed meats (including processed poultry) could contribute to carcinogenesis. The potential for personal factors such as overall diet, cooking methods, genetic variation, and inflammation and infection status to influence these carcinogenic mechanisms is also summarized. Because the intended audience of this review includes those who may be less familiar with current general mechanisms of mutagenesis and carcinogenesis, detailed background on these topics is provided.

Key words: mechanisms of carcinogenesis, mutagenesis, cancer, processed meat

Introduction

Dietary factors have been epidemiologically linked to ~35% of human cancers (Doll and Peto, 1981; Kasai, 2016). In the last 2 decades, numerous international reviews, epidemiological studies, and health organization publications and guidelines have suggested an association between the consumption of processed meats (excluding those from fish) and cancers (Demeyer et al., 2008; International Agency for Research on Cancer, 2018; World Cancer Research Fund/American Institute for Cancer Research, 2018; Clinton et al., 2020). Cancers of the colorectum, stomach, pancreas, prostate, and breast were noted to be associated with processed meats (excluding those from fish) in the International Agency for Research on Cancer (IARC) Monograph on the Evaluation of Carcinogenic Risks to Humans in Red Meat and Processed Meat (International Agency for Research on Cancer, 2018). Although epidemiological factors such as dietary components may correlate with disease, they do not by themselves establish causation. Understanding the mechanisms by which an epidemiological factor might result in an effect supports plausibility and coherence, which are considered essential for establishing causality (Hill, 1965; Fedak et al., 2015).
Cancer is not a monolithic disease; rather, it is a large family of complex diseases in which the proliferation of cells is no longer controlled. Cancer is also not a disease that arises suddenly in response to a single action; rather, it results from a series of genetic changes that act together to allow the inappropriate expansion of a population of cells (Vogelstein et al., 2013). This expansion of cells may progress to dysplasia, carcinoma in situ, cancer, and finally metastatic disease. Many types of cells from various organs and tissues can become malignant (i.e., grow uncontrollably, invade nearby tissues, and metastasize to other parts of the body), and a wide variety of chemical, physical, and biological agents have been associated with cancers. Our understanding of cancer is further complicated by each person having their own unique environment, diet, heredity factors, and microbiome, all of which can influence the initiation and progression of cancer. As a result, many different mechanistic pathways have been proposed to explain how putative carcinogenic agents initiate or promote cancers.

The phrase “processed meats” has been defined differently by different organizations and researchers. This document will use the definitions in the American Meat Science Association (AMSA) Meat Science Lexicon as much as possible (Seman et al., 2018). The term “meat” in that document includes skeletal muscle, associated tissues, and edible offal from mammalian, avian, reptilian, amphibian, and aquatic species. “Processed meats” will include products covered under AMSA’s definition of further processed meat products and thus will include meats products (including poultry and fish) that have undergone “a transformation, beyond minimal processing, containing approved ingredients, and may be subjected to a preservation or processing step(s) through the application of salting, curing, fermentation, thermal processing (smoking and/or cooking), batter/breading, or other processes to enhance sensory, quality, and safety attributes. These products may include ready-to-cook and ready-to-eat products” (Seman et al., 2018). When certain types of meat (beef, pork, poultry, or fish) are known to be excluded in a study or analysis of meats or processed meats, the exclusions will be cited; however, because few studies specifically included meat from reptilian or amphibian species, these exclusions will not be specifically listed. We will use “minimally processed meats” to mean fresh meats (intact or nonin tact) with no added ingredients.

This review summarizes our current understanding of how processed meat consumption might contribute to carcinogenesis. Mechanisms discussed will include molecular, cellular, and physiological pathways by which components of processed meats could promote cancer development. We will also discuss how additional factors related to the consumer, such as the gut microbiome, immune status, genetic and metabolic variations, and additional dietary and environmental factors, may alter carcinogenic potential. Because epidemiologic data suggesting links between processed meats and cancer have been reviewed in detail in recent reports (Mejborn et al., 2016; International Agency for Research on Cancer, 2018), these will not be a focus of this review, nor will attempts be made to quantitatively evaluate risks. Instead, we focus on summarizing and discussing the strengths, weaknesses, and gaps in current knowledge of potential mechanisms of carcinogenesis related to processed meats.

Background

A basic understanding of genotoxicity, mutagenicity, and carcinogenesis is needed to understand the proposed mechanisms by which components of processed meats might contribute to cancer. The following sections contain information for readers who may be less familiar with the general mechanisms by which carcinogenesis is believed to occur.

Agents and mechanisms of genotoxicity, mutagenicity, and carcinogenesis

Chemical, biological, and physical agents can be carcinogens. Most carcinogenic agents that have been associated with processed meats are chemicals (International Agency for Research on Cancer, 2018), although biological or physical agents may also play a role (International Agency for Research on Cancer, 2018). A tremendous variety of carcinogenic mechanisms have been proposed for known human carcinogens (Birkett et al., 2019). Carcinogens can be genotoxic (DNA damaging) or nongenotoxic (working via hormonal effects, epigenetic changes, etc.) (Nohmi, 2018; Hartwig et al., 2020).

Genotoxic and Mutagenic Agents. Genotoxic agents damage DNA through oxidative activities or by covalent attachment to the bases, sugars, or even phosphate groups to form DNA adducts (Liu and Wang, 2015; Hwa Yun et al., 2020). DNA adduct formation can then lead to the removal of bases (abasic sites), crosslinking of adjacent nucleotides or strands, breakage of DNA strands, and other types of DNA damage. Some DNA-damaging agents are intrinsically genotoxic as they enter the body, whereas others must first be
metabolized to an activated form that can react with DNA (Goldman and Shields, 2003). Metabolic activation will be discussed later.

The types and positions of DNA adducts that a genotoxic agent produces (the “adductome”) can be characteristic of an agent (Steinberg, 2019). For example, reactive oxygen species (ROS) formed during oxidative stress can modify guanine bases in DNA, leading to 8-hydroxy-2’-deoxyguanine adducts, and the presence of such adducts in DNA is considered as evidence (and can be used as a biomarker) for oxidative damage (Valavanidis et al., 2009). Similarly, the pattern of mutations that arise from DNA damage by a given agent can result in mutational “signatures” that can also be used to categorize cancers and help identify potential mechanisms of carcinogenesis (Koh et al., 2021). For example, certain agents oxidize guanine, converting it to 8-oxoguanine. The 8-oxoguanine can now pair (or “miss-pair”) with an adenine base, leading the original guanine-cytosine base pair to be converted (mutated) following DNA replication to a thymine: adenine base pair following DNA replication (Figure 1). The presence of this mutation within certain genes in a cancerous tissue suggests that an alkylating agent, perhaps benzo[a]pyrene (BaP), was involved (Kucab et al., 2019).

DNA damage can result in loss of function or regulation of a gene. Any event that alters the DNA structure or sequence inherently poses a threat to a cell’s ability to faithfully interpret and propagate the genomic sequence. Because maintaining DNA integrity is critical to survival and reproduction, organisms from bacteria to humans have evolved highly efficient enzymatic DNA repair systems to detect, remove, or otherwise counteract such lesions (Chatterjee and Walker, 2017). In higher organisms, DNA damage can trigger cell cycle checkpoints, preventing replication of damaged DNA and prolonging the opportunity for repair. Cells with irreparably damaged DNA can also be eliminated by a variety of mechanisms, including apoptosis (Surova and Zhivotovsky, 2013).

Rarely, however, DNA lesions are not repaired, or the repair or attempts at DNA replication of a distorted DNA alkylation may lead to replication mutation

**Figure 1.** Example of how alkylation of a DNA base can lead to mutation.
template create an error in the DNA. This can lead to alterations in the DNA sequence (single-nucleotide changes, insertions, deletions) or chromosomal translocations (Cohen and Arnold, 2008; Kasai, 2016). When such an event leads to a change in DNA sequence that daughter cells will inherit, it is considered a mutation.

Many DNA mutations do not affect the fitness of a cell or organism or lead to cancer. For example, a base pair change may lead to a silent mutation that does not change the amino acid sequence of the encoded protein. Cells in healthy human tissues contain mutations that increase in number with age (Balmain, 2020; Lopez-Bigas and Gonzalez-Perez, 2020). Replication of DNA also can create random mistakes, albeit at exceedingly low rates. However, the more times DNA replication occurs, the greater likelihood that a mutagenic error can be generated (Cohen and Arnold, 2008).

In summary, not all genotoxic agents are mutagens, nor are all mutagenic agents carcinogenic. For a mutation to lead to a cancer, it needs to cause specific effects that result in a growth advantage for the mutant cell that eventually leads to tumor formation.

**Carcinogenesis by Mutagenic Agents.** Cancer is a large family of diseases with several common characteristics: sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis to supply oxygen and nutrients, and active invasion and metastasis (Hanahan and Weinberg, 2011). Cancer formation is known as carcinogenesis (or oncogenesis). Cancer begins with tumor cell initiation, which, if tumor cell promotion occurs, can progress to dysplasia, carcinoma in situ, and eventually invasive cancer and metastasis (Figure 2).

For example, most colorectal cancers begin as a benign adenomatous polyp, which develops into an adenoma before acquiring additional mutations and progressing to invasive cancer (Demeyer et al., 2016). An initial mutation in a “gatekeeper” gene may confer a growth advantage to a cell. Additional mutations in that cell can occur that further increase its growth advantage (Vogelstein et al., 2013). Cancer cells exhibit an elevated rate of mutagenesis (Bielas et al., 2006). Most cancer cells have many mutations; for example, colorectal cancer cells harbor a median of 66 mutations that change the coding sequence of proteins (Vogelstein et al., 2013).

Most acquired mutations do not lead to cancer. Cancers can occur when mutations affect “cancer driver” genes, which are genes essential to tumor development. Mutations in such genes can lead, either directly or indirectly, to a selective growth advantage (Vogelstein et al., 2013; Balmain, 2020). Mutations in genes that promote cell growth (proto-oncogenes), for example, may result in uncontrolled cell proliferation and survival and lead to cancer. Alternatively, mutations that inactivate tumor suppressor genes like p53, which are involved in apoptosis (programmed cell death), or in genes that control abnormal cell growth and tissue infiltration can lead to cancer (Goldman and Shields, 2003; Delbridge et al., 2012). In addition to proto-oncogenes and tumor suppressor genes, other cancer driver genes include genes involved in maintaining genome integrity (DNA repair genes), protecting against genome damaging agents (detoxification enzymes like cytochrome P450 [CYP450] enzymes), or signaling between cells (hormone activation of growth pathways).

Mutations that cause cancer can be inherited (~5% to 10% of cancers), acquired via environmental factors such as diet, or result from random errors during normal DNA replication (which has been estimated to account for 65% of mutations that lead to cancers) (Tomasetti et al., 2017).

![Figure 2. Progression of carcinogenesis from mutation to metastasis.](image-url)
**Inflammation and Reactive Oxygen and Nitrogen Species.** Inflammation is a response by tissue when it is physically irritated, injured, infected, or exposed to a toxic substance (Roberts et al., 2010). During inflammation, both ROS (including superoxide radicals, peroxides, hydroxyl radicals, etc.) and reactive nitrogen species (RNS; including nitric oxide [NO], peroxynitrite, nitrogen dioxide, dinitrogen trioxide, etc.) are produced by the body when it senses infectious agents. These reactive oxygen and nitrogen species play other key roles within the body and are critically involved in signaling and blood vessel function (Di Meo et al., 2016; Reczek and Chandel, 2017).

Although ROS and RNS are important physiologically, they are powerful agents that can damage cells and tissue, with potentially carcinogenic consequences (Roberts et al., 2010; Nakamura and Takada, 2021). Oxidative and nitrative stress occurs in an organism when ROS or RNS levels overwhelm the organism’s ability to regulate such activities (Roberts et al., 2010). ROS and RNS can damage DNA and proteins, including those involved in DNA repair or the control of cell proliferation and survival. ROS can damage lipids, leading to lipid peroxidation, a chain of oxidation reactions that cause additional damage and production of other compounds (for example, malondialdehyde [MDA]) that can further damage DNA (discussed later) (Roberts et al., 2010).

Chronic inflammation can contribute to carcinogenesis in other ways besides oxidative and nitrative stress. Chronic inflammation increases cell division, increasing the possibility for natural mutations arising from DNA replication errors (Vassilev and DePamphilis, 2017; Vincze et al., 2022). Cytokines produced during inflammation can also lead to significant downregulation of chemical detoxification enzymes (e.g., CYP450 enzymes) (Zanger and Schwab, 2013) or have other effects that promote the formation of certain tumors (Dranoff, 2004).

Chronic inflammation is often associated with the development of cancers. For example, inflammatory bowel disease (IBD) often leads to colorectal cancer (Demeyer et al., 2016), with 15% of patients with IBD dying from colorectal cancer (Rossin et al., 2017). However, only a small percentage (1% to 2%) of colorectal cancers are associated with IBD (Munkholm, 2003). Additionally, nonsteroidal anti-inflammatory drugs (e.g., aspirin) have been associated with reduced risks for colorectal and other cancers (Samraj et al., 2014; Shaukat et al., 2017).

**Nonmutagenic Mechanisms of Carcinogenesis.** In the classical genetic model of carcinogenesis (Chial, 2008; Little, 2010), DNA mutations cause carcinogenesis through uncontrolled cell proliferation, inactivation of programmed cell death, or inability to repair DNA correctly or detoxify chemical agents. However, some chemicals (hormones, ligands for receptors, etc.), including those that might be found in foods, may directly trigger such effects without generating mutations. In addition, numerous chemicals cause cancer in laboratory animals but do not show evidence of any mutagenicity (Tennant, 1993).

Nonmutagenic agents may promote carcinogenesis through epigenetic changes to the genome. Epigenetic changes are chemical or physical changes to chromatin that are stable (but potentially reversible) and usually heritable. These changes can include covalent modifications (methylation to DNA, acetylation of histones, etc.) that do not alter DNA sequence but greatly affect gene expression (Esteller, 2008; Gibney and Nolan, 2010; Sharma et al., 2010; Biswas and Rao, 2017).

Although epigenetic changes are essential to many normal functions, sometimes they are deleterious (Weinhold, 2006). For example, hypermethylation of the promoter regions of tumor suppressor genes can effectively silence these genes, providing a selective advantage to tumor cells that promotes carcinogenesis (Jones and Baylin, 2002; Kasai, 2016). Epigenetic changes are pervasive in cancers and are often used as biomarkers for detecting and staging cancers (Baylin and Jones, 2016).

Short (20 to 25 nucleotides), noncoding micro-RNA molecules (miRNAs) are another epigenetic means by which gene expression can be fine-tuned post-transcriptionally. Generally, miRNAs interact with 3’ untranslated regions of messenger RNAs to promote their degradation or prevent their translation (O’Brien et al., 2018). miRNAs have been shown to affect many biological pathways, including those involved with DNA damage response, cell-cycle control, apoptosis, differentiation, and metabolism (all of which have relevance to cancer) (Jansson and Lund, 2012). Changes in miRNA expression, which can occur in response to diet (Humphreys et al., 2014), have been linked to many types of cancers, including colorectal cancer (Gavrila et al., 2016).

Other mechanisms for nonmutagenic carcinogenesis include interference with gene expression, apoptosis, or angiogenesis (Tennant, 1993). It has been hypothesized that some carcinogens facilitate the growth of cells that already contain endogenous mutations that arise from rare errors during normal DNA replication (Dart, 2020; Riva et al., 2020). Such nonmutagenic agents may lead to tissue conditions (such
as inflammation, inhibiting apoptosis, presence of hormones, immune suppression) that allow cells with pre-existing mutations to proliferate (Lopez-Bigas and Gonzalez-Perez, 2020), in some cases preferentially.

**Assays for Genotoxicity, Mutagenicity, Carcinogenicity.** A wide variety of tests using bacteria, animals, or human cells can be used to assess the genotoxicity, mutagenicity, or carcinogenicity of chemicals and in some cases, complex combinations of chemical such as foods. Studies ranging from *in vitro* assays, nutritional epidemiology, human feeding, and biomarker studies may also be employed to assess carcinogenic potential.

Genotoxicity assays look for evidence that a chemical causes genetic damage to DNA such as chromosomal breaks. Common tests include the comet assay, chromosomal aberration tests, micronucleus formation assays, and assays that identify the presence of DNA adducts. Genotoxicity assays alone are not sufficient to demonstrate that an agent is mutagenic or carcinogenic, but they can suggest mechanistic information for those agents shown in other tests to be carcinogenic (Hori et al., 2020). Ideally, *in vivo* genotoxicity in target organs should be observed for genotoxicity tests to have direct relevance to a specific type of cancer (Hori et al., 2020).

The most well-known mutagenicity tests include the bacterial Ames test and the mammalian thymidine kinase or hypoxanthine phosphoryl transferase (HPRT) gene mutation tests. These *in vitro* tests assess the ability of a chemical to cause a mutation that leads to an easily observed phenotype. Mutagenicity tests do not show that an agent is carcinogenic, only that it causes a mutation. These tests are faster, much easier, and cheaper to perform than carcinogenicity tests (Weisburger, 1996). Because many compounds are not carcinogenic until activated within the body (discussed in more detail later) (Cox et al., 2016), a micromosomal liver extract (e.g., S9 extract) can be included in such assays to provide enzymes necessary to metabolically activate procarcinogens.

Various assays have been used to assess the carcinogenic potential of foods, chemicals, environmental, or other agents. Cell transformation assays (Syrian hamster embryo and mouse BALB/c3T3 assays, for example) (Schechtman, 2012) are *in vitro* assays that assess the ability of an agent to cause a specific type of mutation that mimics the initiation step in carcinogenesis. Although these tests do not detect nongenotoxic agents and are associated with a significant false-positive rate (Creton et al., 2012), they are much faster and less expensive than animal carcinogenicity studies.

Animal (usually rat or mouse) carcinogenesis studies are long and expensive and raise ethical concerns regarding animal use. The traditional carcinogen bioassay, in rats or mice, requires significant time (2 y, typically) and large numbers of animals. Although considered a reliable predictor of carcinogenicity in humans (Benigni et al., 2013), positive findings in an animal carcinogenicity study do not always translate into human risks (Weisburger, 1996). Differences between animal and human physiology can make clear extrapolations difficult. For example, humans are omnivores and have developed the ability to absorb dietary heme iron from their small intestine. In contrast, mice, which are herbivores, exhibit poor absorption of heme iron (Coffey and Ganz, 2017), making it difficult to assess the human relevance of studies testing heme carcinogenicity in mice.

Animal carcinogenicity studies can employ various strategies to shorten the study length or decrease the cost or number of animals used. For example, biomarkers (such as certain DNA adducts) or surrogates of cancer (such as the presence of colon polyps) may serve as an endpoint, shortening the duration of the study. However, the validity of the biomarker or surrogate and its link to cancer must be firmly established. Some studies utilize animal models of cancer that develop cancer faster than humans, such as tumor xenograft animals or animals genetically engineered or otherwise predisposed (for example, by treating with a cancer-inducing chemical) to develop cancers quickly. However, these models often do not accurately reflect human physiology (Santos et al., 2008). In addition, animal models of cancer may not accurately recapitulate human carcinogenesis: cancers in models versus humans may feature mutation landscapes, abilities to progress and metastasize, pathological features, and interactions with other factors such as genetics, diet, and intestinal microbiota (Neto et al., 2023).

