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# Perspectives on the Chemical Etiology of Breast Cancer

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Multiple factors, known and unknown, contribute to human breast cancer. Hereditary, hormonal, and reproductive factors are associated with risk of breast cancer. Environmental agents, including chemical carcinogens, are modifiable risk factors to which over 70% of breast cancers have been attributed. Polymorphisms of drug-metabolizing enzymes may influence risk of breast cancer from environmental chemicals, dietary agents, and endogenous steroids. The environmental factors discussed in this review include pollutants, occupational exposures, tobacco smoke, alcohol, and diet. Aromatic amines are discussed as potential mammary carcinogens, with a focus on heterocyclic amine food pyrolysis products. These compounds are excreted into the urine after consumption of meals containing cooked meats and have recently been detected in the breast milk of lactating women. **Key words:** heterocyclic amines, mammary carcinogens, PhIP, risk factors. *Environ Health Perspect* 110(suppl 1):119–128 (2002).

<http://ehpnet1.niehs.nih.gov/docs/2002/suppl-1/119-128debruin/abstract.html>

The possible role of avoidable chemical exposures in human breast cancer attracts much public attention and has significant public policy ramifications. Breast cancer is the most commonly diagnosed malignancy in women living in Canada, the United States, and northern Europe. A generally steady increase in incidence has been recorded since the 1940s. It is now estimated that one in nine women living in Canada will develop this disease within their lifetime. Although the mortality rate of breast cancer has declined because of earlier detection and improved therapy, the loss of lives remains second only to lung cancer among malignancies in women. In Canada, it is estimated that this year over 19,200 new cases will be diagnosed and more than 5,500 women will die of breast cancer (1).

The etiology of human breast cancer remains largely unknown. Risk factors associated with breast cancer can be grouped into three broad determinants: family history (hereditary) factors, hormonal and reproductive factors, and environmental (including lifestyle) factors. A recent epidemiologic analysis concluded that 73% of breast cancers are attributable to environmental factors (2). Over 78% of breast cancer cases occur in postmenopausal women. Late onset is consistent with the long latency periods typically associated with chemical carcinogenesis in humans.

In this review, we will discuss recent research relevant to the possible environmental etiology of human breast cancer. First, we consider genetic and hormonal factors that influence breast cancer biology. Many of these factors may also interact with environmental exposures, for example, by affecting the metabolism of environmental chemicals. Subsequently, we consider several specific

classes of environmental chemicals that may act to initiate human breast cancer. A particular focus of the review is the possible role of environmental chemical mutagens. This emphasis certainly reflects the authors' own interests, but we believe that the focus is consistent with the renewed scientific attention to this long-standing question. An international scientific meeting on the topic of breast cancer and environmental mutagens was held under the auspices of the Environmental Mutagen Society, United States, and with sponsorship by the National Institute of Environmental Health Sciences, in September 2001 (Research Triangle Park, North Carolina). The invited papers presented at that meeting will appear in a forthcoming issue of *Environmental and Molecular Mutagenesis* (2002), and the interested reader is directed to that publication for much additional information.

## Hormonal and Genetic Factors in Breast Cancer

Estradiol binds with high affinity to estrogen receptor alpha. This binding induces DNA synthesis, cell division, and production of growth factors and progesterone receptor proteins. Estrogens and progesterone are essential for normal mammary gland development and function, but their stimulation of breast cell proliferation may be procarcinogenic. Many of the identified risk factors for breast cancer can be explained by their effects on lifetime exposure to estrogen and other hormones (3). These effects include both risk factors (increased exposure: early menarche, nulliparity, late menopause, oral contraceptive use, hormone replacement therapy) and protective factors (decreased exposure: full-term pregnancy at young age, breast-feeding). Estrogens and progesterone

interact with specific receptor proteins in the cell nucleus. Furthermore, estrogens can be metabolically activated to cytotoxic and genotoxic products (4).

Breast cancers develop through a series of premalignant stages to invasive cancer accompanied by multiple steps of oncogene activation and tumor suppressor gene inactivation (5). The *HER-2/neu (c-erbB-2)* protooncogene is a member of the epidermal growth factor receptor family. Overexpression of its gene product, a transmembrane glycoprotein with tyrosine kinase activity, has been detected in 10–40% of human breast tumors (6). This overexpression has been associated with ineffective tamoxifen therapy (7). The *BCL-2* and related gene families regulate tissue development and homeostasis. Two of their protooncogene products, *bcl-2* and *bax*, have been identified as apoptosis-related markers. In patients with breast cancer *bcl-2* overexpression has been associated with a better prognosis, whereas decreased *bax* expression has been linked to poor clinical outcome (8,9).

The tumor suppressor gene *p53* is recognized as the most common somatically mutated gene in human cancers (10). *p53* protein participates in regulation of the cell cycle, DNA repair, apoptosis, and other critical functions. It is estimated that *p53* mutations occur in 40% of sporadic breast tumors (11). The *p53* mutational spectrum of breast cancers resembles that in lung cancer, suggesting that mutagenic xenobiotics may be responsible (11,12). The presence of certain *p53* mutations can indicate poor response to systemic therapy, adverse prognosis for recurrence, and higher mortality in patients with breast cancer (13,14). Other tumor suppressor gene products implicated in human breast cancer include *Rb*, cyclin-dependent kinase inhibitors, and DNA-mismatch repair proteins (15).

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We thank the agencies that have supported our research: Natural Sciences and Engineering Research Council of Canada; Breast Cancer Society of Canada; Canadian Breast Cancer Foundation; Canadian Institutes of Health Research; and Toxic Substances Research Initiative (Health Canada).

Received 7 September 2001; accepted 20 November 2001.

