Review Article

Carcinogenicity of ambient air pollution: use of biomarkers, lessons learnt and future directions

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Abstract: The association between ambient air pollution (AAP) exposure and lung cancer risk has been investigated in prospective studies and the results are generally consistent, indicating that long-term exposure to air pollution can cause lung cancer. Biomarkers can enhance research on the health effects of air pollution by improving exposure assessment, increasing the understanding of mechanisms, and enabling the investigation of individual susceptibility. In this review, we assess DNA adducts as biomarkers of exposure to AAP and early biological effect, and DNA methylation as biomarker of early biological change and discuss critical issues arising from their incorporation in AAP health impact evaluations, such as confounding, individual susceptibilities, timing, intensity and duration of exposure, and investigated tissue. DNA adducts and DNA methylation are treated as paradigms. However, the lessons, learned from their use in the examination of AAP carcinogenicity, can be applied to investigations of other biomarkers involved in AAP carcinogenicity.

Keywords: Carcinogenicity; biomarkers; ambient air pollution (AAP); lung cancer; DNA adducts; DNA methylation

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Exposure to air pollution and lung cancer risk

Environmental pollution encompasses a number of hazardous exposures including air, water, and chemical exposures. Ambient (outdoor) and household (indoor) air pollution is a major environmental health risk, affecting populations in developed and developing countries alike (1).

Ambient air pollution (AAP) consists of emissions of complex mixtures of air pollutants from industries, households, cars and trucks (2). Of these pollutants, fine particulate matter (PM) has been widely shown to have adverse effects on human health. Most fine PM comes from fuel combustion, both from mobile sources such as vehicles and from stationary sources such as power plants, industry, households or biomass burning (2). PM can vary in size from ultra-fine particles (UFP) ≤100 nm in diameter, to fine particles ~100 nm -2.5 μm in diameter ($PM_{2.5}$), to larger particles up to 10 μm in diameter (PM_{10}) (3), and differential PM sizes can affect pathophysiological pathways independently (3).

On the other hand, household air pollution (HAP) is the result of cooking and heating households using solid fuels (i.e., wood, charcoal, coal, dung, crop wastes) on open fires or traditional stoves. In poorly ventilated dwellings, fine PM concentrations in and around the home can exceed acceptable levels for up to 100-fold (4).

Health risks associated with air pollution include but are not limited to stroke, heart disease, lung cancer, and both chronic and acute respiratory diseases, including asthma (5-8). AAP and its health effects are much more frequently studied compared to HAP.

Of the AAP health effects, lung cancer contributes greatly to air pollution associated mortality. The association between exposure to AAP and lung cancer incidence and/or mortality has been evaluated in a number of prospective

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studies, which are summarized in *Table 1*. Despite that formal statistical significance was not always reached, the evidence linking exposure to urban air pollutants, mainly $PM_{2.5}$ or PM_{10}, and lung cancer is generally consistent. Cohorts from the United States as well as from Europe have found increased risks of lung cancer with higher exposure to PM and other substances present in polluted air, with statistically significant risk ratios (RRs) ranging from 1.14 to 5.21 (*Table 1*).

Based on the available epidemiological and molecular evidence, the International Agency for Research on Cancer (IARC) has recently classified air pollution as Carcinogenic to Humans (Group 1) (37).

Incorporation of biomarkers in measuring exposure and evaluating health effects

Biomarkers were introduced in the study of the carcinogenic effects of AAP under the assumption that they could enhance research on the health effects of air pollution, and other exposures, by improving exposure assessment, increasing the understanding of mechanisms (e.g., by measuring intermediate biomarkers), and enabling the investigation of individual susceptibility.

Biomarkers used in the epidemiology of cancer are usually divided into three categories: markers of internal dose, markers of early response, and markers of susceptibility. In fact, each category includes subcategories. For example, protein adducts and DNA adducts are both markers of internal dose, but their biological significance differs. While protein adducts are not repaired (i.e., they reflect external exposure more faithfully), DNA adducts are influenced by an individual's repair capacity. If DNA adducts are not eliminated by the DNA repair machinery, they induce a mutation. Also, markers of early response are a heterogeneous category that encompasses DNA mutations and gross chromosomal damage. The main advantage of early response markers is that they are more frequent than the disease and can be recognized sooner, thus allowing researchers to identify earlier effects of potentially carcinogenic exposures. Finally, markers of susceptibility include several subcategories; in particular, a type of genetic susceptibility related to the metabolism of carcinogenic substances, and another type related to DNA repair.

Because of their ability to highlight mechanisms, improve exposure assessment, and reflect individual susceptibility, biomarkers have been and will continue to play a vital role in the investigation of the carcinogenicity of AAP. In this review, we assess DNA adducts as biomarkers of exposure and early biological effect, and DNA methylation as biomarker of early biological change and discuss critical issues arising from their incorporation in health impact evaluations. DNA adducts and DNA methylation are treated here as paradigms, and the lessons learned from their use in the examination of AAP carcinogenicity, can also be applied to investigations of other biomarkers involved in AAP carcinogenicity.

DNA adducts

DNA adducts are covalent bonds arising from the interaction of cancer causing chemicals such as polycyclic aromatic hydrocarbons (PAHs), or metabolites of such chemicals, with sites in DNA (38). Even though adducts can be removed by repair proteins, some can persist, and can contribute to cancer development by causing nucleotide substitutions, deletions and chromosome rearrangements during replication (38).