Nutritional epidemiology studies are often used to identify potential relationships between diet and disease in humans. However, confounding variables in such studies are often unknown or difficult to measure or adjust for. As a result, such studies cannot be used to infer causation (Hill, 1965; Ohukainen et al., 2022). In addition, nutritional epidemiology studies rely on detailed knowledge of dietary intake over a long period of time, which is extremely challenging to assess accurately (Archer et al., 2018; Klurfeld et al., 2018; Brown et al., 2023). Nevertheless, in the absence of better
methods, nutritional epidemiology data are often used to establish regulatory controls on chemicals used in food production.

Biomarkers are biological molecules present in tissues or bodily fluids that indicate exposure to a carcinogen or whether an abnormal process or disease is present (Henry and Hayes, 2012). Examples of biomarkers include the presence of the carcinogen, its metabolite, or DNA adducts characteristic of a mutagen in a tissue or bodily fluid. Biomarkers are surrogates that must be validated to confirm that their presence or levels correlate with what they are purporting to measure (e.g., exposure to a carcinogen or presence of cancer) (Turesky and Le Marchand, 2011). For example, carcinogenic heterocyclic aromatic amines (HAA) can bind with high affinity to proteins in the hair follicle and remain embedded in hair; HAA content in hair thus can be used as a biomarker to assess HAA exposure and may be more accurate than dietary questionnaires (Turesky and Le Marchand, 2011). As another example, short-term human feeding studies might include collection of colonic biopsy samples to look for intermediate biomarkers of cancer development (e.g., aberrant crypt foci as a marker for colon cancer) (Roncucci et al., 2000; Lampe, 2020). Such intermediate biomarkers of cancer can also help elucidate disease etiology (Merlo et al., 2006).

Fecal water can contain biomarkers with specific relevance to how dietary factors contribute to colorectal cancer risk (Pearson et al., 2009). The aqueous phase of feces can contain a host of bioactive compounds from dietary sources, including N-nitroso compounds (NOCs) and HAAs. These bioactive compounds can be assessed analytically, or sometimes functionally, e.g., the fecal water can be tested for its ability to promote genotoxicity, cytotoxicity, apoptosis, etc. The effects of dietary interventions can be assessed by comparing fecal water contents and/or function between control and intervention groups. Fecal water testing is often used in human nutritional intervention studies because it is inexpensive and not invasive; however, the relevance of the results obtained from fecal water assays for predicting cancer risk has not yet been demonstrated (Ristori et al., 2022).

**Carcinogen Thresholds.** Genotoxic agents (and non-genotoxic agents which foster genome instability) are often considered to be a potential risk at all concentrations. Such “no-threshold” views of cancer risk, however, have been viewed as overly protective (Hartwig et al., 2020). Dose-response curves of most carcinogens are not linear and may be relatively flat at low doses, with the rate of carcinogenesis increasing at higher doses when DNA repair and other control mechanisms become overwhelmed (Hartwig et al., 2020). Low doses (below a certain threshold) of a carcinogen might even result in an adaptive and protective response to it (Calabrese et al., 2021), with a more linear dose-response seen only at doses above the threshold.

**Physiologic considerations relevant to carcinogenic mechanisms**

How does the body itself influence carcinogenesis? Why does a carcinogen promote cancers in some tissues but not others? Understanding how the body absorbs and metabolizes foreign substances, and how these substances and their metabolites are distributed throughout the body, can help explain why ingestion of carcinogens (or their precursors) is associated with cancers in certain tissues, or in some people, but not others. Foreign compounds present in food must first pass through the gastrointestinal (GI) tract. Reactions with the foreign compound can occur in the oral cavity, the acidic environment of the stomach or the enzyme-rich environment of the small intestine. If the foreign compound is not absorbed, it will pass through to the colon, where it is exposed to an array of metabolic activities associated with the anaerobic gut microbiota. Alternatively, the carcinogen or precursor may be absorbed, giving the body’s sophisticated Phase I, II, and III systems (described in the following sections) an opportunity to modify, inactivate, and eliminate it. However, in some cases, these metabolic systems make the foreign substance more reactive, at least initially.

**Metabolic Activation and Inactivation of Carcinogens.** Xenobiotics (foreign chemicals that enter the body, including carcinogens) as well as many potentially toxic endogenous chemicals are metabolized by living organisms as part of the body’s elimination strategy (Pelkonen and Vähäkangas, 1980). The first phase of xenobiotic metabolism (“Phase I”) generally involves modification of the chemical by one of many CYP450 enzymes found in the liver and other cells within the body (Mittal et al., 2015). The active site of CYP450 enzymes includes a heme iron group that catalyzes a variety of different oxidative or other reactions.

The actions of CYP450 enzymes make the xenobiotic more reactive so that it can participate in Phase II reactions, or in some cases inactivate the chemical directly or make it more polar so it can be more easily eliminated and excreted. Many carcinogens (such as
the HAAs) are effectively inert in the body until activated by Phase I CYP450 enzymes (Le Marchand, 2021). Although activation of a xenobiotic is necessary to eventually eliminate a potentially toxic compound, activation also poses a risk because the activated compound may cause damage before it can be eliminated.

CYP450 enzymes are highly reactive and generate ROS that can damage DNA, lipids, and proteins (Veith and Moorthy, 2018). CYP450 enzymes are also involved in the production and metabolism of steroid hormones that modify cell growth. Because of these and other properties (which can themselves contribute to carcinogenesis), CYP enzyme levels are tightly controlled by the host (Mittal et al., 2015; Veith and Moorthy, 2018).

Once a xenobiotic is activated, Phase II enzymes conjugate the xenobiotic or its metabolites to an endogenous molecule such as glutathione or glucuronide, making the chemical more hydrophilic and easier to transport and eventually excrete from the body (van Iersel et al., 1999). Finally, Phase III enzymes are involved in transporting xenobiotics out of cells.

Many polymorphisms exist in the genes encoding CYP450 and Phase II and III enzymes, and the resulting variant enzymes may have greater (or less) activity upon substrates. The presence of such variants may be related to an individual’s ancestral diet and environment (Zanger and Schwab, 2013). Current environmental factors (such as diet, drugs, etc.) can introduce competitors or inhibitors of metabolic enzymes, altering the levels and/or activity of these enzymes between individuals or within an individual at different times (Mittal et al., 2015). The levels and/or activity of these enzymes within an individual can also vary greatly between tissues, which may explain why some carcinogens are associated with certain organs of the body (van Iersel et al., 1999).

Where Are Cancers Found in the Body? Carcinogens in foods do not affect every organ in the body equally. Some carcinogens in foods cause cancers along the route of exposure (i.e., the GI tract), whereas others can cause cancers in distant organs (Goldman and Shields, 2003). The tissues or organs where cancers arise can provide clues as to the causative agent and mechanism by which the agent causes cancer.

What cancers are associated with processed meats? The World Cancer Research Fund concluded that processed meat (which by their definition usually excludes that derived from poultry and fish) consumption is associated with an increased risk of colorectal cancer (World Cancer Research Fund/American Institute for Cancer Research, 2018). Similarly, the IARC Working Group concluded that cancers of the colorectum showed the most consistent relationship with processed meats (excluding those from fish but not poultry), with 12/18 cohort studies and 6/9 case-controlled studies showing a positive association (International Agency for Research on Cancer, 2018).

Fewer data were available for other types of cancers, with only stomach cancers being associated with processed meats (excluding those from fish but not poultry) in more than half of the selected studies in the IARC Monograph (International Agency for Research on Cancer, 2018).

The IARC Monograph’s meta-analysis of the 10 cohort studies with statistically significant dose-response results found the relative risk for cancer of the colorectum to be 1.17 (95% confidence interval [CI], 1.05 to 1.31) for an increase in “red meat” (minimally processed beef, pork, or lamb) consumption of 100 g/day. The relative risk of processed meats (excluding those from fish) estimated to be 1.18 (1.10 to 1.28) for increased consumption of 50 g/day (slightly more than 1 standard frankfurter). For gastric cancer, meta-analyses determined a relative risk of 1.17 for an increase in minimally processed beef, pork, or lamb consumption of 100 g/day and a relative risk of 1.15 for cohort studies (but 1.38 for case-control studies) for increased processed meat (excluding those from fish) consumption of 30 g/day. Higher relative risks were associated with bacon and sausage (up to 1.49 for case-control studies with sausage) (International Agency for Research on Cancer and World Health Organization, 2015).

Other reports suggest that esophageal, pancreatic, prostate, breast, kidney, and lung cancer are associated with processed meat (excluding those derived from poultry or fish) consumption (Wolk, 2017).

**Processed meats vs. minimally processed beef, pork, or lamb**

Based on their 2015 review, the IARC Working Group concluded that processed meat (excluding those from fish) is carcinogenic (Group 1), based largely on its associations with colorectal cancer, whereas “red meat” (minimally processed beef, pork, or lamb) is probably carcinogenic (Group 2A) (International Agency for Research on Cancer, 2018). Poultry (unspecified) consumption, in contrast, is not linked to increased risk of colorectal cancers nor consistently to any other type of cancer (Knappel et al., 2020).
What Are Processed Meats? The term “red meat” has traditionally been used for beef, pork, and lamb, whereas “white meat” has been used for poultry, especially chicken and turkey breasts (Keeton and Dikeman, 2017). However, the terms have been argued to be inadequate in capturing the nutrient compositional differences between products and the variations in product types that exist (Keeton and Dikeman, 2017; Seman et al., 2018). IARC defines processed meat to include cured meat, fresh industrial processed meat products (fresh sausage, for example), precooked ready-to-eat products, fermented sausages, and dried meats that are usually made from pork or beef (but may include poultry or offal) and reserves the term “red meat” for unprocessed beef, pork, lamb, offal, etc. (International Agency for Research on Cancer, 2018).

AMSA considers all meats to be processed, so they categorize meats into those that are minimally processed versus those that are further processed, with the “further processed” category including (1) raw, intact, with added ingredients; (2) raw, nonintact, with added ingredients; (3) further processed, unheated, or mildly heat-treated, not fully cooked; (4) further processed, fully cooked; offal/variety meat further processing (gelatin, lard, pork rinds, blood sausage, etc.); and (5) commercial sterile processing (Seman et al., 2018). As mentioned previously, AMSA’s definition of “further processed” meat does not exclude poultry (Seman et al., 2018).

Processed meats are an extremely heterogeneous group of foods that contain different levels of iron, fats, added nitrite/nitrate, and other ingredients (International Agency for Research on Cancer, 2018). Processed meats are formulated, manufactured, and prepared by the consumer in different ways (e.g., a boiled sausage vs. a grilled sausage) that may affect their risks (Table 1). Heterogeneity exists even for a specific processed meat product, based on ways of raising livestock, methods of carcass processing, and levels and types of added ingredients such as preservatives or antimicrobials, etc. There can even be substantial differences for a given product manufactured by different companies or in different countries (Molognoni et al., 2019).

Many published epidemiological studies do not include the definition of processed meats used for their study (Mejborn et al., 2016). Definitions for specific products may be broad (e.g., “ham” refers to products that are produced in quite different ways) or inconsistent between studies, complicating meta-analyses. Should a hamburger that is seasoned and grilled at home be considered a minimally processed meat or a processed meat? Should chicken sausage be considered “processed meat”? If a product contains cultured celery powder (which is a rich source of nitrite), is it really an uncurled product? There has been some call to consider different types of processes and differing compositions of processed meats when assigning risks (Molognoni et al., 2019), which should improve the ability to compare studies.

<table>
<thead>
<tr>
<th>Processed meat</th>
<th>High-temperature cooking (&gt;300°F)</th>
<th>Nitrite</th>
<th>High fat content</th>
<th>Other factors related to carcinogenic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured bacon</td>
<td>Sometimes (by consumer)</td>
<td>Yes</td>
<td>Yes, but dependent upon cooking</td>
<td>Ascorbate/erythorbate required in formulation in the US to prevent nitrosamine formation; may be smoked</td>
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<tr>
<td>Whole cured cooked ham</td>
<td>Rarely</td>
<td>Yes</td>
<td>Variable</td>
<td>May contain ascorbate to prevent nitrosamine formation; may be smoked</td>
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<tr>
<td>Beef jerky</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>May be smoked</td>
</tr>
<tr>
<td>RTE roast beef</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Grilled marinated chicken breast</td>
<td>Sometimes (by consumer)</td>
<td>No</td>
<td>No</td>
<td>A low pH marinade may reduce nitrosamine or polycyclic aromatic hydrocarbon formation</td>
</tr>
<tr>
<td>Fresh bratwurst or Italian sausage</td>
<td>Sometimes (by consumer)</td>
<td>No</td>
<td>Yes</td>
<td>Potentially higher heme content and lipid oxidation because it is made from ground minimally processed meat</td>
</tr>
<tr>
<td>Frankfurter</td>
<td>Sometimes (by consumer)</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Summer sausage</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Italian dry salami</td>
<td>No</td>
<td>Sometimes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Deli turkey ham</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Chicken nuggets</td>
<td>Yes</td>
<td>No</td>
<td>Variable</td>
<td>If made from dark or mechanically separated meat, will have high heme content</td>
</tr>
</tbody>
</table>

RTE = ready to eat.
What Characteristics Distinguish Processed Meats from Minimally Processed Meats? The difference in IARC’s carcinogenic risk assignments for minimally processed beef, pork, and lamb and further processed meats (excluding fish) prompts questions regarding characteristics of further processed meats that clearly distinguish them from minimally processed meats. In addition (although not the focus of this paper), what differentiates minimally processed meats from beef, pork, and lamb from minimally processed meats from poultry and fish, which are not generally linked to carcinogenesis? Because of the heterogeneity in processed meat products and the lack of consistent differentiation between processed meats and minimally processed meats in many studies, it is difficult to identify a specific compositional factor or set of factors that is characteristic of processed meats. Certain attributes are characteristic of some types of processed meats (Santarelli et al., 2008), but generalizations across the category are difficult, or perhaps impossible, to make.

- Some processed meats (such as bacon) are higher in fat than minimally processed meats. However, other processed meats (such as beef jerky or marinated chicken breasts) are very low in fat.
- Some processed meats (such as bacon) are typically fried or broiled at high temperatures whereas others (frankfurters) are simply boiled or not cooked at all (dry-fermented sausages). Conversely, some minimally processed meats (such as beef steak) may be grilled at home over a very hot fire.
- Some processed meats contain additional ingredients such as nitrite, whereas others do not.

Methods Used to Conduct Literature Review

In the preparation of this narrative literature review, a search of scientific literature related to mechanisms of carcinogenesis and processed meats and minimally processed meats was conducted initially using Web of Science. Searches included all databases in Web of Science (including Medline) and initially included papers published in English through July 2021, with an emphasis on papers published since 2010. Selected papers published before 2010 and more recently than July 2021 have also been included as needed.

Full-text papers were retrieved and reviewed by the authors, and a detailed draft of the manuscript was constructed. Additional literature searches (using Web of Science, PubMed, and Google Scholar) were conducted as needed during the writing process, with more recent papers added when identified. Prior to manuscript submission, independent reviewers with expertise in chemistry, biochemistry, meat biology, meat chemistry, meat processing, food additives, DNA repair and mutagenesis, cancer biology, physiology, toxicology, and microbiome research reviewed the manuscript and provided input.

Evidence Suggesting Components of Processed Meats Are Mutagenic and/or Carcinogenic

Foods, including processed meats, are complex products that contain many individual chemicals (Cobos and Díaz, 2015). The net effect of these dietary chemicals is not easy to predict, especially in the context of environmental variations among people, as illustrated by the French diet and the coffee-acrylamide paradoxes (Nehlig and Cunha, 2020). The French diet is high in fat, yet French people are less likely to die from coronary heart disease, perhaps because their diet is also rich in fiber, fruits, and vegetables (Vendrame, 2013). Similarly, acrylamide is a probable carcinogen, and coffee contains acrylamide. Coffee consumption, however, is inversely associated with cancer, possibly because other components (such as polyphenols) counteract the effect of potential carcinogens, such as acrylamide, that it may contain (DiNicolantonio and O'Keefe, 2018).

The health impacts of individual components within a complex food mixture are not always predictive of the effects of the mixture. The aforementioned examples illustrate the need for a holistic approach when assessing the role of diet in disease. Individual genetic, environmental, and microbiota differences among people further complicate our ability to understand how dietary components influence multifactorial diseases such as cancer. However, a reductionist approach to understanding the ways in which individual dietary and food components might contribute to carcinogenesis is a necessary foundation to developing a more holistic understanding that “transcends reductionism” (Hoffmann, 2003).

In the following sections of this paper, we will provide general background information and discuss potential mechanisms of carcinogenesis that have been proposed in the literature for intrinsic components and contaminants of minimally processed meats, ingredients and processing aids used in processed meats, and
endogenous agents made within the body in response to eating processed meats with carcinogenic potential.

**Components intrinsic to red meat and poultry**

Processed meats and minimally processed meats share many components (Cobos and Díaz, 2015). In this section, we will consider potential mechanisms by which these components could lead to cancer development. Greater detail is provided for agents that are more strongly associated with cancer or for which more mechanistic information is available.

**Heme Iron.** Heme is an iron-containing prosthetic group, found within a family of proteins (hemoglobin, myoglobin, cytochromes), that is critically involved in oxygen supply and electron transfer reactions (Bastide et al., 2011; Seiwert et al., 2020). The iron atom within the prosthetic group can bind and transport oxygen (O₂) or bind NO to form nitrosyl heme.

Heme’s reactive nature is essential to its key role in many important reactions, including the transportation of oxygen in the body and its ability to serve as an electron sink. The reactivity of free heme can, however, lead to significant toxicities resulting from oxidative stress and lipid peroxidation (Fiorito et al., 2020). As a result, cellular heme levels are carefully regulated, with heme itself playing a role in regulating its expression and degradation (Chiabrando et al., 2014; Fiorito et al., 2020). Besides regulating its own levels, heme also appears to play a more global role in modulating gene expression, cell proliferation and differentiation, and apoptosis (Chiabrando et al., 2014).

Higher levels of heme are present in muscle tissues that contain greater levels of myoglobin (Sasso and Latella, 2018). In general, beef has more heme iron and total iron than pork (International Agency for Research on Cancer, 2018), and beef and pork in turn have greater levels of heme than chicken (Bastide et al., 2011). However, dark meat from poultry has greater levels of heme than poultry breast meat (Keeton and Dikeman, 2017), and poultry liver can have heme iron levels higher than beef (Kongkachuichai et al., 2002). Some seafood (oysters, mussels, clams, sardines) also has significant levels of heme (British Columbia HealthLinkBC, 2020). Despite their pink color (due to their diets of krill and the pigment it contains rather than heme), fish such as salmon or tuna have lower levels of heme iron (Keeton and Dikeman, 2017). Table 2 shows representative heme iron levels in selected meats. There are inconsistent reports in the literature regarding the effect of cooking on the levels of non-heme iron in meats (Cross et al., 2012). A recent study found that cooking at temperatures below 80°C expelled heme iron from beef muscle into its juice, which may or not be consumed (Gandemer et al., 2020).

