



Chemical biomarkers of exposure and early damage from potentially carcinogenic airborne pollutants

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Abstract: Epidemiology and laboratory studies support the conclusion that air pollution is carcinogenic to humans. Chemically specific biomarkers are useful for determining human exposure to, and metabolism of, potentially toxic and carcinogenic components of air pollution, and distinguishing exposures due to air pollution from those resulting from tobacco smoke. Representative chemically specific biomarkers, quantified by mass spectrometry and related techniques, are discussed. The biomarkers are related to exposure to polycyclic aromatic hydrocarbons, volatile toxicants and carcinogens, oxidants, DNA damaging compounds, and metals found in polluted air. Quantifiable chemically specific biomarkers potentially useful in studies of air pollution carcinogenesis include urinary 1-hydroxypyrene, metabolites of benzo[*a*]pyrene, phenanthrene, benzene, 1,3-butadiene, acrolein, and crotonaldehyde, as well as products of oxidative damage such as urinary 8-hydroxy-2'-deoxyguanosine, malondialdehyde, and F₂-isoprostanes, certain DNA adducts, and metals. Tobacco-specific metabolites such as cotinine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol can be used to distinguish tobacco-related exposures from those resulting from air pollution. The following validated chemically specific biomarkers are currently the optimal ones for use in studies of air pollution and cancer: urinary 1-hydroxypyrene, phenanthrene metabolites, S-phenyl mercapturic acid, urinary or blood Cd, 8-hydroxydeoxyguanosine and F₂-isoprostanes such as 8-*iso*-PGF_{2α}. This suite of biomarkers will reliably establish exposure to carcinogenic polycyclic aromatic hydrocarbons, benzene and Cd, and will also provide critical information on oxidative damage and inflammation, both of which are important in carcinogenesis.

Keywords: Air pollution; carcinogenesis biomarkers; polycyclic aromatic hydrocarbons (PAH); volatile toxicants and carcinogens; oxidative damage; DNA adducts; metals

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Introduction

The World Health Organization (WHO) has concluded that air pollution is a major environmental risk to health ([https://www.who.int/news-room/fact-sheets/detail/ambient-\(outdoor\)-air-quality-and-health](https://www.who.int/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health)). More than 90% of the world's population lives in locations where WHO air quality guidelines were unmet. WHO's International Agency for Research on Cancer (IARC) has classified outdoor air

pollution and particulate matter (PM) from outdoor air pollution as carcinogenic to humans based on sufficient evidence of carcinogenicity in humans and experimental animals and strong mechanistic evidence (1). Epidemiology studies and experiments with laboratory animals provide a consistent picture of the carcinogenicity of air pollution. The epidemiology studies in particular demonstrate an increased risk of lung cancer in cohort and case-control studies which included millions of people and thousands

of lung cancer cases from Europe, North America, and Asia (1). Fine PM (PM_{2.5}) is widely used as an indicator pollutant in air pollution studies, and its concentrations range from less than 10 to more than 100 μm^3 worldwide (1). In addition to particles, air pollution is a complex mixture of chemicals and toxicants, some of which are established carcinogens and are involved in cancer etiology due to air pollution. This review discusses quantifiable chemically specific biomarkers which can be used to assess human exposure to carcinogenic airborne pollutants including polycyclic aromatic hydrocarbons (PAH), volatile toxicants and carcinogens, oxidants, DNA damaging compounds, and metals. We also examine biomarkers that can be applied in epidemiological studies to distinguish exposures to air pollution versus tobacco smoke. While previous reviews have discussed the application of biomarkers to air pollution studies (2-7), ours is unique in focusing only on analytically validated chemically specific biomarkers.

PAH

PAH are a group of pollutants generated from incomplete combustion of organic substances and are widespread in the environment. Humans are exposed to PAH from different sources, including occupational exposures, air pollution, cigarette smoke, food and water, soil and dust. A large body of evidence implicates PAH as significant causes of cancer in cigarette smokers and workers with occupational exposure (8). They may also play a potentially important role in the development of lung cancer among lifelong never smokers (9). For the general population, air pollution, cigarette smoke and food are the main exposure pathways. Concentrations of several typical PAH including pyrene, benzo[*a*]pyrene (BaP), and phenanthrene (Phe) in cigarette smoke, indoor and outdoor air, and food are listed in *Table S1*. Urinary metabolites have been extensively used as biomarkers to assess human exposure to PAH. Metabolites derived from 3 PAH—pyrene, BaP, and Phe—are discussed here; representative structures are illustrated in *Figure 1*.

Pyrene metabolites

Pyrene is a relatively abundant and non-carcinogenic component of all PAH mixtures. Metabolism of pyrene leads to formation of 1-hydroxypyrene (1-OHP) which undergoes phase II metabolism to 1-OHP-glucuronide and sulfate. Jongeneelen *et al.* first proposed the use of

1-OHP as a biomarker to assess exposure to PAH in coal-tar treated patients and workers (10-12). After that, it was determined in hundreds of studies to assess human exposure to PAH. Many analytical procedures have been developed, including high performance liquid chromatography (HPLC) with fluorescence detection (HPLC-FLD) (13,14), gas chromatography-mass spectrometry (GC-MS) or GC-high resolution mass spectrometry (GC-HRMS) with silylation (15-17), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) with or without derivatization (18-20). *Table 1* summarizes some representative data from recent investigations of urinary 1-OHP.

This biomarker has several notable strengths. It is the principal metabolite of pyrene, which is always found in PAH mixtures in reasonably high proportions and it has a high detection frequency in human urine (30). While pyrene itself is non-carcinogenic, levels of 1-OHP are representative of carcinogenic PAH exposure. Levels of 1-OHP were 2.5 to 6.9 times higher in various occupationally exposed workers than in controls, and consistently about twice (1.5–2.7) as great in cigarette smokers as in non-smokers (26,29,33). Levels in the urine of the general population from the U.S. and several Asian countries vary considerably and are likely to be strongly influenced by airborne pollutants (*Table 1*), although diet and genetic polymorphisms in metabolizing enzymes due to ethnic/racial differences may also contribute (30,31). In one 10-year study [2003–2014] in the U.S., children aged 6–11 years had higher urinary 1-OHP levels than adults aged 21+ years (30). Possible causes for this are the unique activity patterns and behavior of children, as well as physiological differences between them and adults. Exposure to air pollution and secondhand smoke have been associated with the development of childhood asthma, lower respiratory infections and leukemia (34-37).

All of the studies described above have measured 1-OHP after treatment of urine with deconjugating enzymes, β -glucuronidase and/or aryl-sulfatase, since 1-hydroxypyrene glucuronide conjugate (1-OHPG) was the major pyrene metabolite in human urine (38). Methods for direct measurement of 1-OHPG have been established without enzymatic hydrolysis using immune-affinity chromatography with synchronous fluorescence spectroscopy (IAC-SFS) (38,39), HPLC-FLD (40,41), and LC-MS/MS (42,43). Urinary concentrations of 1-OHPG were about 5.6 times higher in occupationally exposed workers (2.16 pmol/mL, mean value) than among workers (0.38 pmol/mL) with no or low exposure (44).

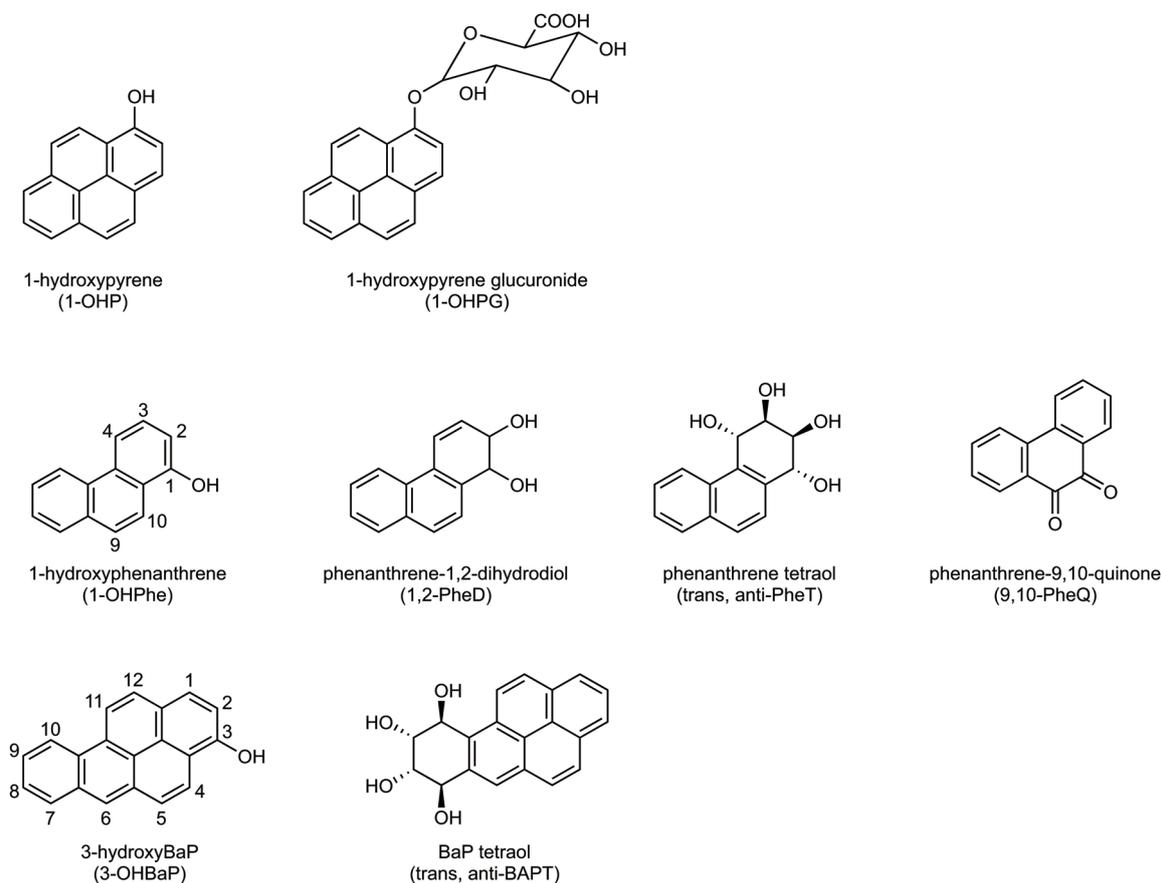


Figure 1 Structures of main PAH metabolites discussed in this review. PAH, polycyclic aromatic hydrocarbon.