Hereditary factors are associated primarily with early-onset premenopausal breast cancer. During the 1990s genetic analysis of breast cancer-prone families identified the *BRCA1* and *BRCA2* genes. Women with mutations in either *BRCA1* or *BRCA2* (over 250 mutations have been identified) are at a significantly elevated lifetime risk (55–85% compared with 12% for the general population) for developing breast or ovarian cancer (16). Approximately 5–10% of breast cancer cases can be attributed to germline mutations in *BRCA1* or *BRCA2*. Recent evidence suggests that *BRCA1* and *BRCA2* may have important but distinct roles in sporadic breast cancer (17,18). *BRCA1* and *BRCA2* encode multifunctional protein products implicated in transcriptional regulation, cell cycle control, apoptosis, and DNA-repair pathways (19). The detection of the protein products of the *BRCA1* and *BRCA2* genes in milk fat globules also suggests a possible role in lactation (20).

### Polymorphisms of Drug-Metabolizing Enzymes That May Influence the Risk of Breast Cancer

Polymorphisms have been identified in the genes encoding many enzymes involved in the bioactivation and detoxication of environmental chemicals, dietary agents, and endogenous steroids. A mutation may give rise to a bioactivating enzyme with increased activity or to a detoxifying enzyme with decreased activity; either scenario could increase susceptibility to mammary carcinogenesis. Most of the major families of drug-metabolizing enzymes have been examined in this regard, including cytochrome P450 (CYP), glutathione *S*-transferase (GST), arylamine *N*-acetyltransferase (NAT), catechol-*O*-methyltransferase (COMT), sulfotransferase (SULT), and uridine diphosphate-glucuronyltransferase (UGT). These results will now be discussed briefly.

CYP enzymes catalyze reactions involved in the oxidative metabolism of xenobiotics and steroids. Many classes of chemical carcinogens are bioactivated to DNA-reactive species by P450 enzymes. Potential mammary carcinogens such as polycyclic aromatic hydrocarbons (PAHs) and aromatic amines are activated to reactive species by P450 1A1. Several CYP1A1 variants have been studied. For example, the *MspI* polymorphism may be a significant risk factor for breast cancer in Chinese women in Taiwan (21). However, in a case-control study nested within the Nurses' Health Study, no increase was associated with variant CYP1A1 genotypes (22). CYP1A1 expression is highly variable, independent of the

genetic polymorphism: over a 400-fold difference was noted in 58 samples of non-tumor breast tissue (23).

P450 1B1 bioactivates carcinogens and hydroxylates 17 $\beta$ -estradiol at the C-4 position, forming genotoxic 4-hydroxyestradiol (24). Identified polymorphisms of human CYP1B1 include codon 119 Ala $\rightarrow$ Ser and codon 432 Val $\rightarrow$ Leu. Epidemiologic associations of these variants with breast cancer are still controversial (25),(26).

GST enzymes, which inactivate reactive electrophilic intermediates by catalyzing glutathione conjugation, are important enzymes in the detoxication of chemical carcinogens. In humans, the GST gene superfamily is divided into at least four classes: alpha (GSTA), mu (GSTM), pi (GSTP), and theta (GSTT). Only about half the population carries the *GSTM1* gene (27). The *GSTM1* null genotype has been associated with increased risk of breast cancer in a South Korean study population (28) but not in an Australian Caucasian population (29). Meta-analysis of 46 studies found a slight increase in risk of breast cancer among postmenopausal women with the GSTP1 Ile $\rightarrow$ Val polymorphism and the *GSTM1* deletion (30).

COMT catalyzes the methylation of endogenous catechol compounds such as biogenic amines (dopamine) and catechol estrogens (2- and 4-hydroxyestrogens). Variations in COMT activity will affect the bioavailability of endogenous estrogens. The low-activity *COMT* allele may contribute to both the risk (31) and progression (32) of breast cancer.

*N*-Acetyltransferases (encoded by the human *NAT1* and *NAT2* genes) are involved in both the bioactivation and the detoxication of aromatic amines (33). These enzymes possess three activities: *N*-acetylation, *O*-acetylation, and *N,O*-acetyltransfer activity. The *O*-acetylation and *N,O*-acetyltransfer activities can lead to formation of unstable acetoxy compounds that decompose to DNA-reactive nitrenium ions. The classic *NAT2* polymorphism accounts for slow versus fast acetylation of drugs such as isoniazid. About half of individuals in Caucasian populations are slow acetylators; Inuit and Oriental populations are predominantly fast acetylators (up to 90%). The controversial question of *NAT1* and *NAT2* associations with breast cancer will be discussed later in this review.

SULT and UGT catalyze conjugation of endogenous compounds and xenobiotics to form sulfates and glucuronides, respectively. Human mammary tissue expresses SULT (34) and UGT (35) activities, and the roles of polymorphisms of the genes encoding these enzymes in breast cancer are now under investigation (35,36).

### Demographics of Breast Cancer

Studies of migrant populations and geographic variations in incidence of breast cancer suggest that lifestyle and environmental influences are involved in the etiology of the disease. The rates of breast cancer in Asian and Mediterranean countries are significantly lower than in North America (37), yet, within several generations, the rate of breast cancer in female offspring of Asian immigrants to the United States approaches the American rate (38). Recent studies indicate that the rate of breast cancer in Japan is increasing, coincident with the westernization of the Japanese lifestyle (39). Generally, the incidence of breast cancer in American Indian and Alaskan Native women has been lower than in most of the other racial/ethnic groups in the United States. Migration of these Native American families has led to an increase in the breast cancer rates. An elevated incidence of breast cancer has been noted in the U.S. northeast, especially the New York–New Jersey–Pennsylvania area (40), which is among the most heavily polluted regions on the continent in terms of industrial and vehicular emissions. An increased risk of breast cancer was also reported for postmenopausal women who lived for more than 10 years near an industrial facility in Long Island, New York (41).