Numerous *in vitro* and animal studies have associated dietary heme iron with colorectal cancer, as reviewed in Bastide et al. (2015), Kruger and Zhou (2018), Turner and Lloyd (2017), and Seiwert et al. (2020), although caveats to some studies should be considered. Some studies administered levels of heme much higher than in typical human diets (Turner and Lloyd, 2017). Mice, which do not typically eat meat, do not absorb dietary heme iron well; this may have implications for interpreting this and other mouse studies (Fillebeen et al., 2015). It is also important to note that the use of isolated heme or heme-containing test agents such as hemin in feeding studies may not accurately reflect heme iron within minimally processed meat such as beef, pork, or lamb (Pierre et al., 2008). One 14-d rat study demonstrated that administration of freeze-dried cooked cured ham or hemin resulted in similar increases in biochemical markers associated with carcinogenesis; however, these effects were not observed in a hemoglobin diet despite all 3 diets formulated to deliver comparable levels of heme, sodium chloride, nitrite, and phosphate (Pierre et al., 2010). Many of the rodent studies of heme used the chemical carcinogen azoxymethane (AOM) to induce colonic lesions (and shorten study durations); however, some studies conducted in other rodent models without genotoxic agents suggested that heme levels in a typical Western diet might be insufficient to initiate or promote carcinogenesis (Kruger and Zhou, 2018; Seiwert et al., 2020).

In humans, heme is usually absorbed in the small intestine; however, if large amounts are consumed,
some unabsorbed heme will travel to the colon, where it can remain for some time (Gamage et al., 2018). Colorectal cancer is the cancer most often associated with minimally processed meats (excluding poultry and fish), although consumption of these products has also been linked in some studies and meta-analyses with cancers in organs both inside and outside of the GI tract, including the rectum, pancreas, bladder, breast, endometrium, liver, kidney, and lung (Keller et al., 2020; Aveta et al., 2022). Some, but not all, prospective epidemiological studies in humans found an association between high heme intake and colorectal cancer (Bastide et al., 2011; Bastide et al., 2016; Demeyer et al., 2016). Heme intake has also been associated with esophageal and stomach cancers (Lee et al., 2005; Ward et al., 2012) but not prostate cancer (Blysma and Alexander, 2015).

A variety of mechanisms have been proposed for how heme could promote colorectal cancer, with most falling into 3 main categories: (1) promoting endogenous formation of NOCs; (2) enhancement of lipid oxidation; (3) direct effect on colon cells (Demeyer et al., 2016; Mejborn et al., 2016; Kruger and Zhou, 2018); or (4) alteration of the colonic microbiota (Demeyer et al., 2016; Mejborn et al., 2016; Turner and Lloyd, 2017; Kruger and Zhou, 2018; Sasso and Latella, 2018; Fiorito et al., 2020). As will be discussed later, many NOCs are considered potent carcinogens. As reviewed by others (Turner and Lloyd, 2017; Kruger and Zhou, 2018), dietary heme increases NOC production and the presence of NOCs in the colon in both animal and human studies. NOCs can directly alkylate DNA, leading to mutagenesis, as will be discussed later in this review. Epidemiological support for an NOC-related mechanism for heme in colorectal cancer comes from a cohort study that found heme intake was associated with colorectal cancers that had specific types of mutations (G to A transitions), suggesting alkylative rather than oxidative DNA damage (Gilsing et al., 2013). A prospective human study demonstrated that a “red meat diet” (420 g/day of minimally processed beef or pork for 15 d) resulted in significantly higher O6-carboxymethyl guanine adducts in colorectal cells when compared with a vegetarian diet (Lewin et al., 2006). Similarly, a human intervention study demonstrated that dietary addition of 300 g/day of minimally processed beef or lamb for 4 wk significantly increased O6-carboxymethyl guanine adducts in rectal cells (Le Leu et al., 2015). Of note, this increase in adducts was eliminated when a dietary fermentable carbohydrate (which generates butyrate, a short-chain fatty acid (SCFA) with antitumor effects, in the colon) was added to the diet (Le Leu et al., 2015).

The reactivity of heme iron also plays a role in the peroxidation of proteins and dietary lipids within the colon (Turner and Lloyd, 2017; Kruger and Zhou, 2018). Peroxidation of dietary and endogenous lipids catalyzed by heme can lead to the formation of cytotoxic and genotoxic products such as the thiobarbituric acid reactive substances (TBARS) MDA or 4-hydroxy-2-nonenal (HNE) in the intestinal lumen (Bastide et al., 2011; You and Henneberg, 2018; Keller et al., 2020; Zhang et al., 2023). Some lipid peroxidation products such as HNE are absorbed in the intestine and can potentially reach organs beyond the intestine (Keller et al., 2020). Lipid peroxidation and protein oxidation are discussed in more detail later in this review.

Dietary heme (in the form of purified hemin) intake has been associated with chronic intestinal inflammation and suppression of apoptosis, possibly leading to colorectal cancer (Seiwert et al., 2021). However, it is not clear if this inflammation is due to hemin alone or through its catalysis of lipid peroxidation. Heme participates in formation of oxidative free radicals/ROS, which are cytotoxic (Casella et al., 2018). Heme’s amphiphilic nature allows it to infiltrate the lipid layers of the plasma membrane (Gamage et al., 2018), where it can participate in the generation of ROS. These ROS cause cytotoxic damage to colonic epithelial cells, leading to reactive epithelial hyperproliferation, aggravation of colitis (Constante et al., 2017), and increased risk of colon cancer (Sasso and Latella, 2018; Vernia et al., 2021).

The consumption of heme and the ROS generated by heme can induce heme oxygenase (HO-1), an enzyme involved in the catabolism of heme (Hemmati et al., 2021). HO-1 has immunomodulatory and anti-inflammatory activities, and some drugs that induce HO-1 have shown efficacy in IBD (Funes et al., 2020). However, HO-1 also has anti-apoptotic and angiogenic activities, increases the survival of colon cancer cells in vitro, and has been associated with various cancers, including colorectal cancer (Chiang et al., 2019; Hemmati et al., 2021; Lu et al., 2021).

Experimental results in mice have demonstrated that dietary heme (a Westernized diet supplemented with 0.5 μmol/g heme) impacts the gut microbiota and can alter colorectal epithelial cell homeostasis (Ijssennagger et al., 2012; Sasso and Latella, 2018). Iron is a key nutrient required for bacterial growth. As a result, many bacteria have iron uptake systems
that allow bacteria to acquire heme from hemoproteins or free heme (Tong and Guo, 2009; Constante et al., 2017). The presence of heme in the colonic lumen is therefore expected to affect the microbial community in the colon, favoring the growth of those bacteria that are efficient at using heme. High levels of dietary heme (a Westernized diet supplemented with 0.5 μmol/g heme) increased the ratio of Gram-negative to Gram-positive organisms in the colon of mice (Ijssennagger et al., 2012). In particular, the relative abundances of Bacteroides, Prevotella, Helicobacter, Akkermansia, and Mucispirillum increased, whereas levels of lactic acid bacteria decreased (Ijssennagger et al., 2012). This study also demonstrated a significant increase in the nitrate-reducing capacity of the microbiota of heme-fed mice. This activity could lead to higher levels of carcinogenic NOC production (Ijssennagger et al., 2012). The presence of nitrite in the diet increases but is not required for endogenous heme nitrosylation, whereas prior cooking of beef significantly decreases endogenous heme nitrosylation in an artificial digestion system (de La Pomélie et al., 2019).

Another study found that dietary heme (delivered as 50 mg/kg iron in the form of hemin in a commercial diet) or colitis both resulted in similar perturbations of the gut microbiota of mice (Constante et al., 2017). The changes in the gut microbiota in mice consuming dietary heme were accompanied by the development of adenomas (Constante et al., 2017). In a separate study, rats fed a heme iron-rich diet (1.5 μmol/g hemin in a low-calcium powdered diet) had an altered gut microbiota that closely paralleled changes in lipid peroxidation markers in the lumen when compared with rats receiving ferric citrate at the same level of iron in the low-calcium diet (Martin et al., 2019). Other components of the diet can affect the changes that heme induces in the colon. Dietary antioxidants, such as ascorbate or those found in olive oil or green vegetables containing chlorophyll (a magnesium porphyrin), can interfere with heme’s cytotoxicity and its impact on the colonic epithelium (de Vogel et al., 2005). Added dietary calcium (450 mg, about half the current daily recommended intake in the US; National Institutes of Health, 2022) reduces heme iron absorption in humans consuming moderate (360 mg) or low (60 mg) calcium meals (Roughead et al., 2005). In rats, high (1.5% or 2% vs. a standard 0.5%) calcium diets reduced biomarkers of colorectal cancer (Pierre et al., 2013; Gamage et al., 2018; Martin et al., 2019). Calcium (1,000 mg/d, similar to the recommended daily amount for adult men and women and current daily average intakes in the US; National Institutes of Health, 2022) also reduced the levels of nitroso compounds and lipid peroxidation markers in the stool of humans fed cured meat (180 g cooked ham) for 4 d (Pierre et al., 2013).

Heme can be nitrosylated in processed meats cured with nitrite or nitrate (Kostka et al., 2020). The mechanism for nitrosylation involves the reduction of nitrate to nitrite and then NO, which binds to the iron in the heme moiety. Nitrosylation of heme occurs more readily at low pH. Nitrosylated heme can also be formed endogenously (Lunn et al., 2007; de La Pomélie et al., 2018a). It is unclear how heme versus nitrosylated heme compare in their contributions to carcinogenesis (Kruger and Zhou, 2018; Kostka et al., 2020). Although some researchers have speculated that nitrosylated heme might be a key difference explaining the stronger link between processed meats than red meats with cancer, mechanistic, and other data seem to dispute this idea (Kostka et al., 2020). Nitrosylated heme can release NO and act as a nitrosating agent during digestion (Kuhnle and Bingham, 2007; Bonifacie et al., 2021). Nitrosylated heme causes double-stranded DNA breaks in a comet assay and is mutagenic in the HPRT assay. However, nitrosylated heme did not induce malignancy in the BALB/c3T3 cell transformation assay, whereas non-nitrosylated heme did (Kostka et al., 2020). Both nitrosylated and non-nitrosylated heme have been associated with colorectal adenomas (considered a precursor to cancer) (Bastide et al., 2016).

Non-heme Iron and Other Metals/Minerals Naturally and Normally Present in Meats. Non-heme iron may exist in both oxidized and reduced forms, in which reduced iron can react with oxygen to produce mutagenic and potentially carcinogenic products (Manz et al., 2016). Iron is also necessary for the proliferation of cancer cells, which require greater levels of iron than do normal cells (Manz et al., 2016; Phipps et al., 2021). High levels of iron may therefore favor growth of cancer cells over normal cells. However, the effects of iron ingestion are not limited to damaging DNA or fostering proliferative cell growth. Higher levels of total dietary iron, particularly non-heme iron, appear to reduce the risk of gastric cancer in healthy individuals (Tran et al., 2021; Collatuzzo et al., 2022). Conversely, low iron intake or low iron levels are associated with colorectal cancer (Aksan et al., 2021; Phipps et al., 2021). One possible explanation is that iron, as iron sulfide (Fe-S) clusters, is essential for many enzymes involved in DNA replication and repair. Low levels of iron may hamper...
assembly of active enzymes, contributing to genome instability, which might lead to mutagenesis (Aksan et al., 2021).

Besides iron, other metals are present in animals and their meats, including those that play a physiological role in the living animal and those that are present as a result of environmental contamination (Tchounwou et al., 2012; Hassan Emami et al., 2023). Zinc, selenium, copper, manganese, and other metals that play biological roles in the animal are thus normally found in low levels in meats. The reactivity of these metal ions can be enhanced or reduced by chelation with other molecules or proteins (Welch et al., 2002). Some metal ions could contribute to genotoxicity by destabilizing DNA, forming free radicals and ROS that damage DNA, inhibiting DNA repair, or catalyzing lipid oxidation reactions within meat products (Bal et al., 2011; Domínguez et al., 2019; Kocadal et al., 2019). However, studies that link the natural presence of non-iron metals at levels normally found in meat products with cancer risks were not found in this review. Metals found as contaminants (vs. naturally present) in meats are discussed in a later section of this review.

Lipids Oxidation Products. Lipids can be oxidized by reactive oxygen and nitrogen species present in animal cells undergoing normal metabolism or experiencing oxidative or nitrosative stress (Guéraud et al., 2010; Gamage et al., 2018). An oxidized lipid becomes a lipid radical, triggering a chain of oxidation reactions (peroxidation) that is not terminated until an antioxidant donates an electron to generate a nonradical lipid product (Ayala et al., 2014).

Lipids in meats can be oxidized during meat processing, storage, and GI digestion (Van Hecke and De Smet, 2021; Zhang et al., 2023), although the generally hypoxic environment of the colon limits lipid peroxidation (Gamage et al., 2021). Enzymes present within meats or processed meats can sometimes catalyze lipolysis, resulting in the release of free fatty acids in the product (Wu et al., 2021). Meat processing such as cutting, grinding, deboning, presence of salt (at least at concentrations up to 3%), metals, lower pH, and cooking are associated with increased lipid oxidation (Min, 2006; Sharedeh et al., 2015; Mariutti and Bragagnolo, 2017; Domínguez et al., 2019; Huang and Ahn, 2019). Storage conditions (light, temperatures, duration, presence of oxygen or heme) can also facilitate lipid oxidation in meats (Kilic and Richards, 2003). Heme iron can initiate lipid oxidation in meats, both during food processing and during digestion (Demeyer et al., 2016; Zhang et al., 2023). Of note, mechanically separated meats are especially prone to lipid oxidation given their relatively high fat content, the presence of heme proteins, the presence of minerals from fragmented bone, and the exposure to light and oxygen during the process (Lee et al., 1975; Mielnik et al., 2003; Bigolin et al., 2013). Mechanically separated poultry products often have much higher levels of hemoglobin, myoglobin, and non-heme iron than hand-boned poultry products (Froning, 1981). The relative rate of lipid hydroperoxide decomposition by methemoglobin was 62.5-fold greater than non-heme iron (Fe$^{3+}$ + ascorbate) and 4.5-, 4.2-, and 1.3-fold greater than myoglobin, oxyhemoglobin, and hematin, respectively (O’Brien, 1969). Lipid hydroperoxide decomposition by these oxidants leads to further lipid peroxidation and formation of aldehydes, as shown in Figure 3A.

Although lipid oxidation is responsible for some of the characteristic pleasant aromas associated with dry-cured meat products, it is also a critical nonmicrobial factor that decreases quality of meats (Domínguez et al., 2019). Lipid oxidation also introduces safety concerns related to the toxicity of lipid oxidation products. Lipid oxidation within an organism is associated with cytotoxicity that can lead to aging, atherosclerosis, and cancer (Schaich, 2020). Consumption of oxidized lipids in foods is subject to metabolism and detoxification during digestion, complicating assessment of their toxicity (Schaich, 2020).

Lipid peroxidation, and oxidative stress, in general, is often measured by the presence of certain reaction products, especially those that can react with thiobarbituric acid. Two key TBARS produced during lipid peroxidation of meats are the aldehydes MDA (a 3-carbon aldehyde) and 4-hydroxy-2-nonenal (HNE) (Figure 3B). MDA and HNE are considered to be the most mutagenic and toxic lipid peroxidation products, respectively (Ayala et al., 2014). MDA can be formed from most fatty acids that have more than 2 double bonds (including some omega-3 fatty acids), whereas HNE is formed from omega-6 polyunsaturated fatty acids (PUFAs; which are more abundant than omega-3 PUFAs in meat from grain or feedlot raised livestock) (Guéraud et al., 2010; Ponnampalam et al., 2021).

Nitrite or nitrate can act as antioxidants that prevent formation of lipid oxidation products in dry-cured fermented sausages, dry-cured loins, and in cured and cooked meat products (Van Hecke et al., 2021). Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), when used in processed meats, both reduce lipid oxidation and have demonstrated chemoprotective effects in rodents (Hocman, 1988). However, the
Toxicology Program finds BHA itself to be “reasonably anticipated to be a human carcinogen” (National Toxicology Program, 2021). Natural antioxidants, including polyphenol-rich extracts from rosemary and other herbs, are also effective at inhibiting lipid oxidation in meat products and may prove more attractive to consumers than synthetic antioxidants (Lee et al., 2006; Raghavan and Richards, 2007; Whalin et al., 2022). Avoiding temperature abuse can also reduce lipid peroxidation (Dominguez et al., 2019).

Epidemiological studies have shown inconsistent and, in some cases, conflicting associations between dietary fatty acids (including n-3 or n-6 PUFAs) and the risk of colorectal cancers (Storniolo et al., 2020).

Figure 3. (A) Lipid peroxidation with radical and (B) toxic aldehyde byproducts of lipid peroxidation.
Adding to the confusion, animal models have demonstrated both tumor-promoting and anticancer activities for dietary fatty acids (Nguyen et al., 2021b), although feeding a high-fat diet together with heme increases colon cancer in rats (Kasai, 2016), and dietary fat can worsen colitis and has been linked to colonic tumor formation in mice (Park et al., 2012).

Products of lipid oxidation, especially reactive aldehydes such as MDA and HNE, have demonstrated genotoxic, mutagenic, and carcinogenic activities. These reactive aldehydes can bind covalently to proteins and DNA, forming exocyclic DNA adducts (Guéraud, 2017). MDA and HNE are mutagenic in bacterial and mammalian systems (Guéraud et al., 2010; Demeyer et al., 2016) and increase colonic inflammation in animals (Turner and Lloyd, 2017). Byproducts of lipid oxidation have been linked to precancer and cancerous states in humans and in animals as well. MDA, for example, was carcinogenic in a 2-y rodent bioassay (Marnett, 1999). DNA adducts associated with MDA (MDA-deoxyguanosine adducts) are found at greater frequency in colorectal cells from colonic adenoma biopsy samples than in biopsies from healthy controls (Demeyer et al., 2016). Levels of lipid peroxidation products are elevated in colonic tissue in animal models of colorectal cancer and in humans with IBD or colorectal cancers (Lei et al., 2021).

HNE is associated with the apoptotic death of healthy, but not precancerous, cells, suggesting it may provide a selective advantage to precancerous cells (Demeyer et al., 2016). Lipid peroxides can also bind to heme forming a cytotoxic heme factor that appears to mediate changes in cell turnover and proliferation (Ijssennagger et al., 2015; Gamage et al., 2018).

Reactive aldehydes such as 4-hydroxy-2E-hexenal (HHE) can also arise from oxidation of n-3 PUFA in fish (Guillén and Goicoechea, 2008). Although less extensively characterized than HNE, HHE increases ROS and RNS levels, can form adducts with proteins and DNA and is genotoxic (Long et al., 2008).

**Other Roles for Lipids Related to Carcinogenesis.** Other roles for dietary fats in carcinogenesis that do not involve lipid oxidation have been proposed. High-fat diets increase inflammation and oxidative stress and disrupt hormone and insulin activities, potentially contributing to carcinogenesis and cancer progression (Park et al., 2012; Oczkowski et al., 2021). However, the World Cancer Research Fund found limited evidence with no conclusion as to whether dietary animal fat affected the risk of colorectal cancer (World Cancer Research Fund/American Institute for Cancer Research, 2018).

Cancer cells may use fatty acids as an important fuel source for cell proliferation (Storniolo et al., 2020). Some fatty acids (such as the monounsaturated fatty acid oleic acid) have been shown to stimulate growth of colorectal cancer cells in vitro (Storniolo et al., 2020). Dietary fat also increases the growth of prostate cancer cells, whereas low availability of fat inhibits cancer development, lowers serum androgen levels, and reduces the sensitivity of cancer cells to androgens, all of which may influence malignancy (Oczkowski et al., 2021).

High-fat diets also stimulate the secretion of secondary bile acids, which act as strong surfactants in the gut, leading to cell loss and subsequent proliferation that potentially favors tumor formation (Demeyer et al., 2016). Mechanisms of carcinogenesis related to bile acids are discussed in a later section of this review. High-fat diets are also associated with changes to the gut microbiota, gut barrier dysfunction, and colonic inflammation which may contribute to colorectal cancer development or progression (Yu et al., 2022).