Several studies have considered DNA adducts as biomarkers of exposure to genotoxic carcinogens, such as PAHs, present in AAP, employing cross-sectional and case-control study designs, some nested within prospective cohorts. Studies which compared the mean DNA adduct levels in individuals with estimated high or low external exposures are summarized in *Table 2*, whereas studies which carried out correlation and regression analyses on all subjects are summarised in *Table 3* (52-66). The majority of studies and two reviews demonstrated positive associations between exposure to air pollution or chemicals in polluted air and the formation of DNA adducts in exposed individuals. Subjects in these studies included a wide range of occupationally and residentially exposed individuals, such as policemen in Bangkok (47), Genova (45), and Prague (49,66), school children in Thailand (50), residents in an industrial area and rural controls in Poland (39), bus and taxi drivers in Stockholm (40), bus drivers in Copenhagen (41), students in Denmark and in Greece (42), as well as street vendors, taxi drivers, gasoline salesmen and road side residents in Benin (51). Only two studies reported no association (54,67).

DNA adducts in children

Fetal exposures and DNA adducts in newborns also showed positive associations (44,53,65). Experimental evidence indicates that developing fetuses are more susceptible than adults to the carcinogenic effects of PAHs. To assess

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Table 1 Prospective study results on the relationship between exposure to air pollution and lung cancer incidence and/or mortality, listed by study or cohort

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
American studies							
American legion study							
Buell, 1967 (9)	USA	>10 years in LA county *vs.* other counties	Lung cancer mortality	Age, sex, smoking, size of birthplace	336,571 person-years	2.5	Not reported
		>10 *vs.* <10 years in LA county	Lung cancer mortality	Age, sex, smoking, size of birthplace		1.26	Not reported
ASHMOG study							
Mills, 1991 (10)	USA	Total suspended particulate (exceedance frequency of 200 μg/m^3)	Cancer in females incidence	Age, sex, education, ex-smoking, ETS, and occupational exposure	6,000	1.72	0.81-3.65
		Ozone (exceedance frequency of 10 pphm)	Lung cancer incidence	Age, sex, education, ex-smoking, ETS, and occupational exposure		2.25	0.96-5.31
Beeson, 1998 (11)	California, USA	Ozone (100 ppb increase)	Lung cancer incidence—males	Pack-year of past cigarette smoking, educational level, and current alcohol use	6,338	3.56	1.35-9.42
		PM_{10} (IQR increase)	Lung cancer incidence—males	Pack-year of past cigarette smoking, educational level, and current alcohol use		5.21	1.96-13.99
		SO_2 (IQR increases)	Lung cancer incidence—males	Pack-year of past cigarette smoking, educational level, and current alcohol use		2.66	1.62-4.39
		PM_{10} exceedance frequencies of 50 mg/m^3 (IQR increase)	Lung cancer incidence—females	Smoking, age		1.21	0.55-2.66
		PM_{10} exceedance frequencies of 60 mg/m^3 (IQR increase)	Lung cancer incidence—females	Smoking, age		1.25	0.57-2.71
		SO_2 (IQR increases)	Lung cancer incidence—females	Smoking, age		2.14	1.36-3.37

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Abbey, 1999 (12)	USA	PM_{10} (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use	6,338	3.36	1.57-7.19
		PM_{10} (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		1.33	0.60-1.96
		Ozone (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use		2.10	0.99-4.44
		Ozone (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		0.77	0.37-1.61
		SO_2 (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use		1.99	1.24-3.20
		SO_2 (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		3.01	1.88-4.84
		NO_2 (IQR increase in mean conc.)	Lung cancer mortality in males	Years of education, pack-years of ex smoking, alcohol use		1.82	0.93-3.57
		NO_2 (IQR increase in mean conc.)	Lung cancer mortality in females	Years of education and pack-years of past smoking		2.81	1.15-6.89
McDonnell, 2000 (13)	USA	$PM_{2.5}$ (IQR increase =24.3 μg/m^3)	Lung cancer mortality		6,338	2.23	0.56-8.94
		$PM_{2.5-10}$ (IQR increase =9.7 μg/m^3)	Lung cancer mortality			1.25	0.63-2.49
		PM_{10} (IQR increase = 29.5 μg/m^3)	Lung cancer mortality			1.84	0.59-5.67
American cancer prevention study II							
Pope, 2002 (14)	USA	NO_2 (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, race, smoking, education, marital status, body mass, alcohol comsumption, occupation, and diet	409-493 thousand	1.14	1.04-1.23
Jerrett, 2005 (15)	USA	PM_{10} (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, race, education, smoking, marital status, BMI, alcohol consumption, occupational exposure, diet, and other ecological variables	22,905	1.2	0.79-1.82

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
		Ozone (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, race, education, smoking, marital status, BMI, alcohol consumption, occupational exposure, diet, and other ecological variables		0.99	0.91-1.07
		Distance to freeways (<500 *vs.* >500 m)	Lung cancer mortality	Age, sex, race, education, smoking, marital status, BMI, alcohol consumption, occupational exposure, diet, and other ecological variables		1.44	0.94-2.21
Turner, 2011 (16)	USA	$PM_{2.5}$ (10 mg/m^3 increase) ACP	Lung cancer mortality	Age, sex, smoking, educational attainment, BMI, chronic lung disease	188,699	NA	1.15-1.27
Pope, 2011 (6)	USA	$PM_{2.5}$ (10 mg/m^3 increase)	Lung cancer mortality	Age, sex, education, marital status, body mass, alcohol consumption, occupational exposures, smoking duration, and diet	1.2 million	1.14	1.04-1.23
Harvard six cities study							
Dockery, 1993 (17)	USA	Inhalable particles: Most polluted *vs.* least polluted city	Lung cancer mortality	Age, sex, smoking, education, and BMI	8,111	1.27	1.08-1.48
		Fine particles: most polluted *vs.* least polluted city	Lung cancer mortality	Age, sex, smoking, education, and BMI		1.26	1.08-1.47
		Sulphate particles: most polluted *vs.* least polluted city	Lung cancer mortality	Age, sex, smoking, education, and BMI		1.26	1.08-1.47
Krewski, 2005 (18)	USA	$PM_{2.5}$ (most *vs.* least polluted city = 18.6 mg/m^3 increase)	Lung cancer mortality	Age, sex, smoking, education, BMI, diabetes, occupational exposure to dust, gases or fumes	8,111	1.43	0.85-2.41
Laden, 2006 (19)	USA	$PM_{2.5}$	Lung cancer mortality	Age, sex, smoking, education, and BMI	8,096	1.27	0.96-1.69