### Cigarette Smoking and Breast Cancer

Tobacco smoke is the major causative agent in the development of lung cancer (42). In contrast, the possible role of smoking in breast cancer has remained controversial. A large number of epidemiologic studies were conducted during the 1980s and early 1990s. Several found a weak association of smoking with breast cancer, many found no difference in risk, and a few even noted reduced risk. Reduced risk might be accounted for on the basis of an antiestrogenic effect of smoking, lowering the age of menopause and total exposure to estrogen. In these early studies, analyses were generally conducted with respect to never-smoker and ever-smoker categories.

During the last 10 years, the effect of environmental tobacco smoke (ETS) (also referred to as secondhand smoke or sidestream smoke) on human health has become an important public health issue. Some recent epidemiologic studies have divided never smokers into unexposed nonsmokers and passive smokers (individuals exposed to ETS). A groundbreaking Swiss study carefully examined the relationship between tobacco smoke exposure and breast cancer (43). In the population-based case-control study, women were categorized as active

smokers, passive smokers, or unexposed nonsmokers. An active smoker was defined as a woman who had smoked at least 100 cigarettes in her lifetime, whereas a passive smoker was any woman exposed to passive smoke for at least 1 hr per day for at least 12 consecutive months during her lifetime. Among ex-active and current active smokers, an increase in the risk of breast cancer was associated with the number of cigarettes smoked per day. The odds ratio (OR) of breast cancer in current active smokers ranged from 1.5 (95% [confidence interval] CI 0.6–3.9) for women who smoked 1–9 cigarettes per day and 2.1 (95% CI 0.9–4.8) for women who smoked 10–19 cigarettes per day, to 5.1 (95% CI 2.1–12.6) for women who smoked more than 20 cigarettes per day. The OR of breast cancer in passive smokers was 3.2 (95% CI 1.7–5.9). It was estimated that exposure to passive smoke for 2 hr per day for 25 years was equivalent to actively smoking 20 cigarettes per day for 20 years. A similar contribution of ETS to disease development has also been observed in heart disease (44).

In a recent Canadian study, data from 2,317 cases of breast cancer (25% premenopausal and 75% postmenopausal women) and 2,438 population controls were analyzed for risk of breast cancer with passive and active (current and ex-) smoking (45). When the data were analyzed without controlling for passive smoke exposure, with never smokers as the referent group, the OR values for breast cancer were 1.0 (95% CI 0.8–1.3) and 1.2 (95% CI 1.0–1.4) for ex-smokers/current smokers in premenopausal and postmenopausal women, respectively. When the referent group included only women who never actively smoked, never lived with a regular smoker for at least 1 year, and never worked for at least 1 year with regular smokers, the results were very significant. For the same cohort of premenopausal women, the OR values were 2.3 (95% CI 1.2–4.6) for passive smokers, 2.6 (95% CI 1.3–5.3) for ex-smokers and 1.9 (95% CI 0.9–3.8) for active smokers. For postmenopausal women, the effects of ETS were also observed; the OR values were 1.2 (95% CI 0.8–1.8) for passive smokers, 1.4 (95% CI 0.9–2.1) for ex-smokers, and 1.6 (95% CI 1.0–2.5) for active smokers.

The gene–environment interaction for tobacco smoke has also been investigated. In 1996 it was reported that postmenopausal women who were slow acetylators and smoked have a significantly elevated risk of breast cancer (OR up to 4.4) (46). This result could not be confirmed in a nested case–control study in the Nurses' Health Study (47). On the other hand, Millikan et al. (48) reported that postmenopausal

women who smoked within the past 3 years and possessed the rapid NAT2 genotype (OR = 7.4; 95% CI 1.6–32.6) had a greater risk of breast cancer than women with the slow NAT2 genotype (OR = 2.8; 95% CI 0.4–8.0). Morabia and colleagues (49) investigated the role of active and passive smoking, this time including the interaction of the NAT2 genotype. The increased risk of breast cancer was observed only in postmenopausal women, not premenopausal women. Fast acetylators who passively smoked had a greater risk (OR = 11.6; 95% CI 2.2–62.2) than fast acetylators who actively smoked (OR = 8.2; 95% CI 1.4–46.0). The effect of smoking was observed with slow acetylators, but the association was weaker: passive smokers (OR = 1.1; 95% CI 0.3–4.3), active smokers (OR = 2.9; 95% CI 0.8–11.2). The previously mentioned nested case–control study in the Nurses' Health Study also analyzed two genetic variants of CYP1A1 (22). A modest increase of risk was observed among women who started smoking before the age of 18 and possessed the CYP1A1 *MspI* variant (T'C transition at nucleotide 6235) or another CYP1A1 variant, the exon 7 polymorphism (A'G transition at nucleotide 4889). No more than a small percentage (<5%) of all cases of breast cancer can be attributed to these two risk factors.

### Breast Cancer and Lifestyle Factors Other Than Smoking; Dietary Factors

The relationship of alcohol consumption to risk of breast cancer has been studied for many decades (50,51). Low consumption levels (1–3 drinks per week) were not found to increase risk of breast cancer (52). The only significant increase of relative risk (1.41–1.70) compared with nondrinkers is associated with high levels of intake, at least 60 g/day (approximately 2–5 drinks) (53,54). Alcohol consumption may increase levels of plasma estrogen and insulinlike growth factors (55,56). An increased risk of breast cancer was observed recently in postmenopausal women who consumed alcoholic beverages and had low folate intake (57,58).

Dietary fat has often been examined as a risk factor for breast cancer (59). Although the question remains controversial, the consensus indicates that there is not a strong association between fat intake during adulthood and risk of breast cancer. The roles of dietary fat intake during childhood and adolescence, and of different types of dietary fatty acids, remain unclear.