One group of fatty acids that deserve additional mention are conjugated linoleic acids (CLAs), which are isomers of linoleic acid (an n-6 PUFA) with at least one pair of carbon-carbon double bonds separated by a single carbon-carbon bond (Pariza et al., 2001). CLA is found in food products derived from ruminants, including beef (particularly from grass-fed animals), in some plant seeds (Dachev et al., 2021), and can also be formed by some bacteria (such as Bifidobacterium spp.) found in the human gut (Gorissen et al., 2015). Although PUFAs (including CLA) can be oxidized (Basu et al., 2000) and potentially contribute to carcinogenesis, CLA demonstrates significant anticarcinogenic activity. This has been demonstrated both in vitro and in animal models of colon, breast, prostate, and liver cancer (Ha et al., 1987; Liew et al., 1995; Moon, 2014; Dachev et al., 2021), but clinical studies investigating anticarcinogenic effects of CLA in humans are needed (Dachev et al., 2021). Various mechanisms have been proposed for the anticarcinogenic properties of CLA, including prevention of DNA damage via antioxidant activity, anti-inflammatory effects, or anti-proliferative actions (Pariza et al., 2001; Dachev et al., 2021).

**Protein Oxidation Products.** Like lipids, the amino acids found in proteins can be oxidized, leading most commonly to carbonylation of basic amino acids, thiol oxidation, and aromatic hydroxylation (de La Pomélie et al., 2018b). These modifications can lead to protein
aggregation, fragmentation, and loss of function (Li et al., 2023). Protein oxidation can occur during processing, storage, or digestion of minimally processed or processed meats when reactive species (ROS, RNS, hydrogen peroxide, oxidized lipids, ozone, etc.) are present to initiate the reaction (Soladoye et al., 2015). Meat protein oxidation has been a topic of interest because it affects the quality and nutritional value of meat (Estévez and Luna, 2017). Higher levels of protein carbonyls are reported for processed meat (80 nmol/mg protein for smoked bacon) compared with vegetable protein (10 nmol/mg protein for heat-treated soy protein isolate) (Estévez and Luna, 2017). Other studies evaluating protein oxidation by protein carbonyls and other markers (assessed by various analytical methods) found less clear differentiation in marker levels between different types of processed and minimally processed meats (including fish) (Domínguez et al., 2022). High fat-to-protein ratios, low protein levels, and intense mincing or cooking methods were associated with higher levels of protein oxidation products in minimally processed and processed meats, with greater levels found in processed pork than in cooked minimally processed pork (Goethals et al., 2020). Of note, this same study found lower lipid oxidation products in processed pork products than in cooked minimally processed pork.

More recently, increasing awareness that oxidation of host proteins is associated with age-related diseases (including Alzheimer’s disease, Parkinson’s disease, IBD, and cancers) has raised the question of whether dietary protein oxidation (consumption of oxidized protein or endogenous oxidation of dietary protein during digestion) is a health concern (Chang et al., 2008; Soladoye et al., 2015; Estévez and Luna, 2017). Among the cancers associated with protein oxidation are colorectal cancers. Oxidative stress and inflammation are associated with colorectal cancers, and significantly higher serum levels of protein carbonyl and advanced oxidation protein products were observed in patients with colorectal cancer than in controls (Chang et al., 2008; Murlikiewicz et al., 2018). However, these markers alone do not determine causation as they might result from stimulation of the immune system’s response to a developing tumor (Murlikiewicz et al., 2018).

Several potential mechanisms have been envisioned for how dietary protein oxidation could promote pathologies related to carcinogenesis, although more work is needed in this relatively unexplored field (Díaz-Velasco et al., 2022). Oxidized proteins can aggregate, decreasing their digestibility, and leading to their accumulation in the colon where they can alter the gut microbiota and its metabolites (de La Pomélie et al., 2018b). Microbial fermentation of nonhydrolyzed proteins in the colon has been shown to generate mutagens such as phenols and cresols (de La Pomélie et al., 2018b). Carbonyl groups that arise in protein oxidation could serve as substrates for the generation of HAA or advanced glycation endpoints (Dominguez et al., 2022). One modified amino acid (α-amino adipic acid, which arises from the oxidation of lysine) has been shown to impair human enterocyte viability and homeostasis (Díaz-Velasco et al., 2022).

Amino acids in dietary proteins can also be nitrosated during digestion (de La Pomélie et al., 2017). N-nitroso tryptophan is mutagenic and is considered the most important source of endogenous nitrosamines (de La Pomélie et al., 2017).

**Other Components of Minimally Processed Meats.**

**Sialic acids.** Sialic acids, especially N-glycolylneuraminic acid (Neu5Gc), are found in higher levels in pork and beef than chicken (Gamage et al., 2018), with trace levels in fish (except caviar) and none in plants (Samraj et al., 2014). Although many mammals produce Neu5Gc, humans do not (Kooner et al., 2019). However, Neu5Gc from dietary sources can be incorporated into human tissues, with higher levels found in some human cancers. In fact, anti-Neu5Gc antibodies have been used for immunohistopathology markers of tumors (Samraj et al., 2014).

Most healthy humans develop antibodies against Neu5Gc, possibly because of early exposure to bovine products (milk or ingredients used in common vaccines) (Tangvoranuntakul et al., 2003). The incorporation of dietary Neu5Gc into human tissues can elicit an immune response, leading to chronic inflammation that might contribute to carcinogenesis (Samraj et al., 2014). This response may be modulated by the gut microbiome, whose composition is altered upon consumption of a diet rich in Neu5Gc, resulting in the production of enzymes that can digest and eliminate Neu5Gc before it accumulates in human tissues (Zaramela et al., 2019).

Animals that produce Neu5Gc would not be expected to have immunological reactions to it and thus might not be appropriate models for assessing the carcinogenic potential of Neu5Gc (Steppeler et al., 2017). However, Neu5Gc-deficient mice have been engineered and, when immunized against Neu5Gc and fed Neu5Gc, shown to accumulate Neu5Gc and exhibit a much higher incidence of hepatocellular tumors (Samraj et al., 2015).

**Cholesterol.** Cholesterol is involved in cell membrane structure and fluidity, cell signaling, immune
system modulation, and regulation of cell survival and is a precursor for bile acid and steroid hormone biosynthesis (Vona et al., 2021). Cholesterol is found in animal-based foods at approximately equal levels in beef, pork, lamb, chicken, and turkey and at higher levels in egg yolks (Daley et al., 2010; Spence et al., 2021). Cholesterol is also found in fish and at high levels in some seafoods and liver (beef or chicken) (UCSF Health, 2023).

Disruption of cholesterol homeostasis has been suspected to play a role in cancer induction (Vona et al., 2021). Epidemiological studies have linked high dietary cholesterol intake with increased risk of colorectal cancer, and conversely, cholesterol-lowering drugs (statins) reduce the risk of various cancers including colorectal cancer (Mok and Lee, 2020). Higher low-density lipoprotein cholesterol levels are associated with some cancers, whereas high levels of high-density lipoprotein (HDL) cholesterol are associated with greatly reduced risks of cancer (Mok and Lee, 2020). The Word Cancer Research Fund concluded that evidence that cholesterol was carcinogenic was limited, resulting in a “limited-no conclusion” decision (World Cancer Research Fund/American Institute for Cancer Research, 2018), similar to an earlier evaluation of cholesterol by IARC which categorized cholesterol in Group 3 (“not classifiable”) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1987).

Cholesterol is oxidized in the body by endogenous enzymes involved in steroid metabolism (Rossin et al., 2017). Cholesterol can also be nonenzymatically oxidized during food processing (especially thermal processing) or storage (Liu et al., 2022), although the presence of nitrite inhibits cholesterol oxidation (Santarelli et al., 2008). Dysregulation of cholesterol can affect certain oncogenic pathways and appears to play a role in inflammatory and miRNA-mediated carcinogenesis (Mok and Lee, 2020). Cholesterol oxidation products (COPs) have been associated with IBD; their presence in the lumen has been proposed to disrupt the intestinal barrier, potentially by activating enzymes that degrade tight-junction proteins (Liu et al., 2022). The resulting translocation of lumen microorganisms across the epithelial barrier can trigger host inflammatory responses. In addition, some COPs bind liver X receptors (LXR), which serve as master transcription factors regulating cell proliferation, inflammation, and immunity. Binding of COPs to LXRs can create an immunosuppressive tissue microenvironment that permits a tumor to escape the immune system (Rossin et al., 2017).

**L-carnitine and Choline.** L-carnitine is a quaternary ammonium compound that is involved in fatty acid metabolism (Longo et al., 2016) and is relatively abundant in muscle tissues. Minimally processed beef, pork, and lamb are rich in L-carnitine, although it is present in much lower levels in other foods, and healthy individuals normally synthesize adequate amounts of it.

Choline is another quaternary ammonium compound made by the human body, but at insufficient levels; therefore, dietary choline is essential (Derbyshire, 2019). There are high levels of choline in animal-based foods, particularly beef liver and eggs, with similar levels in beef steak and salmon and lower levels in pork chops and chicken (Derbyshire, 2019). Choline serves as a dietary methyl group donor, and such nutrients are essential for a key DNA repair pathway (the methyl-directed mismatch repair system) (Kim et al., 2022). Nutritional epidemiology studies have linked dietary choline intake to decreased risk of cancer; however, a more recent study concluded that higher intakes of choline were associated with higher rates of colorectal cancer (Kim et al., 2022).

The gut microbiome converts L-carnitine to trimethylamine (TMA) (Rajakovich et al., 2021; Buffa et al., 2022). TMA subsequently is absorbed and oxidized to trimethylamine-N-oxide (TMAO) in the liver (Figure 4) (Buffa et al., 2022). Abundance of a key gene involved in TMA production from carnitine correlates with plasma TMAO levels and a “red meat” (unspecified)-rich diet (Buffa et al., 2022). In humans,
increased intake of fish increases TMAO levels (greater than eggs or beef), although the degree of response was driven by the distribution of gut microbes in each individual (Cho et al., 2017). Diet is considered a minimal contributor to elevated plasma TMAO levels (Krüger et al., 2017; Kühn et al., 2017; Hamaya et al., 2020) when compared with individual genetic factors, age, and genetic factors (Canyelles et al., 2018), although plasma TMAO levels are influenced by the microbial response to diet.

Like L-carnitine, choline is also converted by the intestinal microbiota to TMA that is transported to the liver for oxidation to TMAO (Chan et al., 2019). A potential role for TMAO in colorectal cancer has been proposed, as serum levels of TMAO are higher in patients with colorectal cancer than in healthy controls (Jalandra et al., 2021). A variety of mechanisms by which TMAO could be involved in colorectal cancer have been suggested, including inflammation, oxidative stress, DNA damage, epigenetic alteration via DNA methylation, and protein misfolding (Chan et al., 2019; Jalandra et al., 2021). However, rats given L-carnitine orally at relatively high doses for 1 y did not develop preneoplastic lesions in their colon (Empl et al., 2015). In addition, TMAO itself has been shown to boost responses to immune checkpoint blockade therapy in patients with pancreatic cancer (Mirji et al., 2022). These findings suggest TMAO is likely a marker of disease rather than a risk factor (Cho and Caudill, 2017; Zeisel and Warrier, 2017); however, this is an active area of research, with new insights anticipated in coming years (Jalandra et al., 2021).

**Contaminants of meat and poultry products**

Chemicals that inadvertently contaminate minimally processed meat also can be found in processed meat products. Although some of the potential contaminants described in the following sections have carcinogenic potential, their presence in processed meats is inconsistent across product types and is often geographically limited. Levels for many of these contaminants are monitored by regulatory agencies, reducing the likelihood that such contaminants will reach many consumers at levels high enough to contribute to carcinogenesis across large populations.

**Veterinary Drugs Including Growth-Promoting Agents and Hormones, Antibiotics, Etc.** Some animal drugs (arsenic-based coccidiostats, diethylstilbestrol, nitrofurans, nitroimidazoles, chloramphenicol, formalin) can elicit cancers by a variety of potential mechanisms. For example, before their recent ban, the phenylarsenic additives in poultry feed used in China were estimated to cause 1,160 human cancers each year (Hu et al., 2019). The use of many cancer-causing drugs in food animals is now banned in many countries and regions, including the European Union and the US. Although violations can occur, these drugs are not commonly found during routine government meat testing (Bedale, 2019). Therefore, contribution of veterinary drug residues to the cancer risks posed by processed meats is considered to have little impact in regions where these drugs are effectively regulated.

The use of synthetic or natural steroid hormones for growth promotion in food animals is prohibited in the European Union and is not allowed in hogs or poultry in the US, except in gilts and sows for reproductive purposes (Stephany, 2010; Pork Information Gateway, 2012). In the US, beef cattle and sheep can be treated with hormones (natural and/or synthetic estrogen, progesterone, and testosterone) for growth promotion; however, residual levels present in beef from treated animals are typically very low and below Food and Drug Administration (FDA) recommended limits (Doyle, 2000; Shappell et al., 2019; US Food and Drug Administration, 2022b). However, it has been argued that existing hormone residue surveillance programs are inadequate, with more data needed to estimate dietary hormone exposure from treated beef (Nachman and Smith, 2015).

Growth of some cancers, including certain prostate or breast cancers, is hormone dependent. The cells of these cancers express the nuclear receptor for androgens or estrogens. Together with other factors, binding of the corresponding hormone to its receptor turns the latter into a pro-proliferative transcription factor, driving tumorigenesis (Jozwik and Carroll, 2012). However, colorectal cancer, the cancer most closely associated with processed meats, has not been linked to endogenous sex steroid hormone levels in humans (Bouras et al., 2021). In fact, anti-androgen therapy in patients with prostate cancer is associated with an elevated risk for colorectal cancer, and women treated with estrogen plus progesterin demonstrated lower rates of colorectal cancers (Lin and Giovannucci, 2010).

Ractopamine is beta-adrenergic agonist that is used in finishing swine and cattle in the US but is not allowed in the European Union (Fan, 2022). One recent paper hypothesizes that ractopamine residues in meat could facilitate tumor growth or metastasis in patients with Kirsten rat sarcoma viral oncogene homolog (KRAS)-mutation related cancers (including colon...
cancer); however, there is no evidence that use of this growth enhancer in food animals has a carcinogenic effect (Fan, 2022).

An estimated 7% of US beef comes from culled dairy cows, and about 22% of US dairy cows receive bovine somatotropin to increase milk production (USDA Animal and Plant Health Inspection Service, 2003; Geiser and Boetel, 2019). The safety of meat from animals receiving this hormone has been affirmed by numerous health, food safety, and regulatory agencies around the world (US Food and Drug Administration, 2022a).

**Environmental Pollutants and Toxins.** Animals consume plant material, water, and even soil while spending time in environments that might be contaminated with herbicides, pesticides, and other toxic chemicals. Are these chemicals also found in meat produced from these animals, and does consumption of meat from such animals constitute a cancer risk for humans?

Human exposure to pesticides via animal products is small compared with pesticide exposure via plant material (International Agency for Research on Cancer, 2018). A recent study in Brazil demonstrated some correlation between pesticide sales and colon cancer rates (Uyemura et al., 2017), and a 2018 study from Brazil found pesticides in nearly all of fish, beef, and chicken samples, but pesticide levels were generally at levels below regulatory thresholds (Dallegrege et al., 2018). Similarly, recent studies and regulatory surveys in the European Union Oman, Bosnia and Herzegovina, and Korea found low levels of certain pesticide residues in some minimally processed and processed meat samples that were almost always below the maximum residue limit (USDA Food Safety and Inspection Service, 2019; Kartalović et al., 2020; Al-Amri et al., 2021; Lee et al., 2022; European Food Safety Authority et al., 2023).

At relatively high concentrations, the herbicide glyphosate can cause DNA and chromosomal damage in mammalian cells and has been classified as a probable carcinogen by IARC (Guyton et al., 2015); however, this classification is not accepted by the European Food Safety Authority (EFSA; Kolakowski et al., 2020). Glyphosate residues found in foods of animal origin were not detected or were well below regulatory thresholds in numerous recent surveys (Kolakowski et al., 2020; Vicini et al., 2021).

Dioxins and polychlorinated biphenyls (PCBs) are highly toxic environmental pollutants that arise from industrial and natural processes (World Health Organization, 2016). Absorption of these compounds from soil or deposition from the air leads to their presence in vegetables, feed crops, and pastures, leading to food contamination (European Commission, 2001). These chemicals can concentrate and accumulate in fatty tissues of food animals and fish and be found in their milk, eggs, and meat (European Commission, 2001; Weber et al., 2018). Dietary animal fat consumption can contribute to human exposure to these compounds (International Agency for Research on Cancer, 2018), as can dietary exposure to fish (Cao et al., 2008). Dioxins and PCBs have been associated with increased rates of total human cancers but not with any specific cancer types (Knutsen et al., 2018). Dioxins and PCBs do not appear to be directly genotoxic in animal studies, although they can act as promoting agents for skin, ovary, and liver cancers that are initiated by known genotoxins (Knutsen et al., 2018).

Brominated flame retardants, which include some bromophenols, are sometimes found in meats (Schecter et al., 2008). Because some bromophenols are natural metabolites in marine organisms, assessment of contamination in foods of marine origin can be difficult [EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012]. Because some of these compounds are mutagenic (Blum and Ames, 1977), function as endocrine disruptors, and reduce the lytic function of human natural killer cells, they have been proposed to increase the risk of cancer, especially breast cancer (Mancini et al., 2020; Feiteiro et al., 2021). However, inconsistent results have been observed in clinical studies attempting to link these chemicals to breast cancer (Mancini et al., 2020).

Heavy metals, naturally present in the environment from industrial waste or in animal feed, are sometimes detected in foods, including meats, with the highest levels found in seafood, rice and rice products, mushrooms, and poultry (American Cancer Society, 2023). In some cases (e.g., various metals in seafood and arsenic in beef), levels may exceed thresholds associated with cancer risk (Di Bella et al., 2020). However, heavy metals rarely contaminate meat products at high levels (Meurillon et al., 2018). Various mechanisms of carcinogenicity for heavy metals have been proposed and reviewed (Chen et al., 2019). Arsenic, for example, can inhibit DNA repair, leading to chromosomal alterations, and alter DNA methylation and impact genome integrity. Arsenic also can react with ROS, leading to DNA structural damage, defects in apoptosis and alteration in the function of immune cells (Wu et al., 2022b).

**Infectious Agents from Meat or Poultry Products.** Predator mammals that frequently consume mammalian
prey die more often from cancer than those that do not (Vincze et al., 2022). One proposed explanation is that pathogenic processes moving between the prey and predator drive an increased cancer mortality risk (Vincze et al., 2022).

Indeed, various infectious agents (viruses, bacteria, parasites) have been associated with cancers (Hatta et al., 2021), and IARC has estimated that ~18% of cancers are associated with infectious agents (Sahan et al., 2018). Certain viruses have long been known to integrate into the host genome, disrupting genes that control signaling and growth (Alizadeh et al., 2018). More recently, recognition that the bacterium *Helicobacter pylori* is intimately involved in gastric cancers has prompted researchers to consider microbial causes for other cancers.

Many infectious agents cause chronic inflammation, which creates a promutagenic environment. If pathogens are present in meat and survive processing (heat treatments, fermentations) at levels that can cause infection, theoretically, they might stimulate inflammation with the potential to promote cancer development (discussed in a later section). In addition, a variety of microorganisms found in the gut microbiota that are neither associated with meat nor infections have been linked with colorectal cancers (also discussed in a later section).