Cigarette smokers had a 2–3-fold higher concentration of 1-OHPG compared with nonsmokers (44-46). Exposure to secondhand smoke and air pollution would also contribute to the urinary 1-OHPG in children (39,47).

BaP metabolites

BaP is the prototypic carcinogenic and mutagenic PAH and is classified by IARC as carcinogenic to humans (8). BaP requires metabolic activation to exert its carcinogenic and other adverse effects. Phase I reactions of BaP are catalyzed primarily by CYP1A1 and CYP1B1 and produce a series of metabolites including phenols in the detoxification pathway, whereas epoxides, dihydrodiols, quinones and tetraols are produced in the bioactivation pathways, with BaP diol epoxide (BPDE) as a major ultimate carcinogenic metabolite which can attack DNA (Figure 2). These metabolites are mainly excreted in feces and are present in extremely low levels in urine. Thus, direct analysis of urinary BaP

metabolites can be challenging. 3-hydroxyBaP (3-OHBaP) has been proposed as a good surrogate for assessing exposure to carcinogenic PAH, since its excretion, but not that of 1-OHP, correlated significantly with the levels of DNA adducts in humans. Methods for quantification of 3-OHBaP have been developed based on HPLC-FLD (14,48), GC-MS or GC-HRMS with or without silylation (15,49,50), and LC-MS/MS with or without derivatization (20,51,52). Urinary levels of 3-OHBaP in some recent studies are summarized in Table S2. In general, urinary 3-OHBaP concentrations were 2 to 4 orders of magnitude lower than those of 1-OHP, not only because urinary metabolites account for a very low proportion of total BaP, but also due to the more complex metabolism of BaP. A biological limit value (0.45 nmol/mol creatinine), higher than most of the reported levels, was proposed by Lafontaine *et al.* based on the correlation between urinary 3-OHBaP and atmospheric BaP (53).

3-OHBaP represents a detoxification pathway of

Table 1 Representative urinary 1-OHP concentrations in various subjects in recent studies

Country	Subject categories	1-OHP level	Reference
China	Coke oven workers (N=122)	35,120 [19,870, 61,930] pg/mg creatinine [median (25 th , 75 th percentile)]	(21)
South Korea	Office workers (N=137)	85.6 (2.09) pg/mg creatinine [GM (SD)]	(22)
	Painting workers (N=82)	587.9 (3.45) pg/mg creatinine	
Nigeria	Charcoal workers (N=25)	4,280±2,450 [675–9,320] pg/mg creatinine [mean (range)]	(23)
	Charcoal workers (N=23)	2,550±1,250 [656–6,230] pg/mg creatinine	
	Non-charcoal workers (N=20)	617±502 [19.3–792,000] pg/mg creatinine	
Ghana	E-waste recycling workers (N=72)	1,330 [780–2,520] pg/mg creatinine [median (25 th , 75 th percentile)]	(24)
	Controls (N=40)	540 [290–800] pg/mg creatinine	
Canada	Firefighters pre-fire (N=27)	100 [10] pg/mg creatinine [GM (SE)]	(25)
	Firefighters post-fire (N=31)	270 [20] pg/mg creatinine	
	Controls (N=13)	70 [10] pg/mg creatinine	
U.S.	E-cigarette users (N=28)	82.9 (56.7–120) pg/mL [GM (95% CI)]	(26)
U.S.	Cigarette smokers (N=17)	192 [120–308] pg/mL [GM (95% CI)]	(27)
U.S.	Cigarette smokers (N=165)	212 [175–255] pg/mL [GM (95% CI)]	(28)
Iran	Never tobacco users (N=58)	412.0 (344.2–493.1) pg/mg creatinine [GM (95% CI)]	(29)
	Exclusive cigarette smokers (N=33)	636.0 (504.0–802.4) pg/mg creatinine	
	Exclusive waterpipe smokers (N=37)	960.8 (725.1–1273.0) pg/mg creatinine	
	Exclusive nass smokers (N=30)	441.9 (340.2–574.0) pg/mg creatinine	
U.S.	Cigarette smokers (N=885)	259 [240–280] pg/mg creatinine [GM (95% CI)]	(30)
	Nonsmokers (N=1,296)	96.7 (90.8–103) pg/mg creatinine	
U.S.	Mexican Americans (N=450)	125 [117–133] pg/mL [GM (95% CI)]	(30)
	Non-Hispanic blacks (N=578)	182 [168–198] pg/mL	
	Non-Hispanic whites (N=979)	128 [118–138] pg/mL	
	All Hispanics (N=698)	129 [120–139] pg/mL	
	Asians (N=352)	93.2 (83.6–104) pg/mL	
China	General population (N=84)	667 [378] pg/mL [mean (median)]	(31)
Vietnam	General population (N=23)	641 [463] pg/mL [mean (median)]	
Japan	General population (N=34)	183 [75] pg/mL [mean (median)]	
India	General population (N=38)	699 [424] pg/mL [mean (median)]	
Malaysia	General population (N=29)	186 [65] pg/mL [mean (median)]	
Korea	General population (N=60)	167 [103] pg/mL [mean (median)]	
Kuwait	General population (N=38)	320 [220] pg/mL [mean (median)]	
China	Children aged 7–13 years (N=1,206)	184 [212–237] pg/mL [GM (95% CI)]	(32)

Table 1 (continued)

Table 1 (continued)

Country	Subject categories	1-OHP level	Reference
U.S.	Children aged 6–11 years (N=415) [2009–2010]	147 [125–174] pg/mL [GM (95% CI)]	(30)
	Children aged 6–11 years (N=397) [2011–2012]	130 [111–153] pg/mL	
	Children aged 6–11 years (N=399) [2013–2014]	131 [118–145] pg/mL	
	Adults aged 20+ years (N=1,911) [2009–2010]	113 [103–122] pg/mL	
	Adults aged 20+ years (N=1,703) [2011–2012]	107 (96.2–119) pg/mL	
	Adults aged 20+ years (N=1,802) [2013–2014]	128 [120–137] pg/mL	

1-OHP, 1-hydroxypyrene; GM, geometric mean; SD, standard deviation; CI, confidence interval.