Meat consumption is a major source of dietary fat. In addition, genotoxic substances are formed during the cooking or processing of meat. A case–control study in Uruguay in

1996 found an increased risk of breast cancer with intake of red meat (60). A later study found a positive correlation between consumption of well-done meat and risk of breast cancer (61). Two classes of carcinogenic compounds can be formed in the grilling of meats: PAHs and heterocyclic aromatic amines (62). PAHs are formed from the pyrolysis of fats during charcoal grilling and are deposited on the meat by smoke from the fat dripping onto the coals of the grill. The presence of PAH–DNA adducts in human breast tissue (63) is consistent with involvement of these compounds in breast cancer, but epidemiologic evidence in this regard is very limited. Mutagenic heterocyclic aromatic amines (discussed in more detail later in this chapter) are formed by various high-temperature cooking methods, including broiling, frying, roasting, and barbecuing.

Diets with five or more daily servings of fruits and vegetables may be protective against breast cancer (64), but a later study did not support a protective role for dietary fruits and vegetables in adulthood (65). An inverse association between dietary folate intake and breast cancer risk was observed in the Shanghai Breast Cancer Study (66). Consumption of fruits and vegetables rich in  $\alpha$ -carotene,  $\beta$ -carotene, vitamin A, vitamin C, and lutein/zeaxanthin is a protective factor reducing risk of breast cancer in premenopausal women, including women with family history of breast cancer (67). In an earlier study, another vitamin,  $\alpha$ -tocopherol, was suggested to be a protective factor for premenopausal women with family histories of breast cancer (68).

### Occupation and Risk of Breast Cancer

The participation of women in the work force has increased steadily since the 1950s. Research on occupational exposure to carcinogens has focused predominantly on the male worker. Only recently has there been special interest in occupational exposure to hazardous agents as a factor in the risk of breast cancer in women (69,70). Women exposed to benzidine or  $\beta$ -naphthylamine while employed in a dye factory in Moscow had approximately double the risk of breast cancer (71). Labreche and Goldberg (72) have examined occupational exposure to organic solvents. The solvents benzene, 1,1-dichloroethane, 1,2-dichloroethane, 1,2-dichloropropane, and dichloromethane are rodent mammary carcinogens and may be encountered in the petrochemical, dry cleaning, shoe manufacturing, and chemical industries. There is evidence for a positive association of several occupations with risk of breast cancer, including nurses, teachers, laboratory technicians, dental hygienists,



cosmetologists, and aircraft and automotive workers (73–75). The high incidence among professional workers has been partly attributed to reproductive history, including nulliparity and full-term pregnancy at a later age (76).

Estrogenic chemicals represent another possible occupational chemical hazard (77,78). Occupations and potential exposures include nursing and medicine (nonylphenol, bisphenol A, butyl benzyl phthalate); food handlers (butylated hydroxyanisole); electronic industry (bisphenol A); gasoline service station workers; assembly workers; and machine operators (4-octylphenol). Potential exposure to polychlorinated biphenyls (PCBs) was limited to assembly jobs in the electrical equipment industry and clinical/laboratory personnel. Despite these potential exposures, there is little direct evidence that occupational exposures to xenoestrogens are significant hazards.

A recent Danish study found an elevated risk of breast cancer in males with occupational exposure to gasoline and vehicular exhaust (79). This elevated risk increased to an OR of 5.4 in men who were younger than 40 years when first employed in a trade with this type of exposure. The authors proposed that benzene, 1,3-butadiene, 1,2-dibromoethane, and 1,2-dichloroethane, which are found in gasoline, as well as PAHs in the combustion residue may be involved in the etiology, as they are rodent mammary carcinogens.

## Breast Cancer and Organochlorine Pollutants

Causal links between breast cancer and specific environmental pollutants have frequently been proposed (80–83) but remain very controversial. Organochlorine pesticides and related compounds that have been implicated in breast cancer include DDT (bis(4-chlorophenyl)-1,1,1-trichloroethane) and its major metabolite DDE (bis(4-chlorophenyl)-1,1-dichloroethane; chlordane; dieldrin; benzene hexachloride (including  $\gamma$ -lindane); and PCBs. These organic chemicals have long biologic half-lives (up to 12 years) (84) and bioaccumulate in adipose tissue, including the breast. DDT has been widely used as an insecticide throughout the world but has been banned in the United States since 1972. Several preliminary case-control studies found elevated levels of DDT and DDE in breast lipid or blood serum from breast cancer patients (85–88), and these reports heightened public suspicion of a relationship between pesticide exposure and incidence of breast cancer. However, recent reports (including those from the authors of the original studies) have concluded that exposure to DDT or DDE during adulthood is not associated with an increased risk of breast cancer (89–98). Research into the relationship between pesticides and risk of breast cancer has recently been reviewed in these pages (99).

The xenoestrogen hypothesis proposes that environmental pollutants that possess

estrogenic activity stimulate breast cell proliferation and thereby induce or promote breast cancer (100,101). Environmental estrogen mimics are also implicated as endocrine disruptors in wildlife species. Although there is experimental evidence on the estrogenicity of several chlorinated pesticides (including DDT), as mentioned above, recent epidemiologic studies find no increased risk of breast cancer with pesticide exposure (102–104). Furthermore, in view of the presence of natural hormone and antihormone mimics in our diet, Safe and Zacharewski (105) concluded that the estrogenic contribution of organochlorine compounds is small and their role in development of breast cancer is questionable. The role of other xenoestrogens, such as alkylphenols and phthalates, which are used in the synthesis of detergents, plastics, and polymers, is still under investigation (106). The xenoestrogen hypothesis has been extensively debated in other fora and we will not extend the discussion in this review.