A few studies suggest a possible link between several specific microbial agents found in meat animals or their meat products and an increased risk of cancer, outlined as follows:

- *Helicobacter pylori*, found in nearly half of the world’s population, has been associated with stomach cancer, although data linking the organism with colorectal cancer are inconsistent (Burnett-Hartman et al., 2008; National Cancer Institute, 2013). *H. pylori* can be found in sheep and cattle and their meat (Mashak et al., 2020); however, transmission of this organism to humans from consumption of meat products has not been demonstrated (Zamani et al., 2017).

- There is speculation in the literature that certain common bacterial contaminants of minimally processed meats, such as *Streptococcus gallolyticus* (formerly *bovis*), can infect human colonic tissue following consumption of meat, leading to carcinogenesis (Hullar et al., 2014). A potential role for *S. gallolyticus* in human colon cancer has long been debated. Many individuals with *S. gallolyticus* bacteremia or endocarditis simultaneously demonstrate colonic neoplasia, but there is no clear demonstration (or directionality) of cause and effect (Burnett-Hartman et al., 2008). *S. gallolyticus* can adhere to colonic epithelial surfaces, and like many bacteria, binds preferentially to malignant cells (Van Dessel et al., 2015). These observations suggest the organism might promote inflammation, angiogenesis, and proliferation rather than initiate carcinogenesis (Abu-Ghazaleh et al., 2021).

- The cell wall of gram-negative bacteria (which can be found in many environmental sources, including minimally processed meats) contains lipopolysaccharide, a potent inflammatory agent that some have proposed is associated with chronic inflammation and colorectal cancer (Hullar et al., 2014).

- Colibactin is a genotoxin that crosslinks DNA strands. A 54-kb polyketide synthase (*pks*) pathogenicity island that encodes enzymes needed for colibactin synthesis is found in certain strains of the intestinal bacteria *Escherichia coli (pks+ E. coli)* (Arima et al., 2022). A recent large analysis of colorectal cancer samples, combined with information about the patients’ diets, found that consumption of high levels (highest tertile vs. lowest tertile) of “unprocessed red meat” (not specified) and “processed red meat” (not specified), sugar, and refined grains was associated with higher rates (multivariable-adjusted hazard ratio of 3.45; 95% CI, 1.53 to 7.78) of colorectal cancers that contained high levels of *pks+ E. coli* (Arima et al., 2022). *pks+ E. coli* is also enriched in colonic mucosal tissues of patients with IBD (Dougherty and Jobin, 2021).

- *Cryptosporidium* spp. is a zoonotic protozoan parasite that is prevalent in and causes acute disease in a wide range of animals, including livestock; it has been suggested as a potential cause of colon cancer (Chalmers et al., 2020; Sawant et al., 2020). However, viable oocysts of *Cryptosporidium* spp. are not generally associated with meats unless contaminated with fecal material (or washed with contaminated water), and the risk of infection occurs only when meat products are not cooked thoroughly (Duffy et al., 2003; Chako et al., 2010).

- *Toxoplasma gondii* is another protozoan parasite commonly found in raw minimally processed meats and in some ready-to-eat cured products (Warnekulasuriya et al., 1998; Guo et al., 2015). However, the types of cancers associated with *T. gondii* infection (leukemias, lymphomas, myeloma) are different from those associated with consumption of meats and processed meats (Cong et al., 2015).
Higher rates of colorectal cancers occur in geographic regions where Eurasian Aurochs-derived domesticated cattle breeds (*Bos taurus*) are used for beef (Zur Hausen and de Villiers, 2015). These breeds have been shown to carry certain plasmid-like DNA species, which has led some researchers to speculate that these DNA species are associated with infectious agents (termed bovine milk and meat factors or BMMF) that trigger chronic inflammation. ROS associated with BMMF-induced chronic inflammation have been proposed to lead to increased mutational events, some of which might be associated with growth advantages and malignant transformation (Zur Hausen et al., 2017, 2019; Bund et al., 2021).

**Mycotoxins.** Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi (molds) that are common in the environment. In much of the world, these toxins are frequently present in corn, cereal grains, soybeans, peanuts, and other crops fed to food animals or ingested by people (Alshannaq and Yu, 2017), and human exposure to mycotoxins comes primarily from foods derived from plants (Fink-Gremmels and van der Merwe, 2019). Meat derived from animals raised on mycotoxin-contaminated feed could theoretically contain mycotoxins, and carryover of the mycotoxins ochratoxin A (OTA) and aflatoxins from feed into porcine tissues and bovine meat and milk has been documented (Pleadin et al., 2021). However, levels of mycotoxin permissible in animal feed are regulated in many countries, and animals consuming mycotoxin-contaminated feed may be too sick to enter the human food chain (Fink-Gremmels and van der Merwe, 2019).

Other sources besides animal feed might account for the presence of mycotoxins in processed meats. Fungi are an important component of the complex microbiota of many cured and fermented meat products, and mycotoxins have been found on the surface of dry-cured meat products and in some fermented meat products (Franciosa et al., 2021; Lešić et al., 2021; Pleadin et al., 2021). Mycotoxins could also be present in ingredients added to processed meats, including spices, soy protein, wheat gluten, breadcrumbs, etc. (Pleadin et al., 2021).

As reviewed by Pleadin et al. (2021), mycotoxins, particularly OTA, have been found in a variety of meat products, although the levels found are much lower than those found in plant-based foods or feeds. OTA levels as high as 28.4 μg/kg (parts per billion [ppb]) in dry-cured hams and 59.8 μg/kg in dry-fermented sausages have been documented. Although maximum allowed levels for OTA and other mycotoxins in meat products are not established by most countries, these levels are well above most acceptable limits for OTA in other foods, which are typically in the range of 0.5 to 20 ppb (van Egmond, 1991; Duarte et al., 2010; Fink-Gremmels and van der Merwe, 2019). Other mycotoxins such as zearalenone, trichothecene, and fumonisins may be less of a concern in processed meats given the very low levels found in meat products (Fink-Gremmels and van der Merwe, 2019).

After consumption, mycotoxins can be absorbed in the GI tract and transported into the blood, from which they can travel to distant organs. As lipid-soluble molecules, aflatoxins can enter liver cells, where they are activated by CYP450 enzymes to become reactive epoxides that can form DNA adducts that lead to mutations (Ahmed Adam et al., 2017). Epidemiological evidence from 9 of 10 studies suggests a positive, dose-dependent association between consumption of aflatoxins and human liver cancer; as a result, aflatoxin B1 is considered to be a Group 1 carcinogen by IARC (Claeys et al., 2020). Associations between other mycotoxins and human cancers are less clear, as fewer studies have been conducted; however, animal data suggest a link between OTA exposure and kidney cancer (Malir et al., 2021).

**Ingredients and processing aids in processed meats**

Processed meat products are a heterogeneous group of foods to which many different agents are added as ingredients or processing aids. Except for nitrate/nitrite’s potential ability to form nitrosamines (discussed in detail in a later section), the ingredients and processing aids found in processed meat products are unlikely to contribute significantly to carcinogenesis. We will review the evidence from a mechanistic perspective in this section.

**Nitrate or Nitrite.** Nitrate and nitrite (sodium or potassium salts) have long been used to provide flavor and color and preserve the quality of various processed meat products, including ham, bacon, and fermented sausages. Nitrite also contributes to the safety of processed meats by preventing microbial growth (and production of botulinum toxin) and reducing lipid oxidation in cured meat products (Tompkin et al., 2020; Bonifacie et al., 2021). The levels of nitrate or nitrite in processed meats have decreased significantly in the last 40 y (Lee, 2019), and currently only 5% of
dietary nitrate and nitrite comes from processed meats (Milkowski, 2011). Furthermore, many processed meat products (for example, deli roast beef or chicken nuggets) do not contain added nitrate and nitrite.

Nitrate, and its reduced form, nitrite, have the potential to generate compounds that may be carcinogenic (International Agency for Research on Cancer, 2018). An analysis of preclinical and clinical studies that specially investigated links between nitrite-containing meats (rather than all processed meats) and colorectal cancer found conflicting results in preclinical studies; however, a more pronounced link to colorectal cancer was observed in clinical studies limited to nitrite-containing processed meats versus processed meats in general (Crowe et al., 2019). Under conditions of low pH and high heat, nitrite can react with secondary amines to generate N-nitrosamines, which are carcinogenic.

Nitrite can also be further reduced to NO and other RNS, some of which can damage proteins, lipids, and DNA, and are mutagenic in Salmonella (Felley-Bosco, 1998). Mechanisms of genotoxicity for NO and its derivatives include direct DNA damage (DNA base deamination, peroxynitrite-induced adducts, single-stranded DNA breaks) or indirect damage (NO reaction with amines, lipids, or thiols that then damage the DNA). However, because NO is endogenously generated as a signaling molecule and also derived from dietary nitrite via the enterosalivary route, the relative contribution of diet to NO levels and potential links to carcinogenesis are unclear (Felley-Bosco, 1998; Bryan et al., 2012).

Most rodent studies in which nitrite or nitrate were administered in drinking water or in the diet without amines or amides showed little or no evidence of carcinogenicity (International Agency for Research on Cancer, 2010b; Bryan et al., 2012). Under certain conditions (such as low pH, high temperatures) when amines or amides are also present, however, nitrite and nitrate can react to form NOCs. NOCs, which IARC cited as mechanistically key to their assessment of carcinogenicity for processed meats (International Agency for Research on Cancer, 2018), are discussed later in 2 separate subsections of this document as process-induced compounds and as endogenously produced compounds.

Salt (NaCl). Salt consumption might play a role in some chronic inflammation-related ailments and has been associated with increased risk of gastric and other cancers (Hu et al., 2011; Ge et al., 2012) and IBD (Kuang et al., 2023). In mice, a high (7.5% vs. 0.25%) salt diet in the presence of H. pylori can damage the stomach lining, increase NOC formation, and facilitate H. pylori colonization (Fox et al., 1999), potentially leading to gastric cancers. High levels of dietary salt (8.75% vs. a 0.75% standard diet control), in the presence of H. pylori in the GI tract, greatly increased the risk of gastric cancers in gerbils (Gaddy et al., 2013).

Salt can be contaminated with heavy metals, some of which might play a role in carcinogenesis. An Iranian report found salt was contaminated with various heavy metals, including the carcinogen arsenic, at levels above the Codex maximum limits (Cheraghali et al., 2010). Salt can also increase the catalytic activity of heme iron, leading to increased production of free radicals (which can damage DNA, oxidize lipids, etc.) (Zhang et al., 2023) and increased lipid oxidation in meats (Mariutti and Bragagnolo, 2017). However, salt has also been shown to decrease levels of lipid peroxidation products such as TBARS in acid marinated meat products (Sharedeh et al., 2015; Du et al., 2022) and to decrease N-nitrosamine formation in processed meats (International Agency for Research on Cancer, 2018). Overall, the current understanding of the role of dietary salt (including salt in processed meats) in carcinogenesis is complicated and unclear (Allu and Tiriveedhi, 2021).

Other Ingredients Used in Processed Meats. Many other ingredients are used in certain processed meats that have carcinogenic (or anticarcinogenic) potential. Ascorbic acid (and its isomer, erythorbate) can inhibit the formation of some carcinogens such as nitrosamines while also enhancing cellular immunity (Glatthaar et al., 1986). At moderate levels, ascorbate is an antioxidant that prevents damage to nucleic acid from reactive oxygen and nitrogen species and reduces lipid peroxidation (Crott and Fenech, 1999). Ascorbate and erythorbate inhibit nitrosamine formation when cooking nitrite-cured processed meat products at high temperatures and therefore are commonly included (and required by US law) in bacon processing (Tompkin et al., 2020). However, at low levels, ascorbate can facilitate ROS formation and lipid oxidation by binding and facilitating metal-redox reactions (Miller and Aust, 1989).

Sorbate, when used with nitrite, is antibotulinal and increases the shelf life of processed meats. Use of sorbate to preserve foods like bacon allows lower nitrite levels to be used, thus reducing nitrosamine formation (Robach and Sofos, 1982). However, the combination of nitrite and sorbate at low pH (<5), in the absence of
antioxidants (ascorbate), produces agents with strong DNA-damaging potential (Sofos, 1981; Binstok et al., 1998). These include compounds such as ethylnitrolic acid and 2-methyl-1,4-dinitro-pyrrole, which are mutagenic in bacterial assays (Molognoni et al., 2020). Many countries, therefore, do not allow nitrite and sorbate to be used together in processed meat products (Molognoni et al., 2019; Motta et al., 2020).

Caramel dyes are complex mixtures made by heating sugars with other ingredients. They can theoretically contain low molecular weight compounds with carcinogenic potential, including 4-methylimidazole (Molognoni et al., 2019). However, caramel dyes have a long history of use in foods and beverages and are manufactured using processes to control the production of such deleterious compounds (Vollmuth, 2018).

Flour is occasionally used in certain processed meat products, such as breaded poultry nuggets. Mycotoxins (discussed previously) are a potential hazard in flour (Elzupir and Abdulkhair, 2020). Other potential hazards associated with cereal grains such as herbicides or pesticides are theoretically possible, although the contribution of such compounds to processed meat is likely to be very low. Azodicarbonamide is a synthetic chemical that may be added to flour as a whitening agent and dough conditioner in the United States, Canada, and China but not in the European Union or Australia (Ye et al., 2011; US Food and Drug Administration, 2018). This compound can decompose to precursors of ethyl carbamate, which is considered a probable carcinogen by IARC (Weber and Sharypov, 2009).

Cooking oil is used in the formulation or cooking of some processed meats (for example, marinated meat products or chicken nuggets). Exposing common cooking oils (such as soybean and corn oil) to high temperatures generates aldehydic lipid oxidation products (discussed previously) that have mutagenic and carcinogenic properties (Wann et al., 2021). The presence of cooking oil in marinades has also been shown to increase levels of polycyclic aromatic hydrocarbons (PAHs) in grilled chicken (Wongmaneeratip and Vangnai, 2017).

Celery powder, cultured celery powder, and other vegetable products are used to replace nitrate or nitrite in meat products labeled as “uncured” or “no added nitrite.” These powders naturally contain nitrate or nitrite at levels similar those used in traditionally cured meat products (Tompkin et al., 2020) and would therefore be expected to contribute to nitrosamine formation similarly to synthetic nitrite.

Carrageenan is a sulfated polysaccharide derived from seaweed that is used in many foods and processed meats, especially low-fat products, to help retain water (Trius et al., 1996). The safety of carrageenan use in foods has been questioned. Intact, food-grade, high-molecular weight (≥200,000 Da) carrageenan is not genotoxic, does not raise carcinogenic concern, and is considered safe for use in foods by the US FDA, the World Health Organization’s Joint Expert Committee, and EFSA (EFSA Panel on Food Additives and Nutrient Sources added to Food [ANS] et al., 2018; Weiner and McKim, 2019). However, oral administration of poligeenan (“degraded” carrageenan with a low molecular weight (≤40,000 Da) promoted intestinal tract lesions and colon cancer when administered orally to rats (EFSA Panel on Food Additives and Nutrient Sources added to Food [ANS] et al., 2018). Various mechanisms for this effect have been proposed, including effects related to gut microbial composition, inflammatory responses, and intestinal barrier functions (Liu et al., 2021).

Concerns have arisen that “degraded” or low molecular weight forms of carrageenan are present in food-grade carrageenan or could potentially form via acid hydrolysis in the stomach or microbial action in the intestine (David et al., 2018; Liu et al., 2021). EFSA has recently re-evaluated the safety of carrageenan as a food additive and concluded that the existing acceptable daily intakes should be considered temporary and will be re-evaluated (EFSA Panel on Food Additives and Nutrient Sources added to Food [ANS] et al., 2018).

Interestingly, carrageenan has also been proposed to have anticancer activities and has been used as an adjuvant in cancer immunotherapy (Liu et al., 2019). Of relevance to processed meats, the incorporation of 1% K-carrageenan into beef patties reduced formation of PhIP (a HAA and potent carcinogen discussed in the following sections) during roasting by 90%, possibly by sequestering precursors of PhIP formation (Yang et al., 2021).

Phosphorus is an essential mineral found naturally in many foods. Inorganic phosphates (in the form of ingredients such as sodium tripolyphosphate, sodium acid pyrophosphate, sodium hexa-meta-phosphate, tetrasodium phosphate, etc.) are commonly added to processed meats as buffering agents, to improve water-holding ability, as antioxidants, and to maintain flavor and color. Rising dietary phosphate intake in the US (which correlates with increased serum phosphorus levels) has been associated with, at least in part, processed meats and fast foods (Calvo et al., 2014; Armit and Beck, 2021). Phosphorus/inorganic phosphate (Pi) is critically involved in many cellular processes,
and Pi requirements increase during rapid cell growth, such as cancers (Arnst and Beck, 2021). In fact, dietary Pi restriction has been suggested to possibly prevent or control cancer development. However, there is a paucity of data linking dietary phosphorus intake to cancer risks. One large observational study found that increased serum phosphorus levels were associated with decreased cancer risks for women and increased cancer risks for men (Wulaningsih et al., 2013). A more recent study using Mendelian randomization (Lv et al., 2022) found an increased risk of prostate cancer in men as serum phosphorus levels increased; these researchers also performed a large meta-analysis of data in the literature and found that high dietary phosphorus intake was associated with increased prostate cancer risk (Lv et al., 2022).

**Process-induced compounds**

Heating, curing, smoking, drying, and other treatments used to manufacture processed meats can generate compounds that may be carcinogenic.

The levels of potentially carcinogenic compounds vary significantly in processed meat products depending on various factors. For example, cooking temperature, time, and methods greatly influence the levels of HAA or PAH found in cooked meat products. Different regulations or regional preferences may influence the addition of ingredients like nitrite or the processing procedures, resulting in differences when the same product is made in different countries (Molognoni et al., 2019). Fermentation and the presence of lactic acid bacteria may alter and potentially reduce the levels of chemical mutagens in processed meats (Molognoni et al., 2020).

Certain process-induced compounds are not only found in processed meats (for example, HAA and PAH can form in fresh meat when cooked at home), whereas others are specific for certain types of processed meats (for example, NOC are more often associated with cured products).

Some process-induced contaminants (HAAs and PAHs) are not unique to processed meats or beef/pork and can be found at comparable levels in poultry or fish (Nadeem et al., 2021; Sampaio et al., 2021). Some of these compounds are naturally present in the environment or may be created during smoking, complicating exposure estimates (Sampaio et al., 2021).

Significant process-induced contaminants in processed meat and poultry products, including NOCs, HAAs, PAHs, and advanced glycation end products are discussed in the following sections.

**N-nitroso Compounds.** NOCs are a large family of compounds that arise from the interaction of secondary amine compounds with a nitrosating agent (Lijinsky, 1999). Examples of NOCs include N-nitrosamines and N-nitrosamides. Because analytical methods cannot always differentiate NOCs from S-nitrosothiols, O-nitrosothiols, and iron nitrosyls, the term “apparent total NOCs” or ATNCs is sometimes used to describe these related compounds. Humans are exposed to NOCs in the environment, in food and other consumer products, and from their endogenous production within the body (discussed in a later section).