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Nurses' health study							
Puett, 2014 (20)	USA	72-month average exposures to: $PM_{2.5}$ (for 10 μg/m^3); $PM_{2.5-10}$ (for 10 μg/m^3); PM_{10} (for 10 μg/m^3)	Lung cancer incidence	Cohort restricted to never or quit smoking ≥10 years ago; adjusted for: BMI, alcohol consumption, physical activity, overall diet quality, smoking status (when not stratified by status) and pack-year, months since quitting smoking, secondhand smoke exposure at home, work, and during childhood, and census-tract median home value and median income	1,203,946 person-years	1.37; 1.11; 1.15	1.06-1.77; 0.90-1.37; 1.00-1.32
		Residential distance to major roads per 100 m	Lung cancer incidence	Cohort restricted to never or quit smoking ≥10 years ago Adjusted for: BMI, alcohol consumption, physical activity, overall diet quality, smoking status (when not stratified by status) and pack-years, months since quitting smoking, secondhand smoke exposure at home, work, and during childhood, and census-tract median home value and median income	1,203,946 person-years	0.99	0.95-1.04

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
European studies							
Cohort of Oslo men							
Nafstad, 2003 (21)	Norway	$NO_{(x)}$ (per 10 μg/m^3—home address)	Lung cancer incidence	Age, smoking habits, and length of education	16,209	1.08	1.02-1.15
		SO_2 (per 10 μg/m^3)	Lung cancer incidence	Age, smoking habits, and length of education		1.01	0.94-1.08
French PAARC study							
Filleul, 2005 (22)	France	Total suspended Particulate (exceedance frequency of 200 μg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education	14,284	0.97	0.94-1.01
		Black smoke (for 10 μg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.97	0.93-1.01
		NO (for 10 μg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.97	0.94-1.01
		NO_2 (for 10 μg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.97	0.85-1.10
		SO_2 (for 10 μg/m^3)	Lung cancer mortality	Age, sex, BMI, smoking, occupational exposure, education		0.99	0.92-1.07

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Genair cohort study							
Vineis, 2006 (23)	Ten European countries	PM_{10} (10 mg/m^3 increase)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine	197 cases; 556 controls	0.91	0.70-1.18
		NO_2 (10 mg/m^3 increase)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine		1.14	0.78-1.67
		SO_2 (10 mg/m^3 increase)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine		1.08	0.89-1.30
		Proximity of residence to major road (exposed *vs.* nonexposed)	Lung cancer incidence	Age, BMI, education, gender, smoking, alcohol use, intake of meat, intake of fruit and vegetables, time since recruitment, country, occupational index and cotinine		1.31	0.82-2.09

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Netherlands cohort study on diet and cancer							
Beelen, 2008 (24)	Netherlands	Black smoke concentration	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status	40,114	1.47	1.01-2.16
		Traffic intensity on nearest road	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status		1.11	0.88-1.41
		Living near a major road	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status		1.55	0.98-2.43
Brunekreef, 2009 (25)	Netherlands	Black smoke (per 10 μg/m^3)	Lung cancer mortality	Age, sex, smoking status, area-level socioeconomic status	120,000	1.03	0.88-1.20
		Traffic intensity (increase of 10,000 motor vehicles/day)	Lung cancer mortality	Age, sex, smoking status, area-level socioeconomic status		1.07	0.96-1.19
		Black smoke (per 10 μg/m^3)	Lung cancer incidence	Age, sex, smoking status, area-level socioeconomic status		1.47	1.01-2.16
Diet, cancer and health cohort study							
Raaschou-Nielsen, 2011 (26)	Denmark	NO_x at residence (per 100 μg/m^3 increase)	Lung cancer incidence	Age, smoking, ETS, length of school attendance, fruit intake, and employment	52,970	1.09	0.79-1.51
		Traffic load at residence (per 10^4 vehicle km/day)	Lung cancer incidence	Age, smoking, ETS, length of school attendance, fruit intake, and employment	52,970	1.03	0.90-1.19

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
ESCAPE project—17 European cohorts							
Raaschou-Nielsen, 2013 (27)	Europe	$PM_{2.5}$ (for 10 μg/m^3); PM_{10} (for 10 μg/m^3)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.18; 1.22	0.96-1.46; 1.03-1.45
		Road traffic within 100 m of the residence: (4,000 vehicle-km per day increase)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.09	0.91.21
		NO_x at residence (per 20 μg/m^3 increase)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.01	0.95-1.07
		Traffic intensity on the nearest street (5,000 vehicles per day increase)	Lung cancer incidence	Age, year of enrollment, sex, marital status, education level, occupation status, smoking status, years of smoking (among ever smokers), cigarettes/day (among current smokers), plus adjusted for area-level variables such as deprivation index, median income rate etc.	312,944	1.00	0.97-1.04