## Aromatic Amines as Mammary Carcinogens

Many aromatic amines are of toxicologic concern to humans (Figure 1). Occupational exposure began with the dye industry in the nineteenth century and continues today in the plastic and chemical industries. The general population can be exposed to various aromatic amines through environmental pollution, tobacco smoke, medicine, and diet. The chemical class of aromatic amines can be subdivided into monocyclic, polycyclic, and heterocyclic amines. Most heterocyclic amines, many polycyclic aromatic amines, and some monocyclic aromatic amines are bacterial mutagens. Several polycyclic aromatic amines, such as benzidine, 4-aminobiphenyl, and  $\beta$ -naphthylamine, have been classified by the International Agency for Research on Cancer as known human carcinogens. Epidemiologic studies have suggested that monocyclic aromatic amines such as *o*-toluidine (2-methylaniline) and 4-chloro-*o*-toluidine are also human carcinogens (107,108). Comprehensive animal bioassays have determined that many aromatic amines are rodent carcinogens producing tumors at various sites including the mammary gland (109). We have detected environmental aromatic amines, including *o*-toluidine, a rat mammary carcinogen, in human milk samples from smoking and nonsmoking mothers (110). This indicates that the ductal epithelial cells of the breast are exposed to aromatic amines. We and others have continued to investigate the etiologic role of aromatic amines in breast cancer and have focused on the dietary heterocyclic amines.

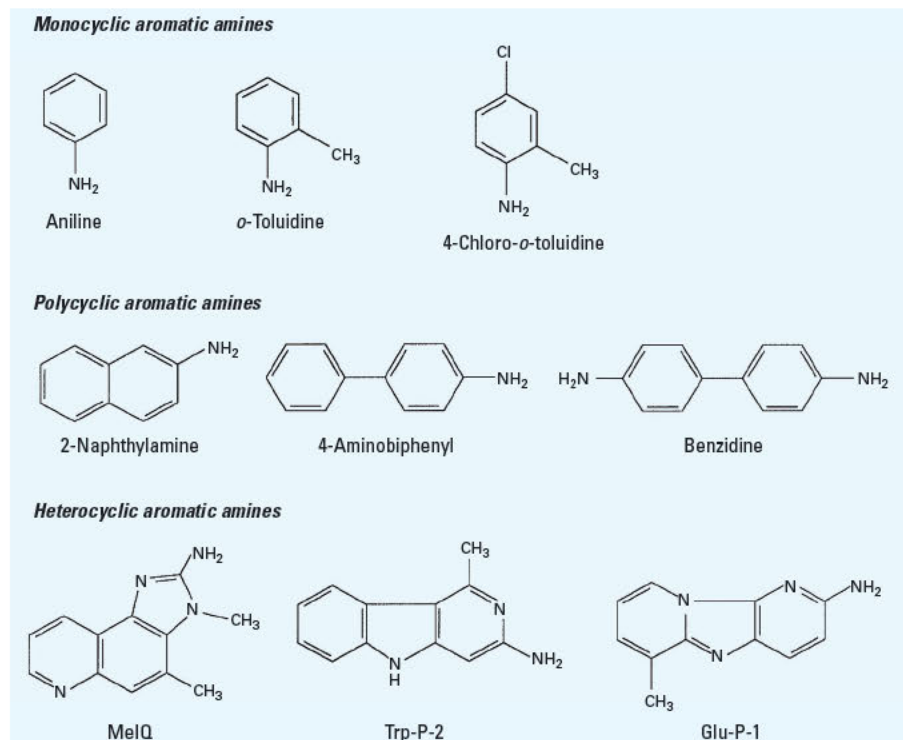


Figure 1. Structures of aromatic and heterocyclic amines.

## Heterocyclic Amines

Twenty-five years ago, Sugimura and colleagues found that organic extracts from grilled fish are potentially mutagenic in the Ames/*Salmonella* mutagenicity assay (111). From various grilled meat extracts and model systems, approximately 20 mutagenic heterocyclic amines have since been isolated and characterized. The genotoxicity of these compounds has also been demonstrated in cultured mammalian cells with various end points, including mutagenesis and induction of chromosome aberrations, sister-chromatid exchanges, DNA strand breaks, and DNA repair synthesis. These food pyrolysis products have been detected in grilled/broiled/fried beef, pork, chicken, lamb, and fish, with levels ranging from <0.1 to >300 ng/g (ppb). The most abundant heterocyclic amines in a typical Western diet are 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP); 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx); 4,8-2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (diMeIQx); 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ); and 2-amino- $\alpha$ -carboline (A $\alpha$ C) (112). PhIP, 4,8-diMeIQx, and MeIQx account for ~60% of the total mutagenic activity of a fried beef sample (113).

In general, higher cooking temperatures and longer cooking times increase the concentration of heterocyclic amines, whereas marinating the meat decreases the concentration of some heterocyclic amines (114,115). Higher levels are found in grill scrapings, meat drippings, and pan residues (116). Some heterocyclic amines can be detected in the cooking fumes (117,118). In contrast, the cooking of meats by stewing, poaching, or microwaving does not lead to the formation of heterocyclic amines.

In addition to dietary exposure, heterocyclic amines have been detected, at very low concentrations (ppt), in environmental samples, including indoor and ambient air (0.07–0.34 pg/m<sup>3</sup>), river water, rainwater (0.13–84.7 ng/L), municipal waste water, cigarette smoke (0.02–47.8 ng/cigarette), smoke from the combustion of wood chips (2.26 ng/g) and rubber (0.15–1.98 ng/g), and diesel-exhaust particles (0.01–14.1 pg/mg) (119–121).

Most heterocyclic amines are hepatocarcinogens in rodents and mammary carcinogens in female rats (122). PhIP induces colon cancers in male rats, mammary cancers in female rats, and lymphomas in mice of both sexes. The incidence of mammary cancer reached 47% in female F344 rats receiving a diet containing 400 ppm PhIP for 52 weeks (123). PhIP induces mammary tumors in female Sprague-Dawley rats in a dose-dependent manner (124). Young female Sprague-Dawley rats treated with PhIP

(75 mg/kg/day, po) for 2 weeks and then placed on a high-fat diet had a higher incidence of tumor formation than rats on a low-fat diet (125). Snyderwine has suggested that the rat model of a high-fat diet in conjunction with a food-derived carcinogen parallels the increased risk of breast cancer in women who are exposed to heterocyclic amines and high levels of fat through the consumption of cooked meats (126).