NOCs can also form during processing of consumer products such as cured tobacco, fire-dried malted barley and nonfat dried milk, certain pharmaceuticals, and some processed meat and poultry products (Scanlan, 1983; Lijinsky, 1999; Thresher et al., 2020). Preformed NOCs (in particular, nitrosamines) have been associated with cured (or smoked) meat products, particularly fried bacon and other heat-treated (especially fried) processed meat and poultry products (Kakuda et al., 1980; Gamage et al., 2018; Lee, 2019), although a recent study found no nitrosamines in cooked cured ham (Bonifacie et al., 2021). The secondary amine required for N-nitrosamine formation within foods can be derived via protein degradation, packaging material, or other ingredients. Biogenic amines, formed by the decarboxylation of free amino acids by certain bacteria or meat enzymes, can also be precursors to NOCs (De Mey et al., 2014). Within foods, nitrite can serve as a nitrosating agent, as can other nitrogen oxides formed from molecular nitrogen at high temperatures or found in smoke (You and Henneberg, 2018) (Mirtwish, 1995; De Mey et al., 2017; Kobayashi, 2018).

High temperatures and low pH potentiate nitrosamine formation in meats, whereas the presence of antioxidants such as ascorbate or erythorbate, and to a lesser extent NaCl, reduces nitrosamine levels in meats and during cooking of cured meat products (De Mey et al., 2017; International Agency for Research on Cancer, 2018; Bonifacie et al., 2021). Nitrosamines are found in processed meats containing nitrite or nitrate, including cured meats, fried bacon, frankfurters, and smoked chicken (Demeyer et al., 2016). N-nitrosamines can also be found at times in fresh and minced meat (De Mey et al., 2017). Among the most common nitrosamines associated with processed meat and poultry are those shown in Figure 5, including N-nitrosodimethylamine (NDMA), N-nitrosopiperidine (NPIP; associated with the use of pepper), and N-nitrosopyrrolidine (NPYR) (De Mey et al., 2014; Lee, 2019).
Nitrosamines, which require metabolic activation to be mutagenic, are generally considered to be potent carcinogens (Demeyer et al., 2016), with one N-nitrosamine (N-nitrosodimethylamine) showing carcinogenicity in more than 40 different animal species (Lijinsky, 1999). However, considerable variation in the mutagenic and carcinogenic potential of nitrosamines exists, with nearly 20% considered noncarcinogenic (Thresher et al., 2020). Some nitrosamines are volatile, and others are not; nonvolatile nitrosamines, which are the main NOCs found in foods, do not appear to be mutagenic or carcinogenic (Demeyer et al., 2016). Many different organs have been associated with nitrosamines, including cancers of the lower urinary tract and gliomas (International Agency for Research on Cancer, 2018).

Following activation by CYP450 enzymes, NOCs can cause mutations through alkylation of DNA (Sasso and Latella, 2018), which results in DNA adducts such as O6-methylguanine (Seiwert et al., 2020) and O6-carboxymethylguanine (Aloisi et al., 2021). DNA adducts resulting from nitrosamines can directly lead to DNA deamination and mutations (International Agency for Research on Cancer, 2010a).

NOCs have been linked to G to A transitions in the KRAS gene, a key proto-oncogene involved in cell signaling. The majority of colorectal cancers contain KRAS mutations, which are associated with oncogenic transformation of colorectal epithelial cells (Gamage et al., 2018).

In contrast to nitrosamines, nitrosamides do not require metabolic activation to damage DNA. Because nitrosamides are very reactive, their presence in foods is difficult to assess (Lijinsky, 1999; Mejborn et al., 2016), and their contribution to carcinogenesis is unknown.

Other nitroso compounds, such as S-nitrosothiols and nitrosylheme, can act as NO donors that can promote tumor cell proliferation and metastasis. Both S-nitrosothiols and nitrosyl heme can also participate in the nitrosation of the amino acid glycine. The resulting N-nitrosoglycine can form highly reactive alkylating agents that produce various DNA adducts (Steinberg, 2019).
**Heterocyclic Aromatic Amines.** Heterocyclic aromatic amines (HAAs or HCAs) were first identified as mutagens present in condensates from cigarette smoke. Molecules in this same class of compounds were subsequently identified in the charred parts of broiled fish and meats (Sugimura, 1997). More than 30 HAAs exist, not all of which are carcinogenic. HAAs are often grouped into 2 categories: aminoimidazoarene (or thermic) HAAs and pyrolytic HAAs (Zamora and Hidalgo, 2020; Bellamri et al., 2021; Du et al., 2022) (Figure 6). The HAAs most often associated with meat products are 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8 dimethylimidazo[4,5-f]quinoxaline (MeIQx), 3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine (4,8-DiMeIQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and 2-amino-9H-pyrido[2,3-b]indole (AαC), with PhIP usually found at the highest levels (Gibis, 2016).

HAAs are formed from creatine, amino acids, other nitrogenous components, and sugars when protein-rich foods are cooked at high temperatures or for long times (Joshi et al., 2012; Lee et al., 2013; Gibis, 2016). The aminoimidazoarene HAAs are formed at somewhat lower temperatures (125°C to ~200°C) than the pyrolytic HAAs, which form at temperatures of 250°C to 300°C or higher (Gibis, 2016; Bellamri et al., 2021).

![Figure 6](image-url)
The greatest exposure to HAAs is from well-cooked protein-rich foods such as pan-fried, grilled and barbequed meat, poultry, and fish (Shirai et al., 1997; Tang et al., 2007; Turesky and Le Marchand, 2011; Adeyeye and Ashaolu, 2021), and the cooking method, rather than the type of meat, contributes more to HAA and PAH exposure in the US (Pouzou et al., 2018). HAA are found at similar levels in heat-treated beef, pork, chicken, and fish, although chicken and fish are not associated with carcinogenesis in epidemiological studies (Demeyer et al., 2016; Neves et al., 2021). Greater levels of HAAs form at higher temperatures or longer cooking times; other conditions such as a lower pH or higher glucose content may increase HAA formation in meat (Bula et al., 2019; Molognoni et al., 2019). Conversely, the presence of certain oligosaccharides, nitrite, antioxidants, and some spices reduces HAA formation in cooked meats (Sugimura et al., 2004; Gibis, 2016; Neves et al., 2021). Conflicting reports exist in the literature regarding the effects of fat content on HAA, with some studies suggesting it may promote their formation and other research suggesting that higher fat levels reduce HAA levels (Adeyeye and Ashaolu, 2021).

Although some processed meats (such as cooked bacon) contain high levels of HAAs, HAA levels were low or undetectable in pan-fried ham and hot dogs, even when cooked “very well done,” whereas greater HAA levels were found in pan-fried pork chops or oven-broiled bacon (Sinha et al., 1998). Pan drippings and scrapings from grilled minimally processed meats, often used for gravies, can have very high levels of HAAs (as much as 10x to 100x higher than levels in the cooked meats) (Gross et al., 1993). HAAs are thus not uniquely and specifically associated with minimally processed or processed meat products.

Certain dietary factors can alter the carcinogenic activity of HAA; for example, lower daily levels of HAA can induce tumors in rodents fed a high-fat diet (Turesky and Le Marchand, 2011). In contrast, dietary dairy products or CLAs can suppress HAA-induced formation of colorectal cancer precursors in rats (Tavan et al., 2002; Sugimura et al., 2004).

Epidemiological studies have linked HAA consumption to cancers of the colon, rectum, breast, prostate, pancreas, lung, stomach, and esophagus, although inconsistent results have been obtained among studies. These epidemiological associations are complicated by the presence of HAA mixtures and other potential carcinogens (National Toxicology Program, 2021). At least 30 HAAs that have been identified, 10 of which (4 thermic and 6 pyrolytic) have been classified as probable or possible carcinogens by IARC (Zamora and Hidalgo, 2020). Four HAAs (MeIQ, MeIQx, IQ, and PhIP) are reasonably anticipated to be human carcinogens by the US National Toxicology Program (National Toxicology Program, 2021).

HAA themselves are relatively unreactive until Phase I or Phase II enzymes in the body convert some (but not all) HAAs into compounds that are strongly mutagenic (Turesky and Le Marchand, 2011; Barnes et al., 2018; Le Marchand, 2021). Metabolic activation of HAAs allows them to react with DNA to form adducts that can lead to mutations (Figure 6). Rodents fed HAA will develop cancers of the liver, colon, pancreas, breast, and prostate (Bellamri et al., 2021). However, only extremely high HAA doses (at least 10^3-fold higher than the average daily human HAA intake) induced tumors in 2-y rodent feeding studies (Turesky and Le Marchand, 2011; Nicken et al., 2016). Nevertheless, DNA adducts characteristic of HAA-induced damage can be found in tissues of interest in humans, suggesting that HAAs can damage DNA even at low levels (Turesky and Le Marchand, 2011). More HAA-DNA adducts were found in the prostate cells of patients with prostate cancer when they consumed grilled beef and pork (but not chicken and fish), especially grilled hamburgers (Tang et al., 2007). It should be noted that different methods of measuring HAA-DNA adduct formation in animal or human tissue, such as ^32P-postlabeling or immunohistochemistry, are not selective and may result in numbers of adducts that are up to 10,000-fold greater than more specific and sensitive methods such as liquid chromatography/mass spectrometry (Bellamri et al., 2021).

Besides directly forming DNA adducts, HAAs can function in other ways to promote carcinogenesis. HAAs also induce prostate inflammation in animal models and increase expression of the androgen receptor, a regulator of cell proliferation, and Ki-67, a marker for cell proliferation; however, the levels of HAAs used in animal studies may be a million-fold greater than the daily human intake (Bellamri et al., 2021). PhIP has estrogenic activity, suggesting a possible mechanistic role in breast cancer, although prospective studies have not linked HAAs with breast cancers (International Agency for Research on Cancer, 2018).

Polycyclic Aromatic Hydrocarbons. PAHs are a family of more than 200 compounds with 2 or more
fused aromatic rings that arise during the incomplete combustion of organic material, including fossil fuels and tobacco (Sahin et al., 2020). Unlike HAAs, which contain nitrogen atoms in their aromatic rings (Figure 6), PAHs contain only carbon and hydrogen atoms (Figure 7), which makes them highly nonpolar and lipophilic (Phillips, 1999).

Although widely distributed in the environment, diet represents the greatest exposure to PAHs for non-smokers (Strait et al., 2005). Plants, fresh vegetables and fruits, and oils contain PAH (sum of the 16 priority PAHs) at levels up to 5 ppm, likely arising from air and soil contamination (Paris et al., 2018). Minimally processed raw meats also contain PAHs, usually below 2 to 3 ppb (sum of 16 PAHs) for ground beef, pork, ham, and chicken, with substantially higher levels noted for frankfurters (~14 ppb) and salami (~365 ppb) (Martorell et al., 2010). Atmospheric PAHs can be deposited in aquatic environments and taken up by fish and mollusks, in which they can reach significant levels (Phillips, 1999). For example, PAH levels (sum of 13 PAHs) in fresh Atlantic salmon were found to be 231.77 ng/g of dry weight (Rascón et al., 2019).

PAHs form in meats and other foods during cooking, typically at higher cooking temperatures (>300°C) than those that generate HAA (Sanz-Serrano et al., 2020). Cooking methods such as charcoal grilling, in which there is direct contact of fat with an open flame and potentially incomplete combustion of charcoal, result in greater amounts of PAHs (Joshi et al., 2015; International Agency for Research on Cancer, 2018), with charcoal grilling generating more PAH levels in meats than gas grilling (Gorji et al., 2016). Grilled and fried meats were found to have PAH levels (sum of 15 PAHs) ranging between 8.23 and 341 ppb (Jiang et al., 2018). Smoking can generate high PAH levels in meat products, with PAH levels dependent on the type of wood used and other smoking parameters (Onopiuk et al., 2021). Higher cooking temperatures, longer cook times, and higher fat contents are associated with elevated levels of PAHs (European Commission Scientific Committee on Food, 2002). Reuse of cooking oils increases PAH levels up to 6-fold (Rajendran et al., 2022).

In addition to cooking methods, formulation of processed meats and poultry products also affects PAH levels. Marinades influence PAH levels in charcoal grilled foods, with less basic marinades or the inclusion of phenolic compounds such as tea, raspberry juice, or dark beer reducing PAH formation (Onopiuk et al., 2022). Conversely, inclusion of oil in the marinade increased PAH levels (sum of 16 PAHs) from 190 to 439 ppm in grilled chicken breasts, and marinade with a higher pH (>7.5 vs. pH 5.3 or lower) further increased the levels of PAHs (sum of 16) in grilled chicken breasts from 485 to 1,781 ppm (Wongmaneepratip and Vangnai, 2017). Partial replacement of sodium chloride with other salts (calcium chloride, potassium lactate, calcium lactate) decreased PAH production in bacon (Li et al., 2021). In smoked meat, the use of liquid smoke or the use of peelable cellulose or collagen (vs. natural sausage casings) can reduce PAH levels within the sausage (Guillén et al., 2000; Pöhlmann et al., 2013; Škaljac et al., 2018). BaP and other PAHs can be reduced on sausages by application of lactic acid bacteria before and/or after smoking (Bartkiene et al., 2017).

Like HAAs, not all PAHs are carcinogenic. IARC has classified BaP as a Group 1 carcinogen, and benzo[b]fluorene (BbF), benzo[a]anthracene (BaA), and chrysene (Chr) as possible carcinogens (Group 2B) (International Agency for Research on Cancer, 2010b). Epidemiological studies have linked dietary PAH consumption with cancers of the stomach, colorectum, breast, and kidney (Kruger and Zhou, 2018).

Like HAA and many other carcinogens, PAHs are chemically inert until they are metabolically...
Activated by Phase I and II enzymes in the body. Activated PAHs have increased polarity and reactivity and can bind covalently to macromolecules like DNA and RNA, forming adducts that can lead to DNA mutations and alter gene expression (Sampaio et al., 2021).

PAH-DNA adducts have been associated with colorectal cancers in humans. Patients with colorectal adenomas were more likely to have PAH-DNA adducts in their blood leukocytes than healthy volunteers (Gunter et al., 2007). Bulky PAH-DNA adducts are found in higher levels in colonic tissue of humans who have a greater dietary intake of PAH from grilled meats (Hansen et al., 2007). However, epidemiological evidence linking PAH intake from meat to colorectal cancer is scant because meat and meat products only account for an estimated 19% of total BaP intake (Demeyer et al., 2016) and exposure estimates are associated with uncertainties (Pouzou et al., 2018).

Because PAHs, as typified by BaP, are common in the environment and in nonmeat foods, the mechanism of their potential carcinogenicity has been studied extensively in other contexts. BaP also been shown to promote epigenetic changes (dysregulation of DNA methylation, histone modifications, etc.) through the formation of DNA adducts at CpG dinucleotide sequences (Bukowska and Sicińska, 2021). BaP also suppresses expression of genes involved in cell cycle progression and DNA repair in prostate cancer cells (Oczkowski et al., 2021). BaP interaction with the aryl hydrocarbon receptor can result in significant alteration of hematopoiesis and the type of immune responses (Hong et al., 2016). BaP and other PAHs have also been shown to repress BRCA-1 protein levels in breast cancer cells, potentially compromising DNA repair abilities and increasing the risk for mutations (Rajendran et al., 2022).

**Advanced Glycation End Products.** Advanced glycation end products (AGEs) are a diverse group of compounds that form when free amino groups in proteins, lipids, or nucleic acids are nonenzymatically glycated as a result of being exposed to reducing sugars in a Maillard reaction (Urribarri et al., 2010; He et al., 2014). AGEs are formed during normal metabolism in humans, but high endogenous levels (which occur with aging as AGEs accumulate) are associated with various pathological conditions including diabetes, heart disease, and other inflammatory diseases (Sharma et al., 2015).

In addition to being formed endogenously, AGEs are found in many types of foods, and their levels can increase upon cooking (Jiao et al., 2015). Cooking methods such as frying, grilling, and roasting generate more dietary AGEs in meats than when meats are cooked in soups/stews or by other moist-heat cooking methods (Urribarri et al., 2010; Jiao et al., 2015; Huang et al., 2021). Higher cooking temperatures are also associated with greater AGE formation (Urribarri et al., 2010), whereas marination of foods or other methods that lower pH reduce AGE formation (Inan-Eroglu et al., 2020). Some phenol compounds have been demonstrated to inhibit AGE formation in cooked meats (Huang et al.). AGEs can also develop during food (including raw meat) storage (Huang et al., 2021). Two common AGEs found in foods are Ne-(carboxymethyl)lysine (CML) and Ne-(carboxyethyl)lysine (CEL). The structure of CML is shown in Figure 8.

Some reports indicate that higher AGE levels are found in cooked meats, poultry, and fish than in other types of cooked foods (vegetables, fruits, grains, milk) (Urribarri et al., 2010; Inan-Eroglu et al., 2020). In contrast, other studies that used different analytical methods and focused on one AGE, CML, found the highest levels in bread crusts, chocolate, and crisp biscuits, with relatively low levels in raw and cooked meat, poultry, and fish (Zhang et al., 2020). The bioavailability of dietary AGEs is not well understood, and their rapid renal clearance makes understanding their contribution to health problems unclear (Zhang et al., 2020). However, consumption of foods with high levels of CML does not lead to corresponding high levels of serum and urinary CML. This unexpected finding has led some researchers to propose that elevations in these parameters result from the production of CML within the body. More specifically, high fructose consumption might allow CML to be formed in the intestine from free amino groups and dietary fructose, a highly reactive sugar that is associated with many of the metabolic and inflammatory conditions that are
linked to high serum or urinary levels of AGEs (DeChristopher, 2017; Gugliucci, 2017). Better understanding of AGE (individual and total) levels in foods and more research into the possibility of endogenous AGE formation from dietary sugars is needed.

AGEs bind to proteins, causing structural deformation and loss of function (Lu et al., 2022). A potential role for AGEs in the initiation or progression of cancers has been proposed, and dietary consumption of AGEs has been positively correlated with the risk of pancreatic (Jiao et al., 2011; Jiao et al., 2015) and breast cancer in humans (Peterson et al., 2020). However, links between individual or combined dietary AGEs and colorectal cancer mortality were not observed (Mao et al., 2021).

CML has been best characterized as the prototype AGE (Peterson et al., 2020) that has receptor-dependent and receptor-independent effects. CML can bind to the RAGE receptor, a cell membrane-bound signal transduction receptor for AGEs that has been proposed to serve as a link between chronic inflammation and cancer (Jiao et al., 2011). Binding of AGEs to RAGE results in prooxidant effects including the release of ROS, promoting oxidative stress and chronic inflammation. A soluble form of the receptor (sRAGE) also binds AGEs, thereby blocking the RAGE-associated inflammatory cascade (Jiao et al., 2011).

**Endogenous agents produced in the body upon processed meat consumption**

Some potentially carcinogenic agents are produced in the body following consumption of processed meat products.

**Endogenous Nitrosamine Formation.** In addition to their formation during cooking of meat at high temperatures, nitrosamines can also form naturally in the body. For example, nitrite derived from the diet (or entero-salivary conversion from nitrate by the oral microbiota) can react with amino acids in the diet to endogenously form nitrosamines in the stomach or intestine (Mejborn et al., 2016). In addition, gut bacteria contribute to endogenous NOC formation in the colon through the action of bacterial nitrate reductase and by bacterial decarboxylation of amino acids (Gamage et al., 2018). Formation of NOCs is catalyzed by acidic conditions (such as those found in the stomach) and by the presence of heme (Dubrow et al., 2010). Endogenous NOC formation is estimated to account for 45% to 75% of total human NOC exposure and has been hypothesized to increase the incidence of various cancers (Dubrow et al., 2010). However, an association of endogenous NOCs with GI cancers was not observed in one large prospective clinical study (Loh et al., 2011).