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
Three prospective cohorts							
Raaschou-Nielsen, 2010 (28)	Denmark	NO_x (30-72 *vs.* <30 μg/m^3)	Lung cancer incidence	Smoking (status, duration, and intensity), educational level, body mass index, and alcohol consumption	679 cases; 3,481 controls	1.30	1.07-1.57
		NO_x (>72 *vs.* <30 μg/m^3)	Lung cancer incidence	Smoking (status, duration, and intensity), educational level, body mass index, and alcohol consumption		1.45	1.12-1.88
Other studies							
Pope, 1995 (29)	USA	Most *vs.* least polluted: sulphates	Lung cancer mortality	Smoking	552,138	1.15	1.09-1.22
		Most *vs.* least polluted: fine particles	Lung cancer mortality	Smoking		1.17	1.09-1.26
Yorifuji, 2010 (30)	Japan	NO_2 (10 mg/m^3 increase)	Lung cancer mortality—non smokers	Smoking	14,001	1.3	0.85-1.93
Katanoda, 2011 (31)	Japan	$PM_{2.5}$ (10 mg/m^3 increase)	Lung cancer mortality	Sex, age, smoking status, pack-years, smoking status of family members living together, daily green and yellow vegetable consumption, daily fruit consumption, and use of indoor charcoal or briquette braziers for heating	63,520	1.24	1.12-1.37
		NO_2 (10 mg/m^3 increase)	Lung cancer mortality	Sex, age, smoking status, pack-years, smoking status of family members living together, daily green and yellow vegetable consumption, daily fruit consumption, and use of indoor charcoal or briquette braziers for heating	63,520	1.26	1.07-1.48

Table 1 (*continued*)

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Table 1 (*continued*)

First author, year	Area/country	Exposure	Outcome	Controlled confounders	Number of subjects	RR	95% CI
		SO_2 (10 mg/m^3 increase)	Lung cancer mortality	Sex, age, smoking status, pack-years, smoking status of family members living together, daily green and yellow vegetable consumption, daily fruit consumption, and use of indoor charcoal or briquette braziers for heating	63,520	1.17	1.10-1.26
Hales, 2012 (32)	New Zealand	PM_{10} (1 mg/m^3 increase)	Lung cancer mortality	Age, sex, ethnicity	50 222	1.015	0.004-1.026
Carey, 2013 (33)	England	$PM_{2.5}$ (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.04	0.99-1.09
		PM_{10} (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.03	0.98-1.08
		NO_2 (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.11	1.05-1.17
		SO_2 (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	1.03	0.99-1.06
		O_3 (IQR increase)	Lung cancer mortality	Age, sex, smoking, BMI, education	835,607	0.94	0.90-0.98
Cesaroni, 2013 (34)	Italy	$PM_{2.5}$ (10 mg/m^3 increase)	Lung cancer mortality	Sex, marital status, place of birth, education, occupation, and area-based socioeconomic position.	1,265,058	1.05	1.01-1.10
		NO_2	Lung cancer mortality	Sex, marital status, place of birth, education, occupation, and area-based socioeconomic position.	1,265,058	1.04	1.02-1.07
Heinrich, 2013 (35)	Germany	PM_{10} (IQR increase = 7 mg/m^3)	Lung cancer mortality	Sex, education, smoking status	4,800	1.84	1.23-2.74
		NO_2 (IQR increase = 16 mg/m^3)	Lung cancer mortality	Sex, education, smoking status	4,800	1.46	0.92-2.32
Yorifuji, 2013 (36)	Japan	NO_2 (10 mg/m^3)	Lung cancer mortality	Not available	14,001	1.20	1.03-1.40

RR, risk ratio; CI, confidence interval; ETS, environmental tobacco smoke; IQR, interquartile range; PM_{10}, particulate matter with diameter of less than 10 microns; $PM_{2.5}$, particulate matter with diameter of less than 2.5 microns; NO_x, nitrogen oxides with unspecified diameter.

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Table 2 Results on the association between air pollution and DNA adducts in exposed individuals; comparison of means analysis

First author, Year	Area/country	Exposure	Controlled confounders	Groups, sample size (total: 1,044)	Mean adducts/10^8 nucleotides ± SD (unless otherwise stated)	P
Perera, 1992 (39)	Poland	Environmental air pollution	NA	• Residents in industrial area, 20 • Rural controls, 21	• 30.4±13.5 • 11.01±22.6	<0.05
Hemminki, 1994 (40)	Stockholm, Sweeden	Traffic related air pollution	Age, smoking	• Bus drivers—urban routes, 26 • Bus drivers—sub urban routes, 23 • Taxi drivers—mixed routes, 19 • Controls, 22	• 0.9±0.35 • 1.4±0.48 • 1.6±0.91 • 1.0±0.32	• Non significant • <0.001 • <0.010
Nielsen, 1996 (41)	Denmark	Environmental air pollution	Smoking, PAH† rich diet	• Bus drivers in central copenhagen, 49 • Rural controls, 60	• Median: 1.214; range: 0.142-22.24 • Median: 0.074; range: 0.003-8.876	0.001
Nielsen, 1996 [2] (42)	Denmark and Greece	Environmental air pollution	Smoking, sex	• Students in urban universities, 74 • Students in agricultural colleges, 29	• Median: 0.205 • Median: 0.152	0.02
Yang, 1996 (43)	Milan, Italy	Traffic related air pollution	Sex, age, smoking habits	• News stand workers at high traffic areas, 31 • News stand workers at low traffic areas, 22	• 2.2±1.0 • 2.2±1.2	0.27
Topinka, 1997 (44)	Teplice & Prachatice, N&S Bohemia	Residence in industrial area	NA	• Placenta samples—industrial polluted area (winter): GSTM—genotype, 15 • Placenta samples—agricultural area (winter): GSTM—genotype, 17	• 1.49±0.70 • 0.96±0.55	0.027
Merlo, 1997 (45)	Genova, Italy	Ambient PAH concentrations	NA	• Traffic police workers, 94 • Urban residents, 52	• 1.48±1.35 • 1.01±0.63	0.007

Table 2 (*continued*)

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Table 2 (*continued*)