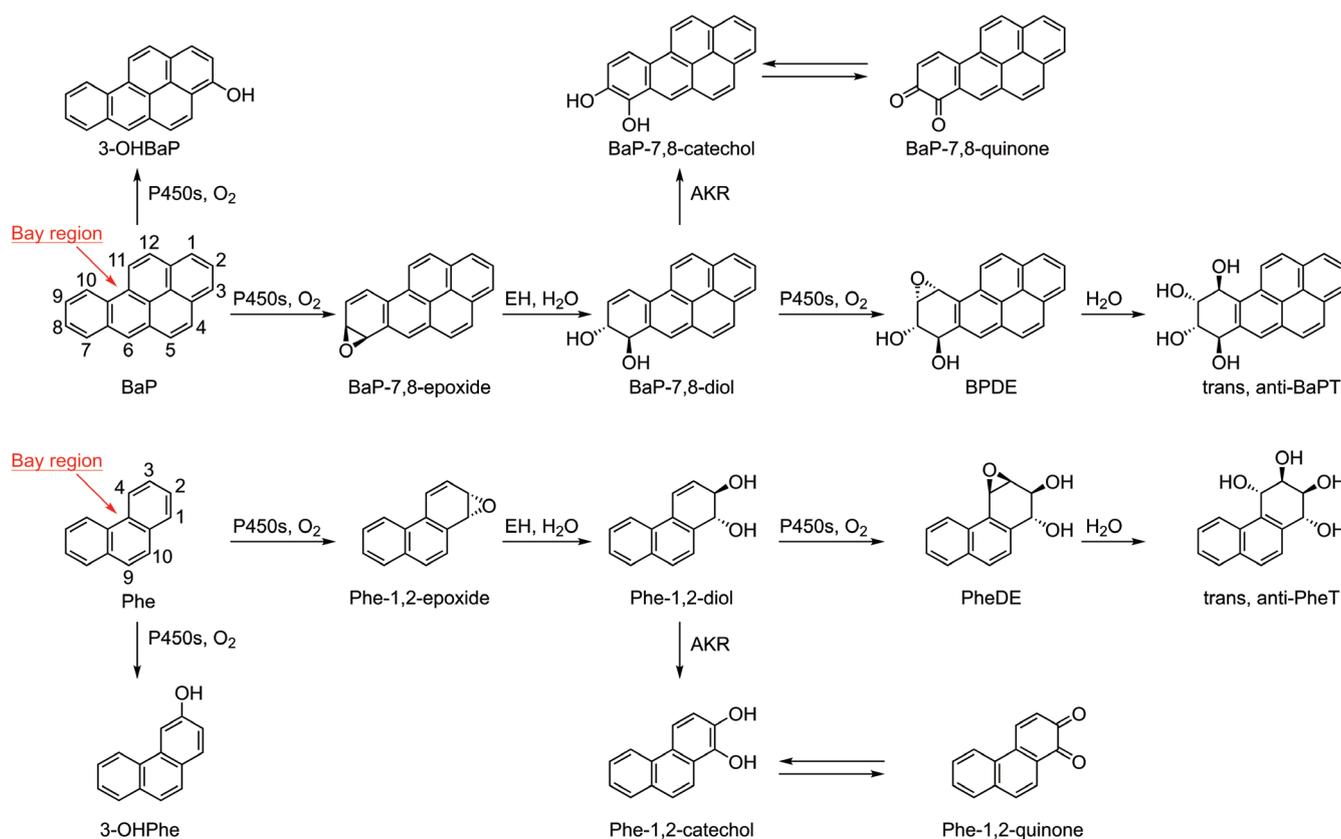


Figure 2 Metabolism of BaP and Phe to dihydrodiols, phenols, quinones and tetraols. P450, cytochrome P450; EH, epoxide hydrolase; AKR, aldo-keto reductases; BaP, benzo[*a*]pyrene; Phe, phenanthrene.

BaP metabolism. Conversely, urinary *r*-7,*t*-8,9,*c*-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans*, *anti*-BaPT), the major end product of the BaP bay region diol epoxide metabolic activation pathway, was proposed to be a more relevant biomarker linked to bioactivation and carcinogenicity (54–56). Methods for quantification

of *trans*, *anti*-BaPT based on GC-negative ion chemical ionization (NICI)-MS/MS or GC-atmospheric pressure laser ionization (APLI)-MS have been described (54–60). For occupational exposure, mean *trans*, *anti*-BaPT was 0.4 ± 0.3 nmol/mol creatinine in coke-oven workers, ~10 times higher than that (0.03 ± 0.03 nmol/mol creatinine)

in referents ($P < 0.001$) (61). An extremely high level of *trans, anti*-BaPT (17.2 ± 13.5 fmol/mL) was observed in creosote workers, ~29 and 66 times higher than that in smokers (0.587 ± 0.311 fmol/mL) and nonsmokers (0.26 ± 0.04 fmol/mL) without occupational exposure to PAH (55,62). The relationship of *trans, anti*-BaPT to exposure to air pollution has not been reported.

Phe metabolites

Phe is the simplest non-carcinogenic PAH with structural features (bay region) and enzymology profile similar to that of BaP (Figure 2). Thus, Phe metabolites may provide information on both exposure and metabolic activation.

As the main metabolites in the detoxification pathway, five phenanthrols—1-OHPhe, 2-OHPhe, 3-OHPhe, 4-OHPhe and 9-OHPhe—have been identified in human urine by using GC-MS (63,64), HPLC-FLD (65), LC-MS/MS (66). Table S3 summarizes some representative data from recent investigations of urinary phenanthrols. Similar to 1-OHP, levels of phenanthrols in the urine of the general population from the U.S. and several Asian countries also vary considerably, ~10 times lower in the U.S. population than those from China, which is likely due to air pollution (30,31). Kuang *et al.* reported that levels of 1-, 2-, and 3-OHPhe were ~3–4 times higher in high exposure coke-oven workers than controls, while 9-OHPhe concentration was only ~1.6 times higher, and 4-OHPhe levels were almost the same and even lower in exposure group than the controls (64). Levels of 1-, 2-, 3- and 4-OHPhe in smokers were 1.7–2.9 times higher than in nonsmokers in the U.S. CDC's National Health and Nutrition Examination Survey (NHANES) 2011–2012 with large sample size (33). And the ratio of 3- plus 4-OHPhe to 1- plus 2-OHPhe $[(3\text{-OHPhe} + 4\text{-OHPhe}) / (1\text{-OHPhe} + 2\text{-OHPhe})]$ increased from 0.39 to 0.57 with cigarette consumption (33), confirming the results obtained by Heudorf & Angerer and Jacob *et al.* (67,68). This phenomenon is thought to be caused by the induction by cigarette smoke of P450 1A2, which plays an important role in 3,4-oxidation of Phe (69,70).

Phe dihydrodiols including Phe-1,2-diol, Phe-3,4-diol and Phe-9,10-diol were also introduced to assess the metabolic pathways of Phe. They have been quantified in human urine by GC-MS (71,72).

While *trans, anti*-BaPT is relevant to bioactivation and carcinogenicity, its concentration in human urine is so low that it cannot be used in large studies. Thus, *r*-1, *t*-2,3,

c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (*trans, anti*-PheT) was proposed as a surrogate measure of carcinogenic PAH metabolic activation by the bay-region diol epoxide pathway (62,73). Urinary *trans, anti*-PheT levels have associated with lung cancer risk in smokers and nonsmokers in cohort studies, and a strong correlation between overall *trans, anti*-PheT and *trans, anti*-BaPT was observed in human urine (9,62,74). A simple high throughput method using one step 96-well format SPE extraction coupled with GC-NICI-MS/MS was developed for the analysis of *trans, anti*-PheT, allowing its measurement in large studies.

Phe quinones (PheQ) are components in airborne PM and generate reactive oxygen species in a redox cycling process. PheQ can also form during P450s and aldo-keto reductases catalyzed metabolism of Phe. 9,10-PheQ and its glucuronide conjugate have been identified in human urine at a quite low level (39.7–128.2 nmol/mol creatinine), 85–275 times lower than total OHPhe, accounting for a small proportion (~1% or less) of Phe metabolites (63,64,75).

Individual susceptibility plays an important role in cancer development in humans exposed to chemical carcinogens, thus identification of high-risk individuals is critical in cancer prevention and therapy. Up to now, Phe is the only PAH which has a bay region and for which most kinds of metabolites can be conveniently analyzed. This provides an opportunity to closely mimic metabolic phenotyping of BaP resulting from bioactivation and detoxification pathways. A ratio of bioactivation metabolites to detoxification metabolites has been proposed to assess human individual sensitivity to carcinogenic PAH exposure. *Trans, anti*-PheT/HOPhe ratios were significantly higher in smokers than in nonsmokers in a longitudinal study, showing that smoking induces the diol epoxide metabolic activation pathway of Phe (76).

Volatile toxicants and carcinogens

Benzene

Benzene is an established cause of acute myeloid leukemia and acute non-lymphocytic leukemia in humans (77). Cigarette smoking is a major source of human exposure to benzene. There are a number of occupational sources of exposure to benzene including in the petrochemical industry, coke oven industry, around gasoline service stations, in urban work settings, and in certain manufacturing industries. Sources of benzene exposure in the general population

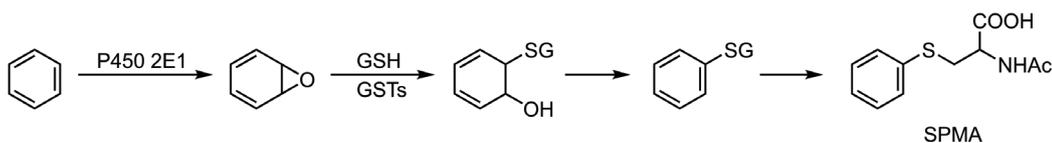


Figure 3 Metabolic formation of SPMA from benzene. Benzene is converted to benzene oxide, catalyzed mainly by cytochrome P450 2E1. Benzene oxide is detoxified by reaction with glutathione (GSH), catalyzed by glutathione transferases (GSTs). Dehydration of the resulting intermediate yields phenyl glutathione, which is metabolized by the mercapturic acid pathway to the urinary metabolite and biomarker SPMA. Some of the undehydrated intermediate from the initial reaction with glutathione may also be processed by the mercapturic acid pathway giving 1-hydroxy-2-*N*-acetylcysteinyl-1,2-dihydrobenzene; acid treatment of the urine sample converts this to SPMA. SPMA, *S*-phenylmercapturic acid.

include areas with heavy traffic, near gasoline filling stations, and consumption of contaminated food and water (77).