Numerous studies have linked minimally processed beef, pork, and/or lamb consumption with increased endogenous NOC formation in humans (Steinberg, 2019). Consumption of high levels (600 g/day vs. 60 g/day) of minimally processed beef, lamb, or pork resulted in a dose-dependent increase in endogenous NOC formation that was not observed when similar levels of poultry and fish were consumed (Bingham et al., 1996). Another clinical study found higher fecal NOC levels after a diet of minimally processed meat (beef and pork) or processed meat (bacon, pork luncheon meat, corned beef, and gammon) when compared with individuals on a vegetarian diet (Joosen et al., 2009). However, the amount of endogenous NOCs produced varies greatly among individuals consuming the same amount of meat (Steinberg, 2019).

Endogenous NOCs could contribute to DNA damage, mutagenesis, and carcinogenesis by mechanisms that are similar to those of NOCs already present in foods (as discussed previously). Consumption of nitrite-containing processed meat products increased colonic expression of p53 (a marker for DNA damage) in mice (Adu et al., 2020). Surprisingly, fecal water from humans on minimally processed meat or processed meat diets induced fewer DNA strand breaks than fecal water from those on a vegetarian diet, although fecal water from the processed meat diet showed increased oxidative DNA damage when compared with minimally processed meat (Joosen et al., 2009).

**Bile Acids.** Bile acids are potentially carcinogenic components produced by the body in response to dietary fats, including those found in processed meat and poultry products. Bile acids including the primary bile acids, cholic acid and chenodeoxycholic acid, are produced in the liver by oxidation of cholesterol (Figure 9). Bile acids can be conjugated with the amino acids glycine or taurine to form conjugated bile acids that facilitate release and passage to the small intestine (Tang and Evans, 2021). Conjugated bile acids are amphipathic molecules whose detergent-like character facilitates absorption of lipids and other nutrients. Conjugated bile acids are reabsorbed in the small intestine and recycled to the liver. A small amount (~5%) are not reabsorbed and pass into the colon, where they are transformed by gut bacteria into secondary bile acids, such as deoxycholic acid and lithocholic acid (Lucas et al., 2021).
In addition to their role in facilitating transport and digestion of dietary fats and cholesterol, bile acids play a signaling role in the enterohepatic system, binding to multiple nuclear receptors to regulate metabolism, immunity, and other physiological processes (Tang and Evans, 2021).

High dietary fat intake results in increased synthesis and secretion of bile acids (Kuhls et al., 2022). Consumption of processed meats (but also of fried potatoes, fish, margarine, and coffee) have been shown to significantly increase human fecal bile acid levels (Trefflich et al., 2020). Bile acid dysregulation has been linked with some cancers associated with processed meat ingestion (colorectal, gastric, and pancreatic) (Ajouz et al., 2014). Disruption of bile acid regulation is often observed during colorectal cancer development, and higher levels of fecal secondary bile acids were associated with higher levels of colorectal cancers (Ajouz et al., 2014; Tang and Evans, 2021). The detergent action of bile acids in the colon may disrupt the lipid bilayer of intestinal epithelial cells, causing damage and eliciting ROS and other defenses that damage DNA (Bernstein et al., 2005). Some secondary bile acids promote the progression of carcinogenesis in the colon, possibly by binding to the farnesoid X receptor (FXR), a nuclear receptor expressed at high levels in the liver and the intestine (Yu et al., 2020; Tang and Evans, 2021). Bile acids also play a role in gut immune system function, and bile acid dysregulation can lead to inflammation and eventual carcinogenesis (Tang and Evans, 2021).

Bile acids can also impact cancer development in the liver. A recent study supported the idea that the gut microbiome uses bile acids as messengers to prevent the host immune system from acting upon liver cancers (Ma et al., 2018). One prospective dietary study identified an increased risk of hepatocellular cancer with increased consumption of minimally processed beef/pork or saturated fat, but not with processed meat or minimally processed chicken/turkey/fish (Freedman et al., 2010).

**Other Endogenous Agents.** miRNAs (introduced previously) have relatively recently been shown to have an important role in modulating gene expression and carcinogenesis. Altered expression of certain miRNAs is commonly associated with various cancers, including colorectal cancer (Parasramka et al., 2012; Pieri et al., 2022). Characteristic changes observed in miRNA levels have even been proposed as potential tumor biomarkers (Link et al., 2019). Tumor-associated miRNAs were shown to be increased in rats and in human colonic mucosal tissue after consumption of minimally processed beef or pork (Humphreys et al., 2014; Nielsen et al., 2019).

miRNA levels also change in response to known carcinogens, including PhIP and BaP that are found in beef, pork, chicken, and fish cooked at high temperatures as well as smoked foods (National Cancer Institute, 2017). In rodents, oral administration of PhIP and BaP results in changes in miRNAs that are associated with estrogenic activity and inflammatory microenvironments, respectively, both of which are pro-carcinogenic for certain cancers (Papaioannou et al., 2014; Malik et al., 2018). The gut microbiome and its metabolites also influence miRNA levels, with...
some researchers envisioning crosstalk among diet, the microbiome, and miRNA as the foundation of epigenetic regulation in colorectal cancer (Guz et al., 2021).

In addition to a potential endogenous effect, some researchers have speculated that miRNAs found in plant and animal tissue could survive cooking and digestion and be absorbed at levels sufficient to elicit a cross-species effect after human consumption (Link et al., 2019). Bovine miRNAs have been demonstrated to survive processing, cooking, and simulated digestion conditions (Pieri et al., 2022). Although it is hypothetically possible that unabsorbed miRNAs could have a direct effect in the lumen on intestinal tissues, currently there are no data that demonstrate that bovine miRNA are absorbed at sufficient levels to alter human gene expression in a way that could then lead to cancer development (Pieri et al., 2022).

Several agents with carcinogenic potential described earlier in other contexts can also form endogenously in the body. Protein and fat metabolism or mitochondrial energy production by the body may result in reactive species (lipids oxidation products, ROS, NOS), which can damage DNA or lead to mutagenesis (Hartwig et al., 2020). These species were discussed earlier. Similarly, lipid oxidation (also discussed earlier) can occur during digestion within the GI tract (Wu et al., 2022a).

Factors that Modulate the Effects of These Components: Other Dietary Factors, the Microbiota, Human Genetic Variations, Inflammation, and Infection

Every individual possesses a unique combination of factors that influence the components discussed previously to affect carcinogenesis. These factors include that individual’s overall diet, how they cook food, the composition of their gut microbiota, the presence of ongoing infections and inflammatory diseases, genetic susceptibilities, etc. The interplay among these factors is difficult to assess. Examples of how some of these factors modulate potential carcinogenic risks associated with processed meats are described in the following sections.

Cooking, dietary, and consumer-related factors

Although nutritional epidemiology studies try to capture as much dietary information as possible from study participants, some important information is inevitably lost. As mentioned earlier, the presence of potential carcinogens in food is dependent upon cooking method (Koszucka and Nowak, 2019). As discussed earlier in this review, the use of marinades and other seasonings, storage conditions and temperature, and time and temperature of cooking can all affect the levels of HAAs, PAHs, lipid oxidation products, and other agents in processed meats.

Inclusion of other foods in the diet might modulate potential risks from processed meats as well. Some epidemiological data suggest that consumption of antioxidants like vitamin C can decrease cancer risks (Block, 1991), although other data suggest high serum antioxidant levels can increase mortality (Peeri et al., 2021). Polyphenols have antioxidant and free radical scavenging activities that protect against cell damage such as DNA damage, lipid oxidation, etc. (Zhang et al., 2023). Diets high in calcium or in oxidation-resistant fats may counteract potential cancer-promoting effects of heme, as suggested by both human and animal studies (Pierre et al., 2003; Pierre et al., 2013; Bouvard et al., 2015; Kruger and Zhou, 2018). Calcium binding to heme reduces its solubility and potential toxic activities within the GI tract (Zhang et al., 2023); it also binds free fatty acids such as stearic acid within the digestive tract, decreasing fat absorption (Stroebinger et al., 2021).

Dietary vitamin D (from sun exposure) can modulate immune responses and affect cancer development. Although some of the earlier touted benefits of vitamin D have been scrutinized and questioned (Theodoratou et al., 2014), multiple clinical studies demonstrated a strong inverse relationship between plasma 25-hydroxyvitamin D₃ levels and colorectal cancer incidence and mortality (Dou et al., 2016). Evidence specifically linking vitamin D intake and processed meat consumption to cancer risk was not found in this review.

Consumption of dietary carbohydrate accessible to microbes (e.g., prebiotics, dietary fiber) leads to the production of SCFAs by gut bacteria. These SCFA, particularly butyrate, have anti-inflammatory and immunomodulatory activities and are associated with a reduced risk of colon cancer (Carretta et al., 2021), at least in part by inhibiting histone deacetylase enzymes (Hajjar et al., 2021). Conversely, a low-fiber diet may slow intestinal transit, allowing potential carcinogenic agents like heme more time to cause damage.

Exposure to a variety of other factors can influence the potential carcinogenic risks from meat and poultry products. For example, alcohol consumption induces cytochrome P450 CYP2E2, which can metabolically activate N-nitrosamines (Goldman and Shields, 2003).
Other foods, drugs, and environmental exposures have the potential to induce or inhibit metabolic enzymes that activate or eliminate potential carcinogens. For example, cruciferous vegetable consumption induces expression of CYP1A2 and UGT1A1, which are involved in activation and subsequent elimination of certain carcinogens, including the HAA PhIP (Kim et al., 2015). Simple extrapolation from such findings to clinical benefit is still difficult, however, given the myriad of other differences between individuals.

Many of these same individual factors also affect the gut microbiota, which interacts with the diet to modulate carcinogenic risks (Genua et al., 2021), as will be discussed in the following sections.

**Gut microbiota**

The human gut microbiome is established early in life and remains remarkably stable and resilient throughout much of life (Faith et al., 2013; Rodriguez et al., 2015; Fassarella et al., 2021). Diet shapes and can sometimes perturb the gut microbiome, which in turn can influence carcinogenesis, especially in the GI tract, where most cancers associated with processed meat and poultry products occur. Historically, a relationship between gut bacteria and colon cancer was postulated more than a century ago by Metchnikoff (Maleki Vareki et al., 2018). Later, the possibility that bacteria such as *Bacteroides* could convert bile salts into carcinogens within the large intestine was proposed (Aries et al., 1969). More than 20 y later, the ability of intestinal lactic acid bacteria to bind and inactivate the HAA PhIP was demonstrated (Orrhage et al., 1994). Tremendous recent advances in microbiome and data analysis methodology have prompted many recent explorations into how the gut microbiome influences the development of cancers, particularly in the GI tract (Nogacka et al., 2019; Cuevas-González et al., 2022; Shao et al., 2022).

In the following sections, we discuss basic relationships between gut bacteria and cancers of the GI tract and consider how diet (especially meat consumption) and gut bacteria may work together to influence potential carcinogenic risks.

**General Associations Between Gut Bacteria and Colorectal Cancer.** Reduced gut microbial diversity is associated with colorectal cancer (Appunni et al., 2021). Additionally, a microbiota enriched with certain microorganisms has been associated with colorectal and other cancers. However, this association does not demonstrate that these organisms promote the development of the cancer, or whether the cancer (or other conditions promoting cancer) creates an environment that favors the growth of certain microbes (Marchesi et al., 2011; Saha and Robertson, 2019).

The 2 dominant bacterial phyla of the human gut microbiome are Firmicutes and Bacteroidetes (Magne et al., 2020). The gut microbiota of patients with colorectal cancer exhibits reduced abundance in the phylum Firmicutes and enrichment of taxa within the Bacteroidetes relative to healthy controls. A similar shift from Firmicutes toward Bacteroidetes is also observed in normal individuals who eat a diet rich in animal products (Tuan and Chen, 2016; Ai et al., 2019). At the genera level, the microbiome of patients with colorectal cancer exhibits a greater abundance of *Anaerostipes, Bilophila, Coprococcus, Desulfovibrio, Flavonifractor, Porphyromonas, Pseudoflavonifractor,* and *Weissella* compared with healthy controls (Ai et al., 2019). Bacterial species in the intestinal microbiota that have been associated with colorectal cancer include *Fusobacterium nucleatum, Streptococcus gallolyticus* (previously *Streptococcus bovis*), *Bacteroides fragilis, Enterococcus faecalis, E. coli, H. pylori,* and *Peptostreptococcus anaerobius* (Marchesi et al., 2011; Konstantinov, 2017; Cheng et al., 2020; Scott et al., 2022). Human colorectal tumors are often infiltrated by *Fusobacterium nucleatum* and *Bacteriodes fragilis,* with the presence of *F. nucleatum* associated with more advanced disease (Rye et al., 2022).

Although the gastric environment limits the growth of many bacteria, some organisms survive within the acidic stomach (Hsieh et al., 2018). Gastric cancer is associated with the presence of certain bacteria, particularly *H. pylori,* and increased abundance of *Clostridium, Fusobacterium,* and *Lactobacillus* species (Hsieh et al., 2018; Stewart et al., 2020).

Certain bacterial taxa are found less commonly in the gut microbiota of patients with colorectal cancer than in healthy controls, including *Clostridium butyricum* and *Streptococcus thermophilus* (Cheng et al., 2020). Oral antibiotic use has been associated with colon (but not rectal) cancer, with a greater impact seen for drugs such as penicillins, which are expected to have an impact on the anaerobes that predominate in the colon (Zhang et al., 2019b). Overall, these findings suggest that components of the gut microbiome can play either a deleterious or protective role in colorectal cancer.

**Effects of Meat Consumption on the Gut Microbiome.** Both individuals whose diets are high in animal products and individuals with colon cancer...
tend to have less diversity in their gut microbiota (Tuan and Chen, 2016; Appunni et al., 2021). Consumption of diets with high levels of animal products alters the gut microbiome in humans, promoting the growth of bile-tolerant organisms (including the genera Alistipes, Bilophila, and Bacteroides) and depleting Firmicutes, which is associated with metabolism of plant-based polysaccharides (David et al., 2014; Tuan and Chen, 2016). Eating high-protein and high-fat diets is associated with a Bacteroides enterotype (Wu et al., 2011), whereas, as discussed previously, patients with colorectal cancer tend to have lower levels of Firmicutes in their gut microbiota.

Perhaps surprisingly, because diets high in animal products tend also to be high in fats, fat-restricted diets are reported to increase Bacteroidetes populations and decrease Firmicutes populations in the gut microbiota of individuals with obesity (Ley et al., 2006; Clarke et al., 2012). Conversely, high-fat diets were associated with decreases in Bacteroidetes and increases in Firmicutes in the gut microbiome of mice (Hildebrandt et al., 2009). The microbiome of children who consumed a Western-style diet with higher levels of animal protein and fat as well as sugar had lower proportions of Bacteroidetes and higher proportions of Firmicutes than children in rural Africa who consumed a fiber-rich diet (De Filippo et al., 2010). Examination of microbiota compositions at the phyla levels is perhaps an oversimplification that does not sufficiently inform how meat consumption alters the microbiome (Magne et al., 2020).

The effects of dietary administration of other meat components on the gut microbiota have been studied in animals and humans. Dietary heme iron decreased microbial diversity in the colon of rats and mice, depleting Firmicutes and promoting Proteobacteria (Constante et al., 2017; Martin et al., 2019). Mice fed a diet rich in the sialic acid N-glycolylneuraminic acid (Neu5Gc, discussed previously) exhibited an altered gut microbiota, with the greatest changes noted for Bacteroidales and Clostridiales (Zaramela et al., 2019). A human study showed that a high-salt diet reduced intestinal survival of Lactobacillus spp. (Fischer et al., 2017).

**Interactions Among Diet, Gut Microbiota, and Cancer Risks.** Diet alters the microbiome, affecting gut microbial composition and activities, which in turn can affect cancer risks. For example, high levels of nitrate in drinking water alters the oral microbiome of human subjects, increasing the abundance of nitrate-reducing bacteria, which might increase the production of NOCs in the GI tract (Sinha et al., 2021). Another example is how consumption of Lactobacillus acidophilus-fermented milk products (or the probiotic organism alone) reduces urinary or fecal markers of HAA mutagenicity in humans after consumption of cooked meat (Hayatsu and Hayatsu, 1993).

Human diet also affects microbial metabolism, which in turn influences levels of other chemicals present in the gut (Loke et al., 2020). Bacterial metabolites (SCFA, TMAO, protein and amino acid metabolites, lipids and lipid metabolites, bacterial cell wall components) in the colon can bind epithelial and immune cell surface and nuclear receptors to activate signaling pathways and alter gene expression, thus modulating the immune system, cell death, and proliferation. These bacterial metabolites can also induce epigenetic changes, including alterations in histone acetylation and DNA methylation patterns by inhibiting or activating certain host enzymes (Bhutia et al., 2017; Thomas and Denu, 2021). These activities in turn can influence cancer risk (Cox-York et al., 2019; Chattopadhyay et al., 2021; Salosensaari et al., 2021).

Human protein metabolism fuels the gut microbiota, which can result in local generation of cytotoxic and carcinogenic compounds (Ma et al., 2017). For example, amino acid catabolism in the gut produces sulfides, phenolic compounds, and amines, which cause inflammation or can serve as precursors to nitrosamine formation (Richardson et al., 2013). A potential role for TMAO (generated by the gut microbiota in response to dietary carnitine and choline) in colorectal carcinogenesis has also been proposed, as was discussed earlier. Other examples of microbial metabolites that have been associated with cancers include lactic acid (colon cancer), NOCs, estrogens and androgens, etc. (Cox-York et al., 2019).

Diet-microbiome interactions can sometimes be protective by modulating the effects of potentially carcinogenic compounds (Gasaly and Gotteland, 2022). Dietary polyphenols and other antioxidants, which are associated with reduced risks of colon cancer, can reduce ROS and protect against damage to DNA and lipids and suppress inflammation (Long et al., 2021). Dietary grape seed extract prevents oxidative stress and colonic DNA damage while also averting undesirable intestinal flora changes associated with the HAA carcinogen PhIP (Zhao et al., 2021).

**Mechanisms for How Meat and Gut Microbes May Alter Gastrointestinal Carcinogenesis Risk.** The examples presented in the previous sections provide several illustrations of how microbiota and diet...
components/metabolites together can influence risks of colorectal cancer, including those related to dietary meat consumption. What mechanisms are responsible for these effects, especially those with relevance to how meat consumption influences colorectal cancer risk?

Numerous studies suggest dietary consumption of meat is associated with perturbation of the gut microbiome relative to a vegetarian, vegan, or “nonmeat” diet, which may foster an inflammatory state and alter multiple signaling cascades that influence cancer development (Saha and Robertson, 2019; Appunni et al., 2021; Wang et al., 2023). Alteration of the gut microbiota plays a key role in the etiology of IBD, which is more prevalent in meat eaters (who also experience more disease relapses) (Ge et al., 2015; Tasson et al., 2017; Hartl and Sigal, 2020). Epithelial inflammation and immune dysregulation are hallmarks of IBD, which is a significant risk factor for colorectal cancer (Konstantinov, 2017). When the gut barrier is damaged, gut bacteria can trigger innate immune defenses (such as ROS) within colonic tissues, or potentially interact more directly and contribute to genotoxicity in the gut (Hartl and Sigal, 2020).