First author, Year	Area/country	Exposure	Controlled confounders	Groups, sample size (total: 1,044)	Mean adducts/10^8 nucleotides ± SD (unless otherwise stated)	P
Georgiadis, 2001 (46)	Greece	Environmental Air Pollution	NA	• Students in Athens (highest PAH concentration), 117 • Students in Halkida (lower PAH concentration), 77	• 1.25±1.19 • 1.54±1.19	<0.001
Ruchirawa, 2002 (47)	Bangkok, Thailand	Environmental air pollution	Smoking, sex	• Traffic Policemen, 41 • Office duty policemen, 40	• 1.6±0.9 • 1.2±1.0	0.03
Marczynski, 2005 (48)	Germany	PAH in air (ambient and personal monitoring)	NA[†]	• Samples from 16 workers(increased PAH exposure) • Samples from 16 workers[¥] (reduced PAH exposure)	Range: 0.5-1.19; range: <0.5-0.09	<0.0001
Topinka, 2007 (49)	Prague, Czech Republic	c-PAH (personal exposure)	Smoking, ocuupational duration	• 109 policemen—January (highest exposure) • 109 policemen—March	• 2.08±1.60 • 1.66±0.65	<0.0001
Tuntawiroon, 2007 (50)	Bangkok and Chonburi, Thailand	c-PAH and B[a]P	Age and lifestyle (i.e., ETS, transportation, medication, diet etc.)	• Bangkok schoolchildren, 115 • Provincial school children (group matching), 69	• 0.45±0.03 • 0.09±0.00	<0.0001
Ayi-Fanou, 2011 (51)	Cotonou, Benin	Environmental air pollution	NA	• Taxi-motorbike drivers, 13 • Intermediate exposure suburban group, 20	• 24.6±6.4 • 2.1±0.6	<0.001
		Environmental air pollution	NA	• Street food vendors, 16 • Intermediate exposure suburban group, 20	• 34.7±9.8 • 2.1±0.6	<0.001
		Environmental air pollution	NA	• Gasoline salesmen, 20 • Intermediate exposure suburban group, 20	• 37.2±8.1 • 2.1±0.6	<0.001
		Environmental air pollution	NA	• Street side residents, 11 • Intermediate exposure suburban group, 20	• 23.78±6.9 • 2.1±0.6	<0.001

NA, not available; PAH, polycyclic aromatic hydrocarbons; c-PAH, carcinogenic polycyclic aromatic hydrocarbons; B[a]P, benzo [a] pyrene; ETS, environmental tobacco smoke; [¥], the sample sizes reported in the summary tables refer to subjects with measurments available both before and after change in work conditions.

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Table 3 Results on the association between air pollution and DNA adducts in exposed individuals; linear regression, logistic regression and correlation analyses

First author, year	Area/country	Exposure	Controlled confounders	Effect measure	Sample size (total: 1,787)	Subject desription	P
Binková, 1995 (52)	Czech Republic	Outdoor air pollution—individual PAH†	Age, active and passive smoking, consumption of fried or smoked food, job category	r: 0.541	21	Non smoking women working outdoors up to 8 hours—gardeners or postal workers	0.016
Whyatt, 1998 (53)	Krakow, Poland	Ambient pollution at mother's place of residence	Smoking, dietary PAH, use of coal stoves, home or occupational exposures to PAH & other organics	β: 1.77	19	Mothers not employed away from home	0.05
		Ambient pollution at place of residence	Smoking, dietary PAH, use of coal stoves, home or occupational exposures to PAH and other organics.	β: 1.73	23	Newborns of mothers (high pollution/low pollution group)	0.03
Sørensen, 2003 (54)	Copenhagen	Personal $PM_{2.5}$	Smoking, diet, season	β: -0.0035	75	Students monitored 4 seasons of a year	0.31
Castaño-Vinyals, 2004 (55)	Review	B[a]P (stationary meas.)	Not applicable	r: 0.6	12	Pairs of data	0.038
Peluso, 2005 (56)	10 European countries	O_3 levels	Age, gender, educational level, country and batch	β: 0.066	564	EPIC cohort subjects	0.0095
Neri, 2006 (57)	Review	Environmental pollutants (including ETS exposure)	Not applicable	Not applicable	178	Newborns-17 years old; 2 studies in total—2 with statistically significant results	Not applicable
Pavanello, 2006 (58)	North-East Italy	B[a]P indoor exposure	Smoking, diet, area of residence, traffic near house, outdoor exposure	β: 0.973	457	Municipal workers (non smoking)	0.012
Palli, 2008 (59)	Florence City, Italy	PM_{10} (from high traffic stations)	Smoking	r: 0.562	16	Traffic exposed workers	0.02
Peluso, 2008 (60)	Thailand	Industrial estate residence	Smoking habits, age, gender	OR†: 1.65	72; 50	Industrial estate residents control district residents	<0.05
			Smoking habits, age, gender	OR: 1.44	64; 72	PAH exposed workers industrial estate residents	<0.05

Table 3 (*continued*)

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Table 3 (*continued*)

First author, year	Area/country	Exposure	Controlled confounders	Effect measure	Sample size (total: 1,787)	Subject desription	P
Pavanello, 2009 (61)	Poland	1-pyrenol	NA	r: 0.67	92	Coke oven workers and controls	<0.0001
Pedersen, 2009 (62)	Copenhagen, Denmark	Residential traffic density	ETS, use of open fireplace, pre-pregnancy weight, folate levels, vitamin B12 levels, maternal education and season of delivery	β: 0.6/0.7	75/69	Women/umbilical cords	<0.01
Eriksen, 2010 (63)	Copenhagen, Denmark	Residence in Copenhagen *vs.* residence in more rural areas	Years of primary and high school attendence and educational level	• OR: 1.00 • OR: 1.09 • OR: 1.16	115 140 120	• Arhus and neightbouring municipali • Suburban municipalities of Copenhagen • Copenhagen	• 0.27 • 0.08
García-Suástegui, 2011 (64)	Mexico City, Mexico	$PM_{2.5}$	Various risk alleles	r: NR	92	Young adults living in Mexico City	0.013
		PM_{10}	Various risk alleles	r: NR	92	Young adults living in Mexico City	0.035
Herbstman, 2012 (65)	USA	PAH exposure—measured in both air and urine	NA	r: NR	NR	152 participants – prenatal exposure, DNA adducts in cord blood	Not significant
Rossner, 2013 (66)	Czech Republic-Prague	• B[a]P (individual monitors) • B[a]P (stationary meas.) • $PM_{2.5}$ (stationary meas.)	Age, BMI, cotinine, vitamins C, A, E, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides	• β: −0.016 • β: −0.065 • β: −0.003		61 to 65 participants, depending on sampling season	• 0.173 • <0.001 • 0.001
	Czech Republic-Ostrava	• B[a]P (individual monitors) • B[a]P (stationary meas.) • $PM_{2.5}$ (stationary meas.)	Age, BMI, cotinine, vitamins C, A, E, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides	• β: 0.001 • β: −0.002 • β: 0.0		98 to 149 participants, depending on sampling season	• 0.429 • 0.012 • 0.104