While benzene itself in blood or urine and *trans, trans*-muconic acid in urine have been used as biomarkers of benzene exposure, they each have disadvantages. Benzene is volatile, so could be lost from blood or urine during collection, and blood collection is invasive. *Trans, trans*-muconic acid is a metabolite of both benzene and sorbic acid, thus detracting from its specificity as a benzene biomarker. The benzene metabolites phenol and catechol also have multiple dietary sources, so cannot be directly linked to benzene exposure (78,79). The most generally useful biomarker of benzene exposure is urinary *S*-phenylmercapturic acid (SPMA, *Figure 3*), which is formed by conjugation of the benzene metabolite benzene oxide with glutathione (78,79). The resulting intermediate spontaneously dehydrates to *S*-phenyl glutathione (SPG) and is processed metabolically by the mercapturic acid pathway (γ -glutamyl transpeptidase and cysteinyl glycylase followed by acetylation) to give SPMA. Some undehydrated product (e.g., 1-hydroxy-2-*N*-acetylcysteinyl-1,2-dihydrobenzene) can also be formed from the intermediate during this process; treatment of the urine sample with acid converts this metabolite to SPMA. SPMA is determined by LC-MS/MS (80,81).

The highest levels of urinary SPMA are observed in cigarette smokers. In one study, levels of SPMA in cigarette smokers from 5 different ethnic groups ranged from 3.05 to 4.56 pmol/mg creatinine (about 1.4 μg per 24 h) while another study found levels of 4.72–7.05 μg per 24 h (81,82). Cessation of smoking resulted in an approximate 90% reduction of SPMA within 3 days to a level of about 0.4 pmol/mg creatinine, consistent with the major effect of cigarette smoking on SPMA levels (82,83). A study carried out in Qidong, China, a region with substantial air pollution, found higher levels of SPMA in urine of

non-smokers (0.98 pmol/mg creatinine) than reported in non-smoking residents of Singapore (about 0.5 pmol/mg creatinine) (84–86). Similarly, urine samples collected from non-smokers in Shanghai, China from 1986 to 1989 when general environmental pollution was likely considerable, had about 1 pmol/mg creatinine SPMA (9). Some studies show elevated SPMA levels in occupationally exposed subjects such as taxi drivers, but the effects are less than those due to smoking or high air pollution (87–90). Genotype can have a substantial effect on SPMA levels in urine (91). In one study, *GSTT1* deletion explained up to 31.6% of the variation in SPMA levels; thus, determination of *GST* genotype is critical in studies using SPMA as a biomarker (81). *GSTT1* null genotype theoretically signifies a higher risk for health effects upon benzene exposure since its detoxification will be decreased, yet the null genotype results in lower SPMA levels, which presents some difficulties in interpretation.

1,3-butadiene

1,3-butadiene is considered carcinogenic to humans by IARC; it causes cancer of the hematolymphatic organs (77,92). Cigarette smoking is the major source of exposure to 1,3-butadiene. Levels of 1,3-butadiene in outdoor air are typically relatively low, with a mean of about 0.1 $\mu\text{g}/\text{m}^3$, although the levels can be somewhat higher in areas of urban industry and high road traffic (77).

The most useful and widely applied biomarkers of 1,3-butadiene exposure are the urinary metabolites 1-(*N*-acetyl-L-cysteine-*S*-yl)-2-hydroxybut-3-ene and 2-(*N*-acetyl-L-cysteine-*S*-yl)-1-hydroxybut-3-ene, collectively termed MHBMA, resulting from glutathione detoxification of 3,4-epoxy-1-butene, as illustrated in *Figure 4*. In one study of more than 1,000 smokers, MHBMA levels determined by LC-MS/MS averaged 4.8 ng/mL urine while another study reported a range of 3.75–5.69

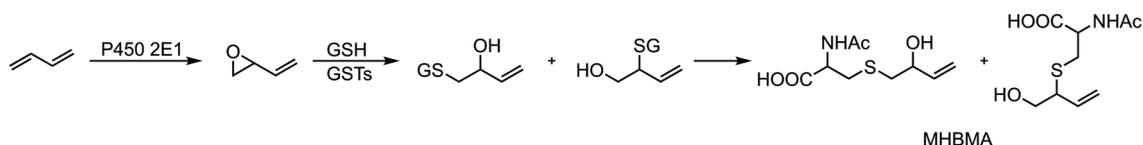


Figure 4 Metabolic formation of MHBMA from 1,3-butadiene. MHBMA, 1-(*N*-acetyl-L-cysteine-S-yl)-2-hydroxybut-3-ene and 2-(*N*-acetyl-L-cysteine-S-yl)-1-hydroxybut-3-ene.

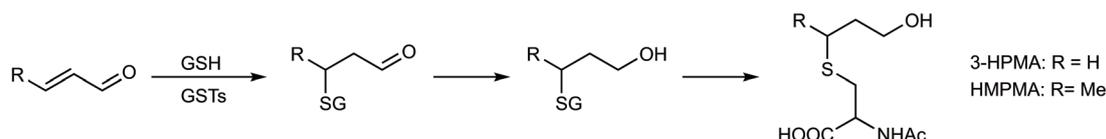


Figure 5 Formation of 3-HPMA and HMPMA from acrolein (R = H) and crotonaldehyde (R = CH₃). 3-HPMA, 3-hydroxypropyl mercapturic acid; HMPMA, 3-hydroxy-1-methylpropyl mercapturic acid.

µg/24 h (82,93). Cessation of smoking resulted in a 77–90% reduction in MHBMA levels (82,83). As is the case with benzene-derived mercapturic acids, *GSTT1* status has a strong effect on urinary MHBMA levels (14). Mercapturic acids of additional 1,3-butadiene epoxide metabolites have been analyzed by LC-MS/MS to demonstrate exposure to this carcinogen in industrial settings (94). LC-MS/MS analysis of MHBMA in non-smoking non-occupationally exposed subjects from the general population resulted in mainly values below the detection limit of the method. Hemoglobin adducts and DNA adducts of 1,3-butadiene have also been developed as biomarkers although not yet extensively used in human biomonitoring (95,96).

Acrolein and crotonaldehyde (Cro)

Acrolein and Cro are strong eye and respiratory tract toxicants causing irritation, inflammation, cell proliferation, and multiple other effects (97–99). Both compounds react directly with DNA forming adducts that may be involved in carcinogenesis although neither is a strong carcinogen (100–102). Acrolein and Cro are also formed endogenously during lipid peroxidation and related processes (97–99).

The mercapturic acid metabolites 3-hydroxypropyl mercapturic acid (3-HPMA) and 3-hydroxy-1-methylpropyl mercapturic acid (HMPMA) (Figure 5), determined by LC-MS/MS, are well-established biomarkers of acrolein and Cro exposure (103). Cigarette smoking is the major source of exposure to acrolein and Cro. In one study of more than 2,200 cigarette smokers, median levels of 3-HPMA and HMPMA ranged from about 2,000 to 3,600 pmol/mL urine

while another reported about 2,000–6,000 µg/24 h (82,104). When cigarette smokers stopped smoking, levels decreased by 70–85% (82,83). In the NHANES study, cigarette smokers and non-users had 1.089 mg/g creatinine and 0.219 mg/g creatinine levels of 3-HPMA, respectively, and 1.61 and 0.313 mg/g creatinine of HMPMA, respectively (105,106). No significant relationship was found between location (urban *vs.* rural) and levels of 3-HPMA and HMPMA in pregnant women in the National Children's Health Study (107).

Ethylene oxide and propylene oxide

Ethylene oxide is considered carcinogenic to humans by IARC based on sufficient evidence for carcinogenicity in laboratory animals and extensive mechanistic data including the induction of sister chromatid exchanges, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers (92). Cigarette smoking is an important source of ethylene oxide exposure; levels of the hydroxyethyl valine adduct of ethylene oxide in hemoglobin, one of the most widely used biomarkers of ethylene oxide exposure, are increased upon exposure to cigarette smoke, and correlated with the number of cigarettes smoked (92). Urinary 2-hydroxyethyl mercapturic acid (HEMA), formed by glutathione conjugation of ethylene oxide followed by normal metabolic processing, is another established biomarker of ethylene oxide exposure. Exposure of hospital workers to ethylene oxide used in sterilization processes resulted in elevated levels of these biomarkers. Pregnant women who were cigarette smokers

had significantly elevated levels of HEMA in the National Children's Study (107). When smokers stopped smoking, their levels of HEMA decreased by 75% within 3 days (83). *N*⁷-(2-Hydroxyethyl)guanine, formed in DNA upon reaction with ethylene oxide, is an additional potential biomarker of ethylene oxide exposure, but is also formed endogenously as are the other biomarkers discussed here (92).

Propylene oxide is considered "possibly carcinogenic to humans" by IARC (108). Urinary 2-HPMA is a biomarker of propylene oxide exposure (109). Similar to the other volatiles discussed here, cigarette smoking results in significant elevations of this biomarker, which decrease upon cessation (109). Air pollution has not been established as a source of elevated 2-HPMA in urine (107).

Other compounds and PM_{2.5}

There is some human exposure to polychlorinated biphenyls (PCBs) even though their production has been banned for decades and their concentrations in the air are quite low. Hydroxylated PCBs in human urine or plasma are commonly used biomarkers (110-112). Oxy- and nitro-PAHs can be formed directly or indirectly by oxidation or nitration of PAH. Among them, 1-nitropyrene, 6-nitrochrysene, 3-nitrobenzanthrone, and anthraquinone have received considerable attention (113,114). Metabolites of 1-nitropyrene have been used to examine personal exposures (115,116).