Various foods, including minimally processed meats (beef, pork, lamb, and hamburger) and processed meats (bacon, hot dogs, and other “processed meats”) are rich in sulfur-containing amino acids that are linked to an increase in sulfur-metabolizing bacteria in the gut (Nguyen et al., 2021a; Wang et al., 2021). Sulfite- and sulfate-reducing bacteria, plus organisms capable of breaking down organic sulfur-containing compounds (e.g., cysteine), produce hydrogen sulfide (H2S), which has been proposed to play a role in the pathogenesis of IBD and cancer (Carbonero et al., 2012). Hydrogen sulfide is genotoxic; it can impair the barrier that separates colonic epithelial cells from the microbial contents of the gut and promote colonic mucosal hyperproliferation (Carbonero et al., 2012; Nguyen et al., 2020). Dietary heme also promotes hydrogen sulfide release by the gut microbiota in mice; however, coadministration with antibiotics prevented the physiological effects observed with heme (Ijsennagger et al., 2015).

Other studies provide clues to potential mechanisms by which consumption of minimally processed meats or processed meats might affect the microbiome and alter carcinogenic risk. In rats, consumption of minimally processed beef versus processed (cured) beef altered the gut microbiota and markers of fermentation; both minimally processed and cured beef resulted in higher fecal acetaldehyde (a Group 1 carcinogen per IARC) levels relative to minimally processed and cured chicken (Na and Lee, 2017; Van Hecke et al., 2021). The gut microbiota can metabolize bile acids (which, as discussed before, are produced in response to a variety of foods, including processed meats but also fish consumption), which may make them more potent carcinogens (Hoyles and Swann, 2019; Trefflich et al., 2020). Enzymatic activity within the gut microbiome can promote endogenous N-nitrosation, leading to formation of NOC within the colon (Hullar et al., 2014). N-nitrosation does not occur in germ-free rats, however, demonstrating the importance of the microbiome in this response (Massey et al., 1988).

Conversely, some residents of the gut microbiome have protective effects against cancer by metabolizing and inactivating carcinogenic compounds (Hullar et al., 2014). For example, gut microbes have been shown to reduce the mutagenicity (and prevent the systemic exposure) of HAAs like MeIQx by converting them to their acrolein conjugates (Zhang et al., 2017; Zhang et al., 2019a).

These examples, by no means exhaustive, demonstrate how the interplay among dietary components (including red and processed meat products) and the gut microbiota may affect the risk for colorectal carcinogenesis and perhaps other cancers.

**Inflammation and infection**

As discussed earlier, specific microorganisms found in fresh and processed meats have been proposed to directly cause carcinogenesis. More generally, however, the human GI tract and other tissues can develop inflammation as a result of colonization by pathogens or commensal bacteria (Hullar et al., 2014; Sahan et al., 2018).

In the absence of such inflammation, the host immune system may be able to control or eliminate cells with carcinogenic potential. The immune system can be suppressed by some infections (American Cancer Society, 2016). Other conditions, such as metabolic syndrome and obesity, are associated with chronic inflammation and with certain cancers (You and Henneberg, 2018). Chronic inflammation in the intestine alters the intestinal microbiome and the accessibility of lumen contents to colonic epithelial cells and blocks antimicrobial activities (Greten and Grivennikov, 2019; Frigerio et al., 2021). Inflammatory conditions such as IBD can therefore influence the ability of carcinogens to initiate or promote cancers.

Higher (highest group vs. lowest group based on distribution of intakes; individual studies used quartiles or tertiles) intakes of red (not specified) and processed meats or processed meats might affect the microbiome and alter carcinogenic risk. In rats, consumption of minimally processed beef versus processed (cured) beef altered the gut microbiota and markers of fermentation; both minimally processed and cured beef resulted in higher fecal acetaldehyde (a Group 1 carcinogen per IARC) levels relative to minimally processed and cured chicken (Na and Lee, 2017; Van Hecke et al., 2021). The gut microbiota can metabolize bile acids (which, as discussed before, are produced in response to a variety of foods, including processed meats but also fish consumption), which may make them more potent carcinogens (Hoyles and Swann, 2019; Trefflich et al., 2020). Enzymatic activity within the gut microbiome can promote endogenous N-nitrosation, leading to formation of NOC within the colon (Hullar et al., 2014). N-nitrosation does not occur in germ-free rats, however, demonstrating the importance of the microbiome in this response (Massey et al., 1988).

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These examples, by no means exhaustive, demonstrate how the interplay among dietary components (including red and processed meat products) and the gut microbiota may affect the risk for colorectal carcinogenesis and perhaps other cancers.
meats (not specified) were associated with obesity in a meta-analysis of 18 studies (Rouhani et al., 2014). A separate meta-analysis found that consumption of processed meats was associated with greater risks of diabetes mellitus, with each additional serving/day associated with a 19% increase in risk (Micha et al., 2010), adding another dimension to how processed meat products might contribute to carcinogenesis.

**Natural human genetic variation**

Unlike mutations that lead to cancer or are associated with genetic diseases, genetic polymorphisms are inherited variations in DNA sequence that do not necessarily cause harm. Some of these variations alter expression of enzymes that activate or detoxify potential carcinogens or the ability to repair DNA damage or modulate cell death (Goldman and Shields, 2003). Sometimes these polymorphisms alter the risk of developing a cancer following exposure to an environmental agent. Knowing how certain polymorphisms that alter expression or activity of certain genes influence cancer risk to a particular carcinogen can provide important mechanistic clues. A few examples are given as follows:

- The human enzymes CYP1B1, CYP1A2, and N-acetyl-transferase (NAT) are believed to be involved in metabolic activation of HAAs, turning them into reactive molecules that damage DNA. Individuals with certain variants in these genes have a greater risk of certain cancers (Chang et al., 2003; Barnes et al., 2018; Le Marchand, 2021), a risk that might be influenced by meat consumption (Egeberg et al., 2008).
- The human XPC gene encodes an enzyme involved in recognizing DNA damage for excision and repair and is essential for the repair of damage caused by HAAs and PAHs (Steck et al., 2014). The XPC Lys939Gln polymorphism reduces the ability of the XPC gene product to participate in recognizing DNA damage. Homozygous carriers of the XPC Lys939Gln polymorphism have a 3.7-fold increase in colorectal cancer risk per 100 g of “red meat” (unspecified) intake/day (Hansen et al., 2007).

By identifying the genotype of individuals in epidemiological studies, it is sometimes possible to associate metabolic gene variants with a particular cancer (Joshi et al., 2012; Le Marchand, 2021). In addition, personalized nutrition based on an individual’s genotype may allow them to avoid hazards that are not problematic for the general population, just as individuals with food allergies can avoid allergens in their diet.

**Summary and Analysis**

**Summary of key mechanistic evidence**

Processed meat and poultry products are complex products that contain or are associated with factors with known or suspected carcinogenic potential. Each factor may promote cancer by a variety of mechanisms (Table 3). None of these factors are uniquely or universally associated with processed meat and poultry products, which are a diverse and inconsistently defined category of foods.

Among the proposed mechanisms (including those well-established and those that are emerging) by which processed meat products might cause cancer, many converge into several common and well-understood pathways (Figure 10 and 11). DNA damage (including oxidative, alkylation, etc.) leading to mutations in key genes is a well-known pathway that can lead to carcinogenesis. Damage to cells and tissue that triggers inflammation and compensatory cell proliferation can establish conditions that favor the growth of cells with mutations that potentially lead to carcinogenesis. Some carcinogenic mechanisms result in cells with specific mutations having growth advantages over normal cells, promoting cancer progression. Many mechanisms involve a complex interplay among diet and other individual factors, such as GI microbiota or host genetics.

**Processed meats vs. minimally processed meats and mechanism: What are the differences?**

Based on evidence in humans (but inadequate evidence in experimental animals), along with strong mechanistic evidence, IARC concluded that processed meats (including poultry-containing products but not fish) cause cancer of the colorectum and noted positive associations between consumption of processed meat and stomach cancer. For the consumption of minimally processed beef, pork, and lamb, IARC concluded that there is limited evidence in humans for carcinogenicity. Minimally processed poultry and fish were not included in their evaluation (International Agency for Meat and Muscle Biology 2023, 7(1): 15762, 1–63 Bedale et al. Cancer Development from Processed Meats).
Research on Cancer, 2018) but are frequently reported to have no or lower carcinogenic risks (Fernandez et al., 1999; Primeu, 2018). Are there differences between processed meats and minimally processed meats that might account for these apparent different carcinogenic potentials?

Neither this review nor others (Mejborn et al., 2016; Turesky, 2018) have identified a "smoking gun" that explain a possible difference in carcinogenic risk between processed meats and minimally processed meats. Some processed meats use nitrite or nitrate in their formulations, but many do not. Heme is found at higher levels in minimally processed beef, pork, and lamb (and processed meats made from this meat) than in most (but not all) poultry and fish. HAAs and PAHs can be found in some processed meats but are also present at comparable levels in some heat-treated minimally processed meats. Some but not all studies found that protein oxidation products were at greater levels in processed meats than minimally processed meats; more work in this area is needed (Goethals et al., 2020; Domínguez et al., 2022).

Perhaps consumption of processed meats is associated with other underexplored or unknown factors or variables that are important in carcinogenesis. For example, the difficulty in studying short-lived reactive nitrosamides makes it currently impossible to assess whether or not they are a risk (Mejborn et al., 2016). Rather than the presence of a carcinogenic factor in processed meat, perhaps we should consider whether the removal or lack of certain putative protective factors (e.g., antioxidants, vitamins, minerals, or CLA) deserves more attention when assessing carcinogenic potential (Ames, 2009). Perhaps supporting this idea, a recent clinical study found that consumption of processed meat products (∼300 g/day for 2 wk) with added natural bioactive compounds resulted in significantly reduced excretion of apparent total NOCs compared with excretion levels in those consuming processed meat products without natural bioactive compounds (van Breda et al., 2021). Another clinical study found that the relationship between heme iron and colorectal adenoma risk was influenced by the total dietary antioxidant capacity (Bastide et al., 2016). Perhaps those individuals who eat more processed meat also eat fewer vegetables that might prevent certain types of cancer (Santarelli et al., 2008).

Cancer risks associated with minimally processed and processed meats are likely due to complex combinations of chemicals together with differences among overall diets, gut microbiomes, genetic variations, disease states, and medications. Risks associated with

### Table 3. Components of processed meat products and potential mechanisms by which they may be carcinogenic

<table>
<thead>
<tr>
<th>Factor</th>
<th>DNA alkylation</th>
<th>Oxidative and other DNA damage</th>
<th>Formation of NOCs</th>
<th>Lipid oxidation</th>
<th>Damage to cells, inflammation, proliferation</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme</td>
<td>X X</td>
<td>X X</td>
<td>X</td>
<td>X X</td>
<td>X X</td>
<td>Changes to microbiome</td>
</tr>
<tr>
<td>Free iron</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Feeds cancer cells</td>
</tr>
<tr>
<td>Lipid oxidation products</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other fat-related factors</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Hormonal effects, promote cancer cell growth, stimulate bile acid production</td>
</tr>
<tr>
<td>N-glycolyneuraminic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X X</td>
<td>-</td>
</tr>
<tr>
<td>Infectious agents</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X X</td>
<td>-</td>
</tr>
<tr>
<td>N-nitrosamines</td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrite alone</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>But reduces lipid oxidation</td>
</tr>
<tr>
<td>Heterocyclic aromatic amines</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X X</td>
<td>-</td>
</tr>
<tr>
<td>Polycyclic aromatic amines</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Epigenetic effects</td>
</tr>
<tr>
<td>Advanced glycation end products</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>X X</td>
<td>-</td>
</tr>
<tr>
<td>Bile acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X X</td>
<td>-</td>
</tr>
<tr>
<td>miRNA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Epigenetic effects</td>
</tr>
</tbody>
</table>

miRNA = microRNA molecules; NOCs = N-nitrosamine compounds.
these various differences are not yet completely understood, nor are they fully captured in epidemiological studies. It is difficult to assess how much the carcinogenic risk differs between the consumption of processed meats versus minimally processed meats, although a difference in risk between the two could provide mechanistic clues. Studies conducted to date may not have provided sufficient information to discern differences between consumption of different categories of products. Because processed meats as a category include many products that differ substantially in composition and manner of processing, the carcinogenic risks attributed to this broad category may be due to a small subset of processed meat products.

**Other considerations**

Processed meats, driven in part by consumer preferences, have changed and continue to evolve (Table 4).

As food production methods change, the associations (and actual risks) between processed meats and carcinogenesis can be affected. Because carcinogenesis is a process that can take years or decades to occur, such
Emerging carcinogenic mechanisms

Figure 11. Less well-established pathways to carcinogenesis from consumption of processed meat products. The top row of tan boxes includes components of processed meats. Gray boxes indicate factors that have been postulated to be involved in or that modulate carcinogenesis. Dark brown boxes indicate processes leading to carcinogenesis, with blue boxes indicating the 2 main mechanisms that lead to carcinogenesis. Green and red text indicates facilitators or inhibitors, respectively, of processes. Note that this simplified diagram does not include all possible pathways.

Table 4. Consumer preferences and related changes made to processed meat production

<table>
<thead>
<tr>
<th>Consumer demand</th>
<th>Changes to processed meat and poultry production</th>
<th>Potential impact on factors affecting carcinogenicity potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional content of products</td>
<td>Animal diet modification to improve fatty acid profile of meat</td>
<td>Potential for changes in lipid oxidation</td>
</tr>
<tr>
<td>More sustainable and animal welfare–friendly food production</td>
<td>More pasture-raised meat vs. feedlot</td>
<td>Potential for changes in lipid oxidation</td>
</tr>
<tr>
<td></td>
<td>Cultured meat, plant-based meat substitutes</td>
<td>Unknown; use of growth factors in cell culture or leghemoglobin in plant products may be a concern*</td>
</tr>
<tr>
<td>Decreased levels of nitrite and other preservatives</td>
<td>Substitution of synthetic preservatives with natural preservatives</td>
<td>Potential decrease in N-nitrosamines, potential for increased antioxidant levels in products because of natural preservatives</td>
</tr>
<tr>
<td>Reduced sodium</td>
<td>Reduced sodium chloride use, potential substitution with other salts</td>
<td>Potential changes to PAH and other process-induced contaminant levels</td>
</tr>
<tr>
<td>Reduced fat</td>
<td>Changes in animal husbandry, methods of food processing</td>
<td>Reduced potential for lipid oxidation or PAH formation</td>
</tr>
<tr>
<td>Fresh foods</td>
<td>Less frozen and canned food, modified atmosphere packaging</td>
<td>A shorter shelf life and different storage conditions might alter the potential for lipid oxidation</td>
</tr>
<tr>
<td>Safe food</td>
<td>Changes in cooking methods and controls</td>
<td>Reduced levels of HAAs and PAHs</td>
</tr>
</tbody>
</table>

*Choudhury et al. (2020); Chriki and Hocquette (2020).

HAA = heterocyclic aromatic amine; PAH = polycyclic aromatic hydrocarbon.
effects are not likely to be seen for many years and may complicate nutritional epidemiology studies.

**Strengths, weaknesses, and gaps**

A large body of data from human, animal, and in vitro studies provide significant insights into the individual components of meat and poultry products and their potential mechanisms for carcinogenesis. These data have already been used to improve the safety of foods. For example, reductions in nitrate levels and addition of ascorbate greatly reduce the potential for nitrosamine formation in cooked bacon. Elimination of arsenic-based feed supplements in China is estimated to reduce over 1000 cancers each year in that country (Hu et al., 2019). These and other “low-hanging fruit” have resulted in significant health benefits in terms of cancer prevention.

Most mechanistic studies have focused on single components of processed meats. However, foods are complex and contain many chemicals that may influence the metabolism and possible carcinogenicity of carcinogens. Individual differences in diet, genetics, environmental exposures, and microbial makeup also play significant roles that need more exploration.

However, the relative cancer risks of these agents in processed meat versus other sources (endogenous production, environment, other dietary factors) is not always easy to determine.

Many other questions remain. Heat-treated poultry and fish contain components associated with cancer (PAH or HAAs), and fish is generally more prone to lipid oxidation than “red meat” (not specified) although the susceptibility to lipid oxidation varies widely depending on the type of fish (Wu et al., 2022a), yet neither poultry nor fish consumption is associated with an increased cancer risk. Why is this? Are endogenous agents within fish, for example, better able to inhibit carcinogenesis in spite of more pronounced lipid oxidation that occurs during storage of the post-mortem muscle or that which occurs during GI digestion? How much of the risk comes from meat itself or from processing and cooking methods, etc. (Gibis, 2016)?

Knowledge gaps exist, and new ideas are emerging that should be explored. Although we possess significant understanding of the carcinogenic mechanisms of some categories of agents (N-nitrosamines, HAAs, PAHs, lipid oxidation products, heme), individual members within each category vary in their levels depending on processing methods and in their ability to promote carcinogenesis. Yet all members of a category are often lumped (and measured) together, making it difficult to discern the impact of individual members within categories. Doing so, although technically challenging, may improve our ability to reconcile conflicting data. In addition, the relative cancer risks of these agents in processed meat versus other sources (endogenous production, environment, other dietary factors) is not always easy to determine.

Other proposed carcinogens in processed meat and poultry products are less well characterized in terms of carcinogenic potential and/or mechanism of action. Do AGEs, implicated in many metabolic disorders, play a significant role in cancers associated with processed meats? How do other dietary components (for example, polyphenols) prevent or otherwise alter risks from these agents? What are the potential roles for protein oxidation products, Neu5Gc, infectious agents, or miRNA in processed meat–associated carcinogenesis? What roles do endogenous agents such as bile acids, N-nitrosamines, and miRNA play? The role of the gut microbiome in influencing cancer risks from processed meats is also expected to be a significant area of research. At a more basic level, how do epigenetic effects modulate carcinogenesis?

Nutritional epidemiology studies can be improved as well. More consistent definition of processed meat and poultry is needed to better estimate intake across studies, and there is movement in that direction (O’Connor et al., 2022). The use of better biomarkers may improve the ability to estimate exposure to potential carcinogens, such as HAAs. Validation of fecal water testing as predictive of future tumors would facilitate prospective nutritional studies. Single-nucleotide polymorphism data will help define mechanistic pathways involved in carcinogenesis. The use of Mendelian randomization to naturally “assign” individuals to nutritional cohorts from birth may be useful despite its limitations (Merino and Tobias, 2022). How does environmental, dietary, or medicinal exposure to other chemicals induce or inhibit metabolic enzymes involved in activating or eliminating carcinogens? There is a need for more holistic approaches to study the interplay among various risk factors, but this will be a difficult and complicated undertaking.

**Conclusions**

Processed meat and poultry products are a heterogeneous group of complex foods that contain many components that have been suggested to have carcinogenic potential. Some agents within processed meat and
poultry products have demonstrated carcinogenicity in animal studies, others have been linked through nutritional epidemiological studies, and the effects of still others have been inferred from their genotoxicity, mutagenicity, cytotoxicity, and other activities.

Many pathways can lead to cancer, and many components of processed meats can conceivably play a role in these mechanisms. DNA damage can cause mutations with the potential for carcinogenic initiation, and substantial evidence indicates process-induced contaminants (NOCs, HAAs, and polycyclic aromatic amines) of processed meat products can lead to such damage. Inflammation or cell damage caused by heme, AGEs, lipids, or other agents such as the sialic acid Neu5Gc can result in the production of reactive species capable of damaging DNA directly or through the formation of compounds with DNA-damaging activities such as lipid oxidation products. Inflammation also creates an environment that can favor the growth of cells harboring certain mutations, and this growth advantage may lead to tumors.

Although mechanisms for individual components of processed meat and poultry products have been studied, it is more difficult to evaluate entire food products, especially in the context of a vast and diverse diet. Knowledge and methodological gaps remain. Factors unique to individuals, including their gut microbiota and genetic and epigenetic makeup, likely influence the impact of processed meat on carcinogenesis.

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