## Assessment of Dietary Exposure to Heterocyclic Amines

Human dietary exposure to heterocyclic amines has been calculated by various methods. From the levels of compounds detected in the urine, it was estimated that a Japanese study group ingested 0.2–2.6  $\mu$ g MeIQx and 0.1–13.8  $\mu$ g PhIP daily (127,128). Other studies have calculated the exposure from the intake of meat, as recorded in questionnaires. Databases have been constructed for estimation of the levels of heterocyclic amines in various types of meat according to the cooking method, temperature, and time. In examining the cancer risk of heterocyclic amines, Layton et al. (129) estimated that the typical U.S. diet entails total heterocyclic amine intake of 1.4  $\mu$ g/day for a 54-kg individual. The rank order of the five most abundant heterocyclic amines consumed is PhIP > A $\alpha$ C > MeIQx > diMeIQx > IQ, whereas the order of carcinogenic potencies of the same group is almost the reverse: IQ > diMeIQx > MeIQx > PhIP > A $\alpha$ C. Based on this study, the greatest incremental risk of cancer through consumption of meats and fish is due to PhIP.

Augustsson et al. (130) estimated that the elderly Swedish population consumes a total of 160 ng heterocyclic amines per day, with the order of the intake PhIP ~ MeIQx > diMeIQx >> 2-amino-2,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) ~ IQ. The estimated dietary intake for New Zealanders who had a general preference for medium to well-done roasted and baked meat and fish was 1  $\mu$ g heterocyclic amines per day (131).

The role of diet in breast cancer risk was discussed earlier. Here we consider specific studies of the hazard presented by intake of meat and associated heterocyclic amines. Several epidemiologic studies found an increased risk of breast cancer among women who consumed meat often (132–134). More recent studies have looked specifically at consumption of well-done meat and heterocyclic amine exposure. Women who usually consumed very well-done red meat had 4.6-fold increased risk of breast cancer compared with those who usually ate meat that was rare or medium (135). The increased risk of breast cancer has been correlated with exposure to PhIP but not to MeIQx or diMeIQx (136).

The possible role of NAT polymorphisms has also been investigated. Several studies that assessed meat intake and estimated heterocyclic amine exposure found that NAT2 genotype, alone or with these factors, was not associated with increased risk of breast cancer (137–139). However, the latest study found a significant dose–response relationship between risk of breast cancer and consumption of well-done meat among women with rapid/intermediate NAT2 genotype (140). The product of the NAT1 gene is a mammary enzyme capable of bioactivating heterocyclic amines. Women who possessed the NAT1\*11 allele (1.2–3.6% frequency) and consumed high levels of red meat or well-done meat had a 6-fold increase in risk of breast cancer (141).

Heterocyclic amine exposure through intake of well-done meats is a biologically plausible risk factor for breast cancer. Epidemiologic studies usually estimate heterocyclic amine exposure from meat intake, as reported in questionnaires, and evaluate recent dietary practices. The period between menarche and first full-term pregnancy, when rapid mammary cell division occurs, may be most critical for the initiation of breast carcinogenesis (142). Therefore, heterocyclic amine intake during adolescence and early adulthood may be most significant in development of breast cancer. Other possible criticisms of the questionnaire-estimated heterocyclic amine levels include recall bias and lack of validation with a biomarker of exposure (e.g., DNA or protein adducts) (143).

## Biologic Monitoring of Heterocyclic Amine Exposure

The bioavailability of heterocyclic amines from the consumption of cooked meat has been demonstrated through the detection of the compounds in the urine, as mentioned earlier (Tables 1 and 2). Urinary heterocyclic amine levels can typically range from 0.01 to 78 pg/mL (144,145) (Table 1). None of these compounds was detected in the urine of colon cancer patients receiving parenteral alimentation (127), which indicates that urinary excretion reflects recent dietary intake.

**Table 1.** Levels of heterocyclic amines in human urine reported in the literature.

Heterocyclic amine	pg/mL	Reference
MeIQx	10.6–17.8 <sup>a</sup>	(144)
Trp-P-1	0.2–0.60 <sup>b</sup>	(127)
Trp-P-2	0.2–0.57 <sup>b</sup>	
PhIP	0.10–1.66 <sup>b</sup>	
MeIQx	0.01–37.0 <sup>b</sup>	
PhIP	5.6–17 <sup>a</sup>	(145)
MeIQx	9–78 <sup>a</sup>	

<sup>a</sup>Mean concentration in 12-hr urine samples. <sup>b</sup>Mean concentration in 24-hr urine samples.



**Table 2.** The variation in urinary excretion of PhIP metabolites in two study groups.

PhIP metabolite	Percentage of total metabolites	
	Three elderly male colon cancer patients <sup>a</sup>	Eight healthy female volunteers <sup>b</sup>
<i>N</i> <sup>2</sup> -OH-PhIP- <i>N</i> <sup>2</sup> -glucuronide	47–61	47–88
PhIP- <i>N</i> <sup>2</sup> -glucuronide	4.34–7.8	10–45
PhIP-4'-sulfate	5.8–12.4	~1–7
<i>N</i> <sup>2</sup> -OH-PhIP- <i>N</i> <sup>3</sup> -glucuronide	7.79–11.6	~1–3

<sup>a</sup>Oral dose of 70–80 µg [<sup>14</sup>C]PhIP in a gelatin capsule. Data from Kulp et al. (170). <sup>b</sup>Ingested a well-done chicken breast containing 19 or 21 µg PhIP. Data from Malfatti et al. (171).