PM_{2.5} refers to PM with an aerodynamic diameter <2.5 μm, which can penetrate deeply into the human lung and even the bloodstream. PM_{2.5} is monitored in the atmosphere as a critical indicator of air pollutant dose, but doesn't have a specific exposure biomarker. Rather, biomarkers of human exposure to PM_{2.5} are mainly based on its bound chemicals, the concentrations of which may have strong positive correlations with PM_{2.5}.

Oxidative damage products

Exposure to airborne pollutants from inhaling polluted urban air, cigarette smoke, and industrial and occupational toxicants is an established cause for cancer and a range of respiratory and cardiovascular diseases, among other health outcomes (117-119). Oxidative stress and inflammation are key mechanisms underlying such diseases. There are several possible pathways by which particles and chemicals in polluted air and cigarette smoke can trigger these processes: the inhaled PM can cause inflammation in the

lung, resulting in the release of various reactive oxygen species by macrophages; inflammatory processes can also be induced by the chemical toxicants and carcinogens present in the polluted air or deposited on the solid particles of PM; or oxidative stress can be induced via Fenton chemistry by transition metals present in PM (120,121). These and other mechanisms are likely to act together to produce persistent oxidative stress, leading to the formation of reactive species capable of oxidizing key macromolecules such as DNA and lipids, and inducing cellular damage (122-125). Products of these interactions can be measured in various biological samples and serve as biomarkers of oxidative stress in populations exposed to air pollution, cigarette smoke, and other inhaled toxic and carcinogenic agents (126-130). Urine is a widely used biological matrix for the analyses of many such biomarkers, including DNA adducts (131-137).

Urinary 8-hydroxy-2'-deoxyguanosine (8-OH-dG)

8-hydroxy-2'-deoxyguanosine (8-OH-dG) is one of the most widely studied urinary DNA-derived biomarkers of oxidative stress. There are several potential mechanisms leading to the excretion of 8-OH-dG in urine, including the removal of the major oxidation adduct 8-oxo-dG from DNA by base excision repair, removal from the nucleotide pool by nucleotide phosphatases, and release from DNA after cell death (138-142). Some studies show that urinary 8-OH-dG correlates with oxidative stress and inflammation, and with the risk of cancer and cardiovascular diseases (135,143-147). A positive correlation between urinary 8-OH-dG and the levels of this biomarker in plasma and saliva has been also reported, supporting its relevance to the overall systemic oxidative status (148,149).

Enzyme-linked immunosorbent assay (ELISA) is one of the most frequently used methods for the analysis of 8-OH-dG in urine and other biological fluids; however, it suffers from a lack of sensitivity and selectivity when compared to mass-spectrometry based assays. Comparison of data obtained by the two methodologies shows much higher levels measured by ELISA than by LC-MS/MS, and a lack of correlation between the results of the two assays, calling into question the accuracy of published data generated by using ELISA (150).

Numerous MS-based assays have been reported for the analysis of 8-OH-dG in urine, including high-throughput techniques (151-154). Studies employing such assays generally show positive associations between exposure to air pollution and urinary 8-OH-dG. For instance, a study examining associations between indoor air quality and health

in participants with chronic obstructive pulmonary disease (COPD) in Boston, USA, found a positive association between traffic-related black carbon exposure and urinary 8-OH-dG (155). The relationship between traffic-related air pollution and urinary 8-OH-dG and other biomarkers of oxidative stress has been also observed in studies outside the U.S. A study of traffic conductors in Taiwan showed that their urinary 8-OH-dG levels and the frequency of DNA strand breaks were significantly higher than in indoor office workers studied as controls (156). The same study showed a significant association of 8-OH-dG with urinary 1-OHPG, which in turn was associated with the levels of PAH in airborne particulates. Other studies assessed changes in urinary 8-OH-dG in response to changes in exposure. In one study in a limited number of subjects traveling from Germany to China, higher levels of urinary 8-OH-dG were observed in these individuals after their trip to China than before the trip, consistent with the higher air pollution levels in China (157). A study conducted in Italy showed that levels of this biomarker in children increased in the evening compared to morning levels, most likely due to day-long exposures to industrial and urban air pollution (158). Increased levels of urinary 8-OH-dG have been reported in smokers, and a correlation between urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a tobacco-specific biomarker, and 8-OH-dG was reported in individuals exposed to secondhand smoke (159-162). Studies that employed HPLC with electrochemical detection are also generally consistent with those based on LC-MS/MS measurements of 8-OH-dG. Such studies generally show increased levels of urinary 8-OH-dG associated with urban air pollution (163-165), exposures to smoke and fumes from cooking (166,167), and occupational toxic exposures (168).

Urinary malondialdehyde

Malondialdehyde is a major product of lipid peroxidation (169). It undergoes oxidative metabolism, and being highly reactive, can lead to DNA and protein adduct formation and is mutagenic (170-172). However, substantial levels of malondialdehyde are still present in the general circulation and excreted in urine, and it has been widely used as a biomarker of oxidative stress caused by air pollution, smoking, and occupational exposures, and has been associated with risk of cancer and atherosclerosis (147,173-175).

While an ELISA method is commonly used for malondialdehyde analysis, many studies employed HPLC-FLD for its analysis in biological samples, including urine,

taking advantage of its reaction with thiobarbituric acid which produces an intensely colored chromogen fluorescent red adduct (176,177). Similar to urinary 8-OH-dG, such studies generally find positive associations between air pollution and urinary malondialdehyde levels. Thus, the Boston study of COPD patients showed a positive association between black carbon exposure and urinary malondialdehyde (155). A study of the impact of Beijing Olympic air pollution control measures showed significant decreases in urinary malondialdehyde during the Olympics, compared to the pre-Olympic period, and subsequent increases in the post-Olympic period, consistent with the trends in air pollution levels (178). In children, urinary malondialdehyde was associated with seasonal and regional variations in air pollution, as well as exposures to secondhand smoke (47,179). Associations with occupational exposures to metals also have been reported (180). However, the thiobarbituric acid-based assay is nonspecific, and MS-based assays are preferred for a more accurate and precise measurement of this biomarker (181). Studies that employed LC- or GC-MS/MS for the analysis of urinary malondialdehyde are less consistent in reporting on the association between its levels and air pollution. For example, the study of individuals traveling from Germany to China showed an increase in the levels of urinary malondialdehyde after the trip, similar to the results for 8-OH-dG (157). However, a randomized crossover intervention study of portable air filter use by Canadian healthy adults showed substantial reductions in indoor fine particle concentrations (60% reduction after 7 days of filter use), but no changes in urinary malondialdehyde levels (182).

*Urinary 8-*epi*-PGF_{2α}*

F₂-isoprostanes are prostaglandin (PG)-like compounds generated through oxidative stress-induced peroxidation of arachidonic acid (183,184). These compounds are mostly stable and robust indicators of oxidative stress present in all human tissues and fluids, and their levels have been related to a variety of chronic conditions including cardiovascular disease, diabetes, obesity, and neurodegenerative diseases (184). Among existing F₂-isoprostanes, urinary 8-*iso*-PGF_{2α} is a commonly used and accepted biomarker of oxidative damage (183,184).

Published studies reporting on the levels of F₂-isoprostanes in various biological fluids, including urine, used analytical techniques such as ELISA, GC, and GC- or LC-MS/MS (185,186). Most environmental exposure studies used ELISA and found positive associations between

air pollution and isoprostane levels in urine (187-190). Environmental exposure studies that employed LC-MS/MS for the analysis of urinary 8-*iso*-PGF_{2α} are limited. The study that assessed oxidative stress markers in travelers returning to Germany after the trip to China showed that the LC-MS/MS-measured urinary 8-*iso*-PGF_{2α} was higher after, than before, the trip to China, consistent with the observations for urinary 8-OH-dG and malondialdehyde (157). However, a study in Peru that investigated the effect of interventions to prevent exposures from wood-burning stoves showed no difference in urinary 8-*iso*-PGF_{2α} between the intervention and the control groups (166). Furthermore, a trend was observed towards negative association between this biomarker and personal PM_{2.5} exposure ($r=-0.21$, $P=0.09$) after controlling for the effect of proximity to the road.

Many studies consistently report the association of 8-*iso*-PGF_{2α} with smoking. The levels of this biomarker were reported to be higher in smokers than in nonsmokers (191-195), associated with smoking intensity (196), and declining after smoking cessation (196,197). A recent study investigated the longitudinal stability of 8-*iso*-PGF_{2α} over a period of 20 weeks in a large multi-site clinical trial (198). The intra-class correlation coefficient for urinary 8-*iso*-PGF_{2α} was 0.51 (95% CI, 0.45–0.57), indicating fair longitudinal stability of this biomarker in cigarette smokers. This is an important result because another recent study reported a significant association between prospectively measured 8-*iso*-PGF_{2α} and lung cancer incidence in cigarette smokers (199).