r, correlation coefficient; β, linear regression coefficient (change in DNA adduct levels (adducts/10^8 nucleotides) for every unit change in exposure). OR, logistic regression odds ratio; PAH, polycyclic aromatic hydrocarbons; PM_{10}, particulate matter of diameter less than 10 microns; $PM_{2.5}$, particulate matter of diameter less than 2.5 microns; B[a]P, Benzo [a] Pyrene; O_3, ozone; NA, not available; NR, not reported; ETS, environmental tobacco smoke.

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fetal versus adult susceptibility to PAHs and second-hand tobacco smoke, a study compared carcinogen-DNA adducts (a biomarker associated with an increased risk of cancer) and cotinine (a biomarker of exposure to tobacco smoke) in paired blood samples collected from mothers and newborns in New York City, USA. The authors enrolled 265 non-smoking African-American and Latina mother–newborn pairs between 1997 and 2001. Despite the estimated 10-fold lower fetal dose, mean levels of B[a]P-DNA adducts were comparable in paired newborn and maternal samples (0.24 adducts per 108 nucleotides in newborns, with 45% of newborns with detectable adducts, *vs.* 0.22 per 108 nucleotides in mothers, with 41% of mothers with detectable adducts). These results indicate an increased susceptibility of the fetus to DNA damage (68).

Dose-response relationship

Lewtas *et al.* [1997] (69) observed that human populations exposed to PAH via air pollution exhibit a nonlinear relationship between levels of exposure and white blood cell-DNA adducts. Among highly exposed subjects, the level of DNA adducts per unit of exposure was significantly lower than those measured after environmental exposures. The observation was confirmed in a meta-analysis of the epidemiological studies (70). The same exposure–dose nonlinearity was observed in lung DNA from rats exposed to PAHs. One interpretation proposed for such an observation is that saturation of metabolic enzymes or induction of DNA repair processes occurs at high levels of exposure (71,72).

DNA methylation

DNA methylation is a biochemical process where a methyl group is added to the cytosine nucleotides mostly found in CpG dinucleotides and this modification influences gene expression (73,74). For example, a high percentage of CpG dinucleotides in repetitive sequences are methylated to inhibit activation and maintain chromosome stability, but CpG sites in CpG islands associated with gene promoters are usually unmethylated. These unmethylated promoter regions allow for active gene transcription (75,76) and also have a role in cell differentiation.

Whole genome methylation is most commonly assessed using surrogate repetitive elements such as long interspersed nuclear element-1 (LINE-1) and Alu repeats (77). Hypomethylation of these endogenous retro-transposons can lead to activation and reposition elsewhere in the genome, causing insertional mutagenesis, transcriptional interference, and genomic instability (77-79). Further to activating repetitive DNA sequences, DNA hypomethylation might also contribute to translocations of these hypomethylated sequences, by loosening chromatin packaging (79-81).

Exposure to AAP, whether short-term or long-term, has been shown to be associated with global hypomethylation. Ten reports (*Table 4*) have recently investigated the effects of AAP exposure on global methylation and a number of them used repetitive elements such as LINE-1, Satα and Alu elements as proxies to whole genome methylation. LINE-1 methylation was frequently found to be altered by exposure to air pollution (82-84). Alu methylation was also significantly altered in three studies (84,85,87) and Satα in one study (88). Lastly, global methylation in healthy adults was decreased following exposure to AAP (86). Despite the small number of available studies, the replication of findings supports AAP's influence on global methylation levels, and these epigenetic changes can contribute to carcinogenesis at least as much as genetic changes.

DNA hypomethylation in children and pre-natal exposures

Two studies investigated global methylation in cord blood and placenta samples and found significant associations with prenatal PAH and PM2.5 exposures (65,89). In addition, when comparing children from the polluted region of Ostrava to children from the non-polluted region of Prachatice, Rossnerova *et al.* [2013] (90) found 9,916 differentially methylated CpGs of which 58 had methylation differences of >10%. All these sites were found to be hypomethylated in Ostrava children demonstrating a significant impact of AAP on the methylation patterns of children.

Critical issues in evaluating the relationship between AAP and biomarkers

Using the pool of evidence on DNA-adducts and DNA methylation as paradigms, a number of critical issues in health impact evaluations using biomarkers arise and several directions for the future of the field can be drawn. The lessons learnt from the experience are critical since the mechanisms through which AAP causes cancer remain to be elucidated and biomarkers of exposure can be incorporated in more accurate exposure assessments.