The majority of ingested PhIP (and other heterocyclic amines) undergo metabolism in the liver, followed by urinary excretion as conjugated products. *N*<sup>2</sup>-hydroxy-PhIP-*N*<sup>2</sup>-glucuronide is the major urinary metabolite. Other metabolites detected include PhIP-*N*<sup>2</sup>-glucuronide, PhIP-4'-sulfate, and *N*<sup>2</sup>-hydroxy-PhIP-*N*<sup>3</sup>-glucuronide, and large differences in the percentages of the metabolites are seen within and between various study groups (Table 2). The individual variation in the ratio of the urinary metabolites of PhIP suggests that there is differential expression of xenobiotic-metabolizing enzymes, possibly because of disease state, medication, environmental chemical exposure, gender, or genetic polymorphisms.

Although protein (in particular, hemoglobin [Hb]) adducts of monocyclic and polycyclic aromatic amines have been used as biomarkers of exposure for the last 20 years, the comparable use of serum albumin and Hb adducts of heterocyclic amines has been delayed because of the very low levels of binding. Studies have been conducted in which cancer patients ingest a single dose of [<sup>14</sup>C]-MeIQx (either 21.3 µg, equivalent to the amount of MeIQx ingested from food sources over 1 week, or 228 µg) (146) or [<sup>14</sup>C]-PhIP (147) (70 µg; equivalent to eating 175 g of very well-done grilled chicken). Very low levels of binding to albumin (0.13 or 1.8% of the dose) and Hb (0.05 or 0.26% of the dose) have been detected by accelerator mass spectrometry (AMS). In a recent report (148), blood samples from 35 Italian volunteers were analyzed for steady-state levels of PhIP protein adducts. By liquid chromatography/MS/MS analysis of the acid-hydrolyzed protein, approximately half the samples had detectable PhIP-serum albumin adducts (5.2 ± 1.3 fmol/mg protein) and PhIP-Hb adducts (2.3 ± 0.6 fmol/mg protein) but not necessarily both. Only 6 individuals, all of whom were vegetarians, had neither albumin nor Hb adducts.

### Heterocyclic Amines as Human Mammary Carcinogens: Mechanisms

The majority of invasive breast cancer is initiated in the mammary ductal epithelial cells. The formation of DNA adducts is a critical step in the cancer initiation process. Most

carcinogens, including heterocyclic aromatic amines, require metabolic activation to form reactive electrophilic species that can covalently bind to nucleophilic sites of DNA bases. With PhIP, for example, hepatic P450 1A2 [or mammary P450 1A1 and 1B1 (149)] can catalyze *N*-hydroxylation of the exocyclic amino group to yield *N*<sup>2</sup>-hydroxy-PhIP. In the liver, as well as in extrahepatic tissues, esterification can occur, catalyzed by various phase II enzymes. *O*-Acetylation or *O*-sulfation produces unstable products that yield highly reactive arylnitrenium ions. The C-8 guanine adduct has been observed as the major DNA adduct, with the *N*<sup>2</sup>-guanine adduct detected less frequently (150,151). To be mammary carcinogens, the reactive species of the heterocyclic amine must reach the genetic material of the mammary epithelial cells.

Over 15 years ago, it was reported that the DNA from the breast tissue of cancer patients displayed significantly higher levels of unidentified adducts than DNA from the tissue from healthy controls (152,153). From the analysis of the <sup>32</sup>P-postlabeling DNA adducts, it was suggested that environmental chemicals, including tobacco smoke and PAHs, are responsible for many of the adducts. Using <sup>32</sup>P-postlabeling analysis, DNA adducts of heterocyclic amines were detected in the mammary gland 6 hr after lactating rats were treated with a single dose of MeIQ, IQ, or PhIP (154). Moreover, the PhIP-DNA adduct levels were higher than MeIQ- or IQ-DNA adduct levels, and the PhIP-DNA adduct levels were approximately 15-fold higher in the mammary gland than in the liver.

PhIP-DNA adducts, ranging from 26–477 adducts/10<sup>12</sup> nucleotides as detected by AMS, have been detected in the normal and tumor breast tissue of cancer patients (155). Although the mammary epithelial cells are the cellular targets of tumorigenesis, both mammary epithelial cells and mammary fibroblasts can metabolize IQ to DNA-binding species (156). The fibroblast may activate the heterocyclic amines to species that are then transferred into the adjacent epithelial cells.