DNA adducts

Many chemical toxicants and carcinogens present in polluted air and cigarette smoke are capable of forming DNA adducts, either directly (e.g., aldehydes) or following metabolic activation to reactive electrophiles (e.g., PAH). Furthermore, oxidative stress and inflammation also result in the formation of a wide range of DNA adducts. Such DNA modifications, if not repaired efficiently, can lead to miscoding and mutations in oncogenes and/or tumor suppressor genes, and therefore play a key role in carcinogenic outcomes associated with exposures to air pollution and cigarette smoke (200). Therefore, analysis of DNA adducts in exposed individuals offers a more relevant measure of harm and subsequent health risk caused by chemical constituents present in polluted air and cigarette smoke, as well as oxidative damage and inflammation triggered by such exposures.

Studies of DNA adduct formation in response to

environmental exposures widely employed the ³²P-postlabeling approach to measure “bulky” DNA adducts formed by a range of chemical carcinogens, particularly PAH (201,202). Such studies generally report increases in the levels of bulky adducts in response to air pollution and smoking (201,203-206). However, ³²P-postlabeling lacks specificity, which undermines the identity and the accuracy of quantitation of the adducts measured by this technique. More selective and sensitive MS-based assays have been developed for the analysis of individual DNA adducts, and studies employing such assays will be discussed here.

DNA adducts derived from specific chemicals

PAH

The most studied PAH-derived adduct is BPDE-*N*²-dG, which is formed by BaP (207-210). One approach to measure this adduct is to analyze *trans*, *anti*-BaP-T released upon DNA hydrolysis (207). As an example, this method was used to show an association of BaP-DNA adduct levels in cord blood and in utero exposure to secondhand tobacco smoke with decreased fetal growth and reductions in cognitive development among children in the World Trade Center Cohort (211,212). However, the specificity of this method has been questioned, given that the frequency of BPDE-*N*²-dG detection in human samples by the more selective and sensitive MS-based assays is very low (213-216). Recently, an ultrasensitive LC-nanoelectrospray ionization (NSI)-high-resolution tandem mass spectrometry (HRMS/MS) method was developed for the analysis of BPDE-*N*²-dG and applied to the analysis of human lung samples obtained during surgery for lung cancer (217). In that study, smokers had approximately 3 times higher levels of BPDE-*N*²-dG in their lungs than nonsmokers, and the measured levels of the adduct were ~1,000-fold lower than those measured in previous studies by the immunochemical technique (208). Another potential approach to the analysis of DNA damage caused by exposure to PAH is the analysis of depurinated adducted DNA bases in urine. For instance, a study of such urinary biomarkers analyzed by LC-MS/MS showed that levels of BaP-DNA bases were much higher in the urine of women exposed to coal smoke than in smokers' urine, and that the two exposures produced very different urinary biomarker profiles (218).

Aldehydes

Human exposure to airborne aldehydes can occur through exposures to automobile exhaust, heated cooking oil,

cigarette smoke, and certain occupational exposures (219). While the presence of DNA adducts induced by acrolein, formaldehyde, acetaldehyde, and Cro in humans has been reported in several studies, application of such biomarkers in environmental exposure studies is relatively limited. Reaction of acrolein with DNA leads to the formation of 1,*N*²-propanodeoxyguanosine adducts α -OH-Acr-dG and γ -OH-Acr-dG, of which α -OH-Acr-dG has higher mutagenicity but γ -OH-Acr-dG is the predominant isomer detected in human DNA from leukocytes, liver, and lung (220,221). Studies that employed mass spectrometry-based techniques showed no significant difference in the levels of these adducts between smokers and nonsmokers (220,222). The most abundant product of the reaction of formaldehyde with DNA is *N*⁶-hydroxymethyldeoxyadenosine (HOMe-dA). Our group was the first to demonstrate the presence of this adduct in human leukocyte DNA, showing much higher levels in smokers (mean \pm SD, 179 \pm 205 fmol/ μ mol dAdo) compared to nonsmokers (15.5 \pm 33.8 fmol/ μ mol dAdo; *P*<0.001) (27). Results from another study showed a similar trend toward higher levels of adducts in smokers, however, the difference was not statistically significant (222). The major acetaldehyde DNA adduct is *N*²-ethylidenedeoxyguanosine (*N*²-ethylidene-dG). In addition, similar to Cro, acetaldehyde can form Cro-dG adducts. Both types of adducts can be measured in human tissues and leukocyte DNA by LC-MS/MS-based methods (223,224). Levels of *N*²-ethylidene-dG have been shown to decrease after smoking cessation (225); however, the difference in the levels in oral cells from smokers and nonsmokers did not reach statistical significance (222). On the other hand, analysis of Cro-dG in human saliva by LC-MS/MS in the same study showed clear differences between smokers and nonsmokers (222). Both adduct measurements have been used in air pollution studies. A study of the effect of Chinese-style wok cooking in nonsmoking Chinese women showed no difference in *N*²-ethylidene-dG between women who engaged in regular home cooking and controls, despite elevated exposures to a range of volatile toxicants (85). In another study, higher levels of urinary Cro-dG were observed in subjects exposed to urban air pollution compared to controls (*P*<0.05) (226).

Other organic compounds

Examples of other DNA damaging toxicants that are present in polluted air or cigarette smoke include aromatic amines, 1,3-butadiene, and acrylamide. The aromatic amine 4-aminobiphenyl (4-ABP) is a known bladder carcinogen

in both animals and humans and forms the DNA adducts 4-ABP-C8-dG (major), 4-ABP-C8-dA, and 4-ABP-*N*²-dG (227,228). In contrast to the reported data generated by ³²P-postlabeling methods, the frequency of detection of these adducts by LC-MS/MS in human tissues is relatively low (215,228-230). The most abundant adduct of 1,3-butadiene is 7-(2,3,4-trihydroxybut-1-yl)guanine (7-THBG). Levels of this adduct are not different between smokers and nonsmokers and do not change in smokers after smoking cessation; however, occupational exposure to 1,3-butadiene leads to higher levels of 7-THBG (231). A major DNA adduct derived from acrylamide exposure is 7-(2-carbamoyl-2-hydroxyethyl)guanine. Similar to 7-THBG findings, measurement of this adduct in human urine did not produce a statistically significant difference between smokers and nonsmokers, but increased levels were measured in workers occupationally exposed to acrylamide (232,233).

DNA adducts derived from oxidative damage

A comprehensive review of oxidative stress-induced DNA damage was published recently (234). The most widely studied DNA adduct is 8-oxo-dG, which can be formed via direct oxidation of DNA by oxidizing agents present in polluted air and cigarette smoke. This highly mutagenic adduct was detected in leukocytes and various human tissues. Studies employing HPLC with electrochemical detection report positive associations between air pollution and 8-oxo-dG. For instance, a study of students living and studying in Copenhagen showed that personal exposure to PM_{2.5} was predictive of 8-oxo-dG levels in lymphocyte DNA, with an 11% increase in 8-oxo-dG per 10 g/m³ increase in PM_{2.5} exposure (163). In another study, the levels of this adduct were significantly higher in asbestos-exposed workers compared to the non-exposed control group (235). A subsequent study in the same population reported no association between 8-oxo-dG and a wide range of potential confounding factors, further supporting the role of asbestos exposure in elevated oxidative DNA damage in exposed workers (236). However, studies employing mass spectrometry-based methods for the investigation of the impact of air pollution or smoking on 8-oxo-dG in DNA (as opposed to urine or plasma) are relatively scarce. A study that investigated the effect of air pollution on 8-oxo-dG measured in human leukocytes by LC-MS/MS observed some associations between exposure and the adduct level; however the effect was different across the three locations included in the study (237).

In addition to interaction of oxidizing agents with DNA, processes such as lipid peroxidation can lead to DNA adduct formation. As an example, 4-hydroxy-2-nonenal, a product of lipid peroxidation, can form the promutagenic adducts 1,*N*⁶-etheno-2-deoxyadenosine (ϵ dA), and 3,*N*⁷-etheno-2'-deoxycytidine (ϵ dC). A study by LC-ESI-MS/MS showed that levels of ϵ dA and ϵ dC in urine of workers exposed to diesel engine exhaust were higher than in the control group, while there was no significant contribution of smoking to the levels of these adducts (238). Another product of lipid peroxidation—malondialdehyde—reacts with DNA to form 3-(2-deoxy- β -d-erythro-pentafulanosyl)pyrimido[1,2-*a*]purin-10(3*H*)-one deoxyguanosine (*M*₁dG) (239). Several studies demonstrated that increased *M*₁dG formation is associated with occupational and environmental exposures to air pollution, smoking, increased cancer risk, and tumor progression (240-244). However, most of these studies used the ³²P-postlabeling technique, and new studies using MS-based assays are needed. A recently developed LC-NSI-HRMS/MS method for the analysis of *M*₁dG in human leukocyte DNA showed no difference in the adduct levels between smokers and nonsmokers (245).