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Table 4 Results on the association between ambient air pollution and Global DNA methylation changes in the cells of exposed individuals

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Baccarelli, 2007 (82)	Boston, USA	Ambient Black Carbon (hourly concentrations measured at a monitoring site approximately 1 km from the site of examination (7-day mean)	LINE-1 methylation	Multiple clinical and environmental covariates	r: −0.11	−0.18, −0.04	718	Subjects from the Normative Aging Study	0.002
		Ambient Black Carbon (hourly concentrations measured at a monitoring site approximately 1 km from the site of examination (7-day mean)	Alu methylation	Multiple clinical and environmental covariates					Not significant
Baccarelli, 2009 (83)	Boston, USA	$PM_{2.5}$ concentrations (7-day mean)	LINE-1 methylation	Age, BMI, cigarette smoking, pack-years, statin use, fasting blood glucose, diabetes mellitus, percent lymphocytes, and neutrophils in differential blood count, day of the week, season, and outdoor temperature	r: −0.13	−0.19, −0.06	718	Subjects from the Normative aging study	<0.001
		$PM_{2.5}$ concentrations (7-day mean)	Alu methylation	Age, BMI, cigarette smoking, pack-years, statin use, fasting blood glucose, diabetes mellitus, percent lymphocytes, and neutrophils in differential blood count, day of the week, season, and outdoor temperature	r: −0.01	−0.07, 0.05			0.71

Table 4 (*continued*)

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Table 4 (*continued*)

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Tarantini, 2009 (84)	Brescia, Northern Italy	PM_{10} (first day of the week and after 3 days of work)	LINE-1 methylation	Unadjusted	0.02%	SE: 0.11	63	Workers	0.89
		PM_{10} (first day of the week and after 3 days of work)	Alu methylation	Unadjusted	0%	SE: 0.08			0.99
		PM_{10} (first day of the week and after 3 days of work)	iNOS promoter methylation	Unadjusted	−0.61%	SE: 0.26			0.02
		PM_{10} (average level of individual exposure)	LINE-1 methylation	Age, BMI, smoking, number of cigarettes/ day	β: −0.34	SE: 0.09			0.04
		PM_{10} (average level of individual exposure)	Alu methylation	Age, BMI, smoking, number of cigarettes/ day	β: −0.19	SE: 0.17			0.04
		PM_{10} (average level of individual exposure)	iNOS promoter methylation	Age, BMI, smoking, number of cigarettes/ day	β: −0.55	SE: 0.58			0.34
Madrigano, 2011 (85)	New York, USA	$PM_{2.5}$ (IQR increase over a 90-day period)	• LINE1 • Alu	Season, time, smoking, BMI, alcohol intake, medication, batch, % WBC type	• 0.03% • 0.03%	• −0.12, 0.18 • −0.07, 0.13	706	subjects from the Normative Aging Study	• Not significant • Not significant
		Black Carbon (IQR increase over a 90-day period)	• LINE1 • Alu	Season, time, smoking, BMI, alcohol intake, medication, batch, % WBC type	• −0.21% • −0.31%	• −0.50, 0.09 • −0.12, −0.50			• Not significant • <0.05
		SO_4 (IQR increase over a 90-day period)	• LINE1 • Alu	Season, time, smoking, BMI, alcohol intake, medication, batch, % WBC type	• −0.27% • −0.03%	• −0.02, −0.52 • −0.20, 0.13			• <0.05 • Not significant

Table 4 (*continued*)

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Table 4 (*continued*)

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
De Prins, 2013 (86)	Belgium	NO_2 (IQR increase) 60 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.05	–0.10, –0.01	48	Non-smoking adults	<0.05
		$PM_{2.5}$ (IQR increase) 30 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.06	–0.11, –0.02			<0.01
		NO_2 (IQR increase) 60 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.18	–0.37, 0.01			Not significant
		$PM_{2.5}$ (IQR increase) 30 days	Global Methylation (%5mdC)	Gender, age and average outdoor temperature during the exposure period, a random factor to correct for correlations between subjects living in the same residence	–0.14	–0.28, 0.00			<0.05

Table 4 (*continued*)

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Table 4 (*continued*)

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Bellavia, 2013 (87)	Toronto, Canada	Fine CAPs for 130 min	LINE1 methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: 0.00	–0.42, 0.44	15	Non-smoking healthy volunteers	Not significant
		Fine CAPs for 130 min	Alu methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: –0.74	–1.18, –0.3			0.0006
		Coarse CAPs for 130 min	LINE1 methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: –0.16	–0.52, 0.24			Not significant
		Coarse CAPs for 130 min	Alu methylation	Not applicable: same subjects compared to postmedical air (control) exposure	β: –0.28	–0.65, 0.10			Not significant
Guo, 2014 (88)	Beijing, China	Personal $PM_{2.5}$ (IQR increase)	SATα methylation		–1.35%		120	Truck drivers & office workers	0.01
		Ambient PM_{10} (IQR increase)	SATα methylation		–1.33%				0.01
		Personal $PM_{2.5}$ (IQR increase)	SATα methylation		–2.34%		60	Truck drivers	0.02
		Ambient PM_{10} (IQR increase)	SATα methylation		–1.44%				0.06
		Personal $PM_{2.5}$ (IQR increase)	SATα methylation		–0.95%		60	Office workers	0.26
		Ambient PM_{10} (IQR increase)	SATα methylation		–1.25%				0.12
Herbstman, 2012 (65)	New York, USA	PAH exposure—prenatal	Global Methylation	Ethnicity	β: –0.11	–0.21, 0.00	164	Cord blood samples	0.05

Table 4 (*continued*)

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Table 4 (*continued*)