Human milk, extracted by methods for isolation of heterocyclic amines, yielded

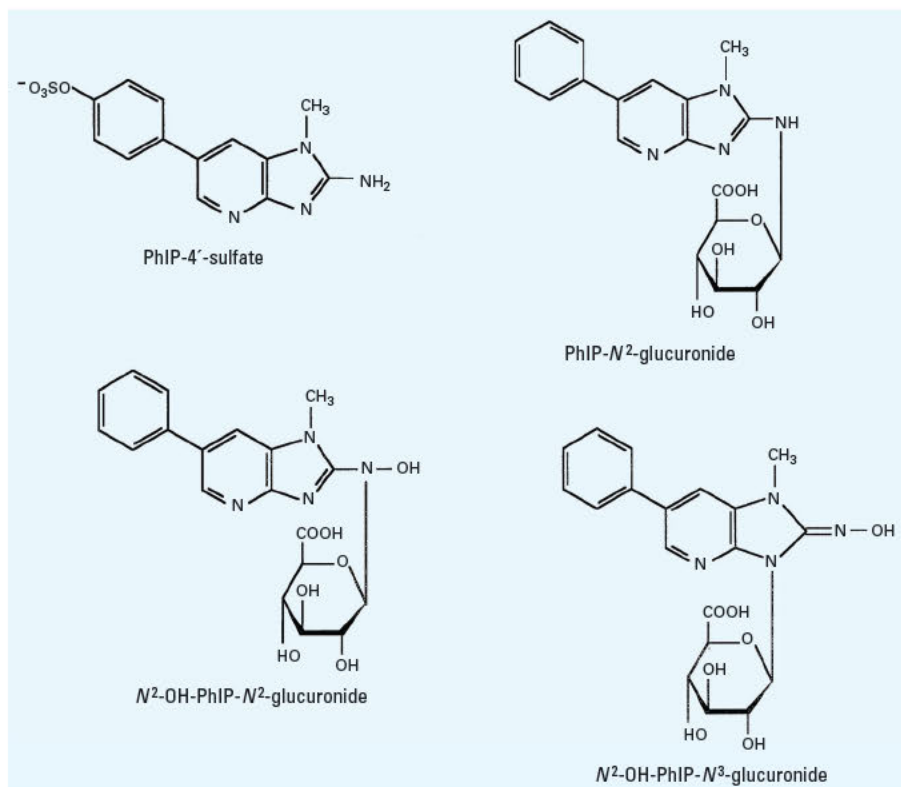
samples that were mutagenic in the Ames assay with TA1538 and YG1019, induced micronucleus formation in MCL-5 cells, and caused single-strand DNA breaks in MCL-5 and exfoliated mammary cells (157). Eleven of 20 milk samples examined were positive in the Ames assay with *Salmonella typhimurium* strain YG1019 (a strain sensitive to aromatic amines). In another study, PhIP, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), *o*-toluidine, and *p*-chloroaniline were activated to DNA-damaging species by exfoliated breast milk cells from various donors (158).

Recently, the bioavailability of PhIP to the breast tissue was demonstrated by giving a dietary equivalent dose of [<sup>14</sup>C]-PhIP in a gelatin capsule to breast cancer patients before surgery (155). Within 4–6 hr, radioactivity was detected in both normal and tumor breast tissue, and DNA adducts were found in both tissue types. PhIP is rapidly distributed to the breast tissue and bioactivated to DNA-reactive species within this short time frame.

We recently examined the levels of PhIP in the milk of healthy lactating women (159). The analysis showed 9 of 11 samples to be contaminated with PhIP, ranging from 13 to 59 pg/mL. These levels are comparable to the amounts of unmetabolized PhIP excreted into urine, as reported in previous studies. No PhIP was detected in the milk of a single vegetarian donor. [Various components of vegetarian diets may affect breast cancer risk (160).] Thus, PhIP is absorbed into the body (most likely from the ingestion of PhIP-containing cooked meat), is distributed to the mammary gland, and is subsequently excreted into the milk. As PhIP is formed by heating creat(in)ine with amino acids, animal meat is the most likely source.

From the circulating blood in the breast, PhIP must be transferred across the ductal mammary epithelial cells into the milk in the lumen of the lobules, which empty into the ductal system. Thus, the mammary epithelial cells are directly exposed to PhIP. Furthermore, mammary epithelial cells can bioactivate PhIP to reactive genotoxic species.

PhIP is transferred via the milk of mice and rats to their offspring (161–164). Unmetabolized PhIP is transferred in a linear dose-dependent manner into the milk of rats treated with PhIP (0.05–5.0 mg/kg iv) (161). PhIP is rapidly cleared from the body; with the lowest dose, PhIP was detected in the blood and milk after 1 hr, and after 4 hr, PhIP was detected only in the milk. The mean milk-to-plasma ratios, at 1 hr for the lowest dose and at 4 hr for the highest dose, were 9.4 and 9.3, respectively. This suggests a high degree of transfer of PhIP into the milk. Female offspring of rats exposed to PhIP during the gestation and lactation



**Figure 2.** Structures of major metabolites of PhIP.

periods only showed a higher incidence of mammary carcinomas than control rats with no exposure (163). The lactating rats were treated with 10 mg PhIP/kg body weight; the pups nursed for a 3-week period. This dose is 400,000 times the estimated human exposure of 25 ng total heterocyclic amines/kg body weight (129). It is not yet known whether breast-fed infants would incur any deleterious effects from this low-level heterocyclic amine exposure. The metabolism of PhIP and other heterocyclic amines has not been fully characterized in humans. However, striking differences in the metabolism of MeIQx and PhIP between rodent models and humans have been found (165). A new metabolite, 2-amino-3-methylimidazo-[4,5-*f*]-quinoxaline-8-carboxylic acid (IQx-8-COOH) is formed only by human P4501A2-catalyzed reactions (166). Glucuronidation of *N*-OH-PhIP by rat liver microsomes forms predominantly *N*-OH-PhIP-*N*<sup>3</sup>-glucuronide, whereas human liver microsomes forms predominantly *N*-OH-PhIP-*N*<sup>2</sup>-glucuronide (167). PhIP and its metabolites PhIP-4'-sulfate, 4'-hydroxy-PhIP, and *N*<sup>2</sup>-hydroxy-PhIP-*N*<sup>3</sup>-glucuronide were detected in the milk of lactating rats (164) (Figure 2). The metabolites in human milk have not yet been determined, but possible major candidates include PhIP-4'-sulfate and *N*-OH-PhIP-*N*<sup>2</sup>-glucuronide.

Because our exposure to the heterocyclic aromatic amines is chiefly dietary, reduction in the intake of cooked meats and avoidance of very well-done meats can minimize exposure. The induction of mammary carcinomas by PhIP in female rats was significantly inhibited by the coadministration of chlorophyllin or a synthetic antioxidant (168), and PhIP-DNA adduct formation in the mammary epithelial cells was lower in rats receiving green tea instead of regular drinking water (169). Thus, it may be possible to reduce the genotoxic effects of heterocyclic amines with the concurrent intake of food and drink high in antioxidants or chlorophyllin.

Further clarification of the significance of specific environmental exposures to mammary carcinogens by epidemiologic, molecular epidemiologic, and biologic analysis may lead to progress in the primary prevention and chemoprevention of human breast cancer.

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