Metals

Particles in polluted air and cigarette smoke can contain metals and metalloids on their surface, including those capable of inducing oxidative stress either directly or through the Fenton reaction (121,246,247). Mechanistic and laboratory animal studies show that such exposures can induce inflammatory processes in lung tissues (247-249). Multiple studies of environmental and occupational exposures to metals investigated the association of such exposures with oxidative stress and inflammation. Metal exposures are typically measured by their analysis in urine or blood by either inductively coupled plasma MS (ICP-MS) or atomic absorption spectroscopy (AAS) methods (250-252). For example, Hu *et al.* studied the effect of occupational exposure to As on oxidative stress in semiconductor workers by analyzing levels of urinary inorganic arsenic (*iAs*³⁺, *iAs*⁵⁺), monomethylarsonic acid, and dimethylarsinic acid by HPLC with flow injection AAS, and urinary 8-OH-dG by LC-MS/MS (250). Their results showed elevated levels of these biomarkers in exposed workers compared to controls, and an association between urinary 8-OH-dG and As biomarkers. The impact of As exposure on inflammatory processes was also observed in copper smelters in Poland (253). Studies of non-

ferrous metal smelter workers who were occupationally exposed to Pb and/or Cd showed an association between the levels of these metals in various biological matrices and α -glutathione-*S*-transferase, a biomarker of proximal tubular injury in nephrotoxicity (254,255). Another study of the effects of As, Cd, Cr, Ni, and Pb exposure in male coke-oven workers in China showed an association between urinary levels of such metals and biomarkers of oxidative stress, as well as an effect of interaction between metal and PAH exposure on oxidative markers (256).

Environmental exposures to metals have also been widely investigated. A cohort panel study involving 97 elderly subjects living in the Los Angeles metropolitan area showed significant positive associations between biomarkers of airway oxidative stress and inflammation and traffic pollution-related metal exposures (257). Metallothionein levels in blood were used as a biomarker of exposure to metals in local fishermen in Abu Qir Bay, Egypt (258). The study showed high levels of metallothionein in fishermen's blood, and its association with the levels of Cd, Cu, Pb, Cr, and Zn. Metabolomics approaches were also employed to investigate the impact of environmental metal exposures on health outcomes. A study of highway commuters applied LC-MS high-resolution metabolomics to analyze the relationship between in-vehicle particulate exposures to Al, Pb, and Fe and metabolomic profiles as well as targeted inflammatory biomarker levels (259). The measured exposures and changes in inflammatory biomarkers were associated with metabolomics-identified molecular alterations that reflected changes in arachidonic acid, leukotrienes, and tryptophan metabolism, suggesting that traffic-associated exposures to metals may induce a broad inflammatory response. Another metabolomics study also showed that increased environmental metal exposure was associated with various oxidative stress-related effects, such as antioxidant depletion, increased lipid peroxidation, and changes in mitochondrial lipid metabolism (260). Other studies are also supportive of the role of environmental metal exposures in inflammatory processes and the associated health outcomes, including cardiovascular and neurotoxic effects (261-263).

Childhood exposures to metals were also studied. The effect of exposure to As and Cd and urinary 8-OH-dG was shown in environmentally exposed pregnant women in rural Bangladesh, indicating that such exposures may play a role in birth outcomes (252). A study of Cr exposure in households adjacent to Cr waste sites in New Jersey showed a statistically significant relationship between

log-transformed urine Cr concentration and Cr dust concentration, and age-stratified analyses revealed that exposures in young children accounted for much of the relationship in the entire population (264). Dust levels of Pb were associated with blood biomarker levels in infants near the Tar Creek Superfund Site (Oklahoma, USA) (265). Deciduous teeth also have been used for the investigation of in-utero and early childhood exposures to Pb, suggesting airborne pollution as the primary source and exposure pathway (266). Analysis of Pb and Cd in blood of preschool children living in the vicinity of industrially contaminated sites in Poland showed higher levels of these biomarkers in children living closer to the sources of exposure as well as in those living with mothers who smoke at home (267). A targeted study of the effect of secondhand smoke exposure on metal uptake in children analyzed urinary levels of 23 trace elements (Li, Be, B, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Rb, Sr, Cd, Sn, Sb, Te, Cs, Tl, Pb, Bi, U) in a sample of Italian schoolchildren, showing an independent association between smoke exposures at home and urinary levels of Li, Ti, V, Co, Ga and Sr (251). A study in Sweden also showed a positive association between urinary Cd and Pb and the levels of urinary cotinine, a biomarker of nicotine intake, in secondhand smoke-exposed children (268).

Distinguishing exposures from cigarette smoking versus air pollution

A particularly important question is how to use biomarkers to separate the potential health impacts of cigarette smoking from those of ambient air pollution in epidemiological studies. Cigarette smoke is frequently the major source of exposure to carcinogenic combustion products such as PAH, benzene, and metals which are also important constituents of polluted air. Furthermore, cigarette smoke is an established cause of oxidative damage. In this section, we discuss several well-established biomarkers—cotinine, total nicotine equivalents, anatabine, anabasine, and NNAL—which are specifically related to cigarette smoking and related practices and can be used to assess the role of smoking in toxicant and carcinogen exposure.

Cotinine

Nicotine is the critical constituent of tobacco products that is responsible for their addictive properties (269). Nicotine is not a particularly useful biomarker due to its relatively short half-life of approximately 2 h. Cotinine is the major

metabolite of nicotine and a useful biomarker because of its relatively long half-life (8–30 h). Cotinine can be quantified in a number of readily available biological fluids including blood, saliva, and urine. Large studies have investigated levels of cotinine that distinguish cigarette smokers from non-smokers who may be exposed to secondhand smoke, usually in indoor environments. For example, the NHANES study from 1990 to 2004 concluded that a serum cotinine level of 3 ng/mL distinguished cigarette smokers from non-smokers, while a study in the United Kingdom determined a level of 12 ng/mL in saliva (270,271). A free cotinine level of about 30 ng/mL urine distinguishes smokers from non-smokers.

There are other sources of nicotine exposure that need to be considered. These include the use of nicotine replacement products and e-cigarettes, which both deliver substantial amounts of nicotine resulting in cotinine levels similar to those observed in cigarette smokers. However, these products do not deliver PAH or most volatiles in significant amounts.

Total nicotine equivalents

Total nicotine equivalents, determined in urine by LC-MS/MS, encompasses nicotine, cotinine, and 3'-hydroxycotinine plus their glucuronides, and in some cases nicotine-*N*-oxide and other minor metabolites as well (272). Total nicotine equivalents are considered the gold standard for determining nicotine dose because all major metabolites are quantified. For the purpose of distinguishing exposure to cigarette smoke versus air pollution, it does not have any significant advantages over cotinine, and has similar limitations such as use of other nicotine containing products.

Anatabine and anabasine

Anatabine and anabasine are minor tobacco alkaloids which are present in all tobacco products but not in nicotine replacement products. Therefore, analysis of these compounds in urine by LC-MS/MS can confirm tobacco product use. Mean levels of anatabine, anabasine, and total nicotine equivalents in the urine of 827 smokers were 39.9 pmol/mL, 52.8 pmol/mL, and 56.4 nmol/mL, respectively. Levels of anatabine and anabasine correlated with those of total nicotine equivalent (273).

NNAL

NNAL is a metabolite of the tobacco-specific carcinogen

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is found exclusively in tobacco products, not in nicotine replacement products or electronic cigarette vapor (274). NNAL and its glucuronides, termed total NNAL, are major metabolites of NNK in all vertebrates including humans. Total NNAL is most commonly measured in urine by LC-MS/MS and can be used to distinguish tobacco use or secondhand smoke exposure from environmental pollution because of its absolute specificity to tobacco products. Another advantage of NNAL as a biomarker is its relatively long urinary half-life of 10–45 days. Secondhand smoke exposure typically results in urinary NNAL levels of 0.03–0.08 pmol/mL (274).

Conclusions

We have presented a summary of the most important and widely used chemically specific biomarkers which can be applied in molecular epidemiology studies of air pollution and cancer. We have focused on biomarkers which can be determined by state-of-the-art analytical chemistry methods, mostly using mass spectrometry. All methods discussed here have been widely applied and are fully validated. We conclude that the following chemically specific biomarkers are currently the optimal ones for use in studies of air pollution and cancer: urinary 1-OHP, Phe metabolites, SPMA, urinary or blood Cd, 8-OH-dG and 8-*iso*-PGF_{2α}. This suite of biomarkers will reliably establish exposure to carcinogenic PAH, benzene and Cd, and will also provide critical information on oxidative damage and inflammation, both of which are important in carcinogenesis. Furthermore, we strongly recommend analysis of the same samples for cotinine or NNAL in order to reliably assess the contribution of tobacco use.

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Table S1 Concentrations of pyrene, phenanthrene and benzo[a]pyrene in cigarette smoke, air and food

Sample type	Concentration [mean ± SD (range)]	Reference
Pyrene		
Mainstream smoke (ng/cigarette)	42±7.1 (8.1±0.9–70±13)	(275)
	37±19 [11–91]	(276)
Indoor/outdoor air (ng/m ³)	0.33 (ND–24.0)/1.59 (0.05–208) (UK, 2011)	(277)
	0.877/0.121 (Los Angeles, US, 2002)	(278)
	1.114/0.8775 (Houston, US, 2002)	(278)
	1.148/0.645 (Elizabeth, US, 2002)	(278)
	108.3 (3.6–272.6)/66.9 (10.4–547.8) (China, 2007)	(279)
Grilled meat (ng/g)	9.23 (ND–82.9)	(280)
Fried meat (ng/g)	2.74 (ND–22.6)	(280)
Phenanthrene		
Mainstream smoke (ng/cigarette)	159±23 (28±2.2–431±39)	(275)
	119±66 [34–307]	(276)
Indoor/outdoor air (ng/m ³)	0.34 (ND–6.42)/1.01 (0.01–30.7) (UK, 2011)	(277)
	6.832/1.01 (Los Angeles, US, 2002)	(278)
	10.019/5.411 (Houston, US, 2002)	(278)
	13.054/6.561 (Elizabeth, US, 2002)	(278)
	351.7 (135.2–963.4)/181.4 (49.3–969.7) (China, 2007)	(279)
Grilled meat (ng/g)	11.1 (ND–80.3)	(280)
Fried meat (ng/g)	5.89 (ND–15.7)	(280)
Benzo[a]pyrene		
Mainstream smoke (ng/cigarette)	10±1.2 (2.1±1.0–22±0.8)	(275)
	15±9 [4–44]	(276)
	8.8–15.8	(281)
Indoor/outdoor air (ng/m ³)	0.1 (ND–4.91)/0.19 (ND–2.52) (UK, 2011)	(277)
	0.0137/0.0061 (Los Angeles, US, 2002)	(278)
	0.0067/0.0068 (Houston, US, 2002)	(278)
	0.0055/0.045 (Elizabeth, US, 2002)	(278)
	95.7 (7.7–380.3)/27.9 (0.9–271.5) (China, 2007)	(279)
Grilled meat (ng/g)	0.33 (ND–2.18)	(280)
Fried meat (ng/g)	0.39 (ND–1.95)	(280)

SD, standard deviation; ND, not detectable.

Table S2 Urinary 3-OHBaP concentrations in various subjects in recent studies

Subjects	Country	3-OHBaP level	Reference
Prebake aluminum electrode production workers	France		(282)
Pre-shift (N=6)		0.29±0.28 nmol/mol creatinine (mean ± SD)	
Post-shift (N=6)		0.23±0.21 nmol/mol creatinine	
Non-occupational exposure population	France		(14)
Smokers (N=13)		0.029±0.024 nmol/mol creatinine (mean ± SD)	
Nonsmokers (N=23)		0.010±0.005 nmol/mol creatinine	
Non-occupational exposure population	Italy		(52)
All subjects (N=200)		0.03±0.05 nmol/L (mean ± SD)	
Smokers (N=39)		0.04±0.07 nmol/L	
Nonsmokers (N=97)		0.03±0.04 nmol/L	
Exsmokers (N=64)		0.03±0.04 nmol/L	
Non-occupational exposure population	Italy		(283)
All subjects (N=1,016)		0.079 (0.0268–32.0) ng/g creatinine [median (5 th –95 th) percentile]	
Smokers (N=269)		0.068 (0.0231–32.0) ng/g creatinine	
Nonsmokers (N=747)		0.084 (0.0282–32.0) ng/g creatinine	
Smokers (N=7)	Germany	0.606±0.220 ng/g creatinine (mean ± SD)	(50)
Nonsmokers (N=7)		0.115±0.066 ng/g creatinine	

3-OHBaP, 3-hydroxybenzo[a]pyrene; SD, standard deviation.

Table S3 Urinary phenanthrol concentrations in various subjects in recent studies

Country	Subject categories	Analytes	Levels	Reference
China	Coke oven workers (nonsmokers)			(64)
		Control group (N=384)	1-OHPhe 0.61 (0.05–3.36) µg/mmol creatinine [median (5–95%)]	
		2-OHPhe 0.25 (0.04–1.48) µg/mmol creatinine		
		3-OHPhe 0.27 (0.03–1.17) µg/mmol creatinine		
		4-OHPhe 0.39 (0.00–1.76) µg/mmol creatinine		
		9-OHPhe 0.60 (0.10–4.27) µg/mmol creatinine		
	Low exposure group (N=545)	1-OHPhe 0.70 (0.11–3.46) µg/mmol creatinine		
		2-OHPhe 0.31 (0.05–1.19) µg/mmol creatinine		
		3-OHPhe 0.33 (0.03–1.19) µg/mmol creatinine		
		4-OHPhe 0.33 (0.00–1.80) µg/mmol creatinine		
		9-OHPhe 0.65 (0.16–4.30) µg/mmol creatinine		
	Intermediate exposure group (N=325)	1-OHPhe 1.11 (0.24–6.13) µg/mmol creatinine		
		2-OHPhe 0.39 (0.10–1.77) µg/mmol creatinine		
		3-OHPhe 0.50 (0.04–1.54) µg/mmol creatinine		
		4-OHPhe 0.32 (0.00–2.44) µg/mmol creatinine		
		9-OHPhe 0.84 (0.22–3.90) µg/mmol creatinine		
	High exposure group (N=79)	1-OHPhe 1.73 (0.26–5.02) µg/mmol creatinine		
		2-OHPhe 0.76 (0.24–2.73) µg/mmol creatinine		
		3-OHPhe 1.13 (0.28–4.45) µg/mmol creatinine		
		4-OHPhe 0.40 (0.03–2.97) µg/mmol creatinine		
9-OHPhe 0.98 (0.16–3.64) µg/mmol creatinine				
China	General population (N=84)	2-OHPhe 581 [323] pg/mL [mean (median)]	(31)	
		3-OHPhe 714 [387] pg/mL		
		4-OHPhe 351 [217] pg/mL		
		9-OHPhe 83 [33] pg/mL		
U.S.	Mexican Americans (N=317)	1-OHPhe 115 (91.3–146) pg/mL [GM (95% CI)]	(30)	
		2-OHPhe 56.3 (43.9–72.1) pg/mL		
		3-OHPhe 54.8 (42.1–71.2) pg/mL		
		4-OHPhe 19.7 (16.2–24.0) pg/mL		
	Non-Hispanic blacks (N=664)	1-OHPhe 141 [129–155] pg/mL		
		2-OHPhe 78.9 (72.3–86.2) pg/mL		
		3-OHPhe 92.6 (84.3–102) pg/mL		
		4-OHPhe 28.4 (26.0–31.0) pg/mL		
	Non-Hispanic whites (N=814)	1-OHPhe 126 [117–136] pg/mL		
		2-OHPhe 58.3 (54.7–62.2) pg/mL		
		3-OHPhe 58.2 (54.3–62.4) pg/mL		
		4-OHPhe 19.4 (18.3–20.5) pg/mL		
	All Hispanics (N=573)	1-OHPhe 125 [108–146] pg/mL		
		2-OHPhe 62.2 (52.7–73.4) pg/mL		
		3-OHPhe 61.3 (51.2–73.3) pg/mL		
		4-OHPhe 21.0 (18.5–23.8) pg/mL		
	Asians (N=352)	1-OHPhe 91.3 (75.3–111) pg/mL		
		2-OHPhe 50.3 (40.5–62.4) pg/mL		
		3-OHPhe 50.0 (39.8–62.9) pg/mL		
		4-OHPhe 16.8 (14.7–19.1) pg/mL		
African Americans (N=358)	3-OHPhe 1.06 (0.984–1.14) pmol/mL	(284)		
	Native Hawaiians (N=321)		3-OHPhe 0.605 (0.559–0.654) pmol/mL	
	Whites (N=432)		3-OHPhe 0.713 (0.667–0.762) pmol/mL	
	Latinos (N=448)		3-OHPhe 0.784 (0.734–0.837) pmol/mL	
Japanese Americans (N=695)	3-OHPhe 0.593 (0.562–0.626) pmol/mL			
Iran	Never tobacco users (N=58)	1-OHPhe 247.1 (213.9–285.5) ng/g creatine [GM (95% CI)]	(29)	
		∑2,3-OHPhe 300.3 (250.4–360.3) ng/g creatine		
	Exclusive cigarette smokers (N=33)	1-OHPhe 264.5 (227.4–307.8) ng/g creatine		
		∑2,3-OHPhe 482.9 (371.5–627.6) ng/g creatine		
	Exclusive waterpipe smokers (N=37)	1-OHPhe 392.0 (309.6–496.4) ng/g creatine		
		∑2,3-OHPhe 753.0 (530.0–1069.8) ng/g creatine		
Exclusive nass smokers (N=30)	1-OHPhe 223.6 (181.6–275.2) ng/g creatine			
	∑2,3-OHPhe 407.7 (275.2–604.1) ng/g creatine			
U.S.	Cigarette smokers (N=885)	1-OHPhe 197 [178–218] pg/mL [GM (95% CI)]	(33)	
		2-OHPhe 112 [102–122] pg/mL		
		3-OHPhe 138 [120–158] pg/mL		
		4-OHPhe 38.2 (35.3–41.3) pg/mL		
	Nonsmokers (N=1,300)	1-OHPhe 114 [106–123] pg/mL		
		2-OHPhe 53.2 (49.5–57.2) pg/mL		
		3-OHPhe 48.0 (44.2–52.2) pg/mL		
		4-OHPhe 17.4 (16.3–18.7) pg/mL		

Phe, phenanthrene; CI, confidence interval; GM, geometric mean.

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