First author, year	Area/ country	Exposure	Outcome	Controlled confounders	Effect measure	95% CI	Sample size (total: 1,499)	Subject desription	P
Janssen, 2013 (89)	Belgium	$PM_{2.5}$ (5 μg/m^3 increase) Trimester 1	Global Methylation	Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature	–2.13%	–3.71, –0.54	240	Placenta tissue mother-newborn pairs	0.009
		$PM_{2.5}$ (5 μg/m^3 increase) Trimester 2	Global Methylation	Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature	–0.43%	–1.81, 0.98			0.55
		$PM_{2.5}$ (5 μg/m^3 increase) Trimester 3	Global Methylation	Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception and trimester-specific apparent temperature	0.74%	–0.85, 2.33			0.36
Rossnerova, 2013 (90)	Czech Republic	Children from Ostrava (highly polluted) *vs.* Prachatice (control)	27K Methylation: 58 differentially methylated regions	Not available	All sites hypomethylated				<0.05

r, correlation coefficient; β, linear regression coefficient [change in DNA methylation levels (%5mC) per unit change in exposure]; % percent difference; CI, confidence interval; LINE-1, long interspersed nuclear element-1; IQR, interquartile range; PM_{10}, particulate matter with diameter of less than 10 microns; tHcy, total homocysteine; BMI, body mass index; $PM_{2.5}$, particulate matter with diameter of less than 2.5 microns; PAH, polycyclic aromatic hydrocarbons; CAP, concentrated ambient particle.

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Confounding

Only 17 of the studies on DNA adducts reviewed here, adjusted for various potential confounders and not all have adjusted for smoking and PAHs in diet, indicating lack of adequate adjustment for confounding. Dietary habits can affect DNA adduct formation, as studies have demonstrated strong negative associations between DNA adducts and consumption of fresh fruit and vegetables, olive oil, and antioxidants as well as positive associations between consumption of charbroiled food and DNA adducts (91,92). In addition, there is almost complete consensus amongst studies in humans, in animals and *in vitro* that smoking, whether active or passive, is associated with DNA adduct formation (93). Also, a recent study has evidenced city-specific spatial and temporal environmental inequalities that relate to the historical socioeconomic make-up of the cities (94). These inequalities become especially important in studies comparing subjects from different cities/rural-urban areas. Considering that PAHs in diet, smoking, exposure to second hand smoke, and socioeconomic status are factors that have an impact on DNA adduct and protein formation, inclusion of these exposures as potential confounders is imperative when investigating the association between exposure to AAP and DNA adducts. It is conceivable, therefore, that the next generation of biomarker studies in relation to AAP could and should address confounding in a more systematic way (e.g., by measuring cotinine as a more accurate reflection of exposure to tobacco). In contrast, almost all studies assessing DNA methylation changes, perhaps due to being more recent, have adequately adjusted for a number of clinical and environmental confounders, including smoking. Further highlighting the inadequate adjustment in the DNA adducts reviewed studies, the confounders considered did not address other carcinogenicity pathways such as inflammation and epigenetics. Considering that these are pathways shown to be influenced by AAP (95-97) and are also shown to be implicated in carcinogenicity (96,98), future studies should use in confounder adjustments markers that are relevant to more than one carcinogenicity pathways.

Reversibility of changes and individual susceptibilities

A second issue that arises from the review of the evidence pool on DNA adducts, is the plasticity and reversibility of the biomarker investigated. Whereas protein adducts cannot be repaired and thus better reflect exposure, DNA adducts can be eliminated by DNA repair mechanisms and are therefore more transient indicators of external exposure. DNA methylation changes have also been shown to be reversible. In addition, other markers of AAP exposure have differential response and step transition times varying at each step with half-lives counted in hours for e.g., 1-hydroxypyrene (1-OHP), oxidized nucleobases, and gene expression, whereas bulky adducts show half-lives of weeks and for chromosomal aberrations (CAs) and micronucei the half-life can be years (95). Hence timing of exposure and the kinetics of the carcinogen and biomarker need to be incorporated in the design of future studies.

In addition, one needs to consider inherited and acquired individual susceptibilities, as DNA adduct levels have been found to be dependent on polymorphisms in metabolic genes involved in adduct formation and DNA repair (i.e., the CYP1A1, MspI, and GSTM1 null genotypes, the XRCC3-241Met homozygote variant allele, and the XPD-Lys751Gln polymorphism with at least 1 variant allele) (99-103). Recently, it was demonstrated the even mitochondrial genetic background can modify the relationship between AAP and biomarkers of inflammation, because of the role of mitochondria in reactive oxygen species production (104). Thus, individual susceptibility can influence different carcinogenicity pathways in different and multi-faceted ways, highlighting the importance of its inclusion in such investigations.

Intensity, duration, and timing of exposure

Furthermore, the issues of intensity, duration, and timing of exposure are of primary importance when evaluating the impact of AAP. As previously discussed, studies show that developing fetuses are more susceptible than adults to the carcinogenic effects of PAHs (44,62,68). Exposure at this critical developmental stage may cause subtle changes that may or may not be repaired. If not repaired, these changes can persist and lead to increased risk of dysfunction and disease later in life (105). Similarly, timing of exposure can be of relevance to other air pollution carcinogenicity biomarkers. For example, exposure to coal and wood smoke after the age of 20 was shown to reduce global DNA methylation levels, but exposure before 20 years was not associated with methylation changes (106).

Studies also show that exposure to PAHs and DNA adduct formation are not linearly associated (69). Instead, among highly exposed subjects the level of DNA adducts per unit of exposure was significantly lower than those at

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lower exposures (70).

There is little evidence in the literature about the impact of duration of exposure on the formation of DNA adducts, since no studies have investigated the impact of short-term exposure on DNA adduct formation. However, with respect to mortality, it has been shown that short-term exposure-mortality associations were substantially lower than equivalent long-term associations, a finding which suggests larger, more persistent cumulative effects from long-term exposures (107).

Lastly, the exact composition of AAP exposures needs to be defined in future studies. According to a recently published study, the size fraction of the particles in air are likely to affect different pathophysiological pathways independently (3), therefore AAP exposures with different fractions of differently sized particles might have different biological effects.

In order to add biological credibility and certainty to the impact assessment of AAP, future studies need to aim in bridging current gaps in knowledge about the timing of air pollution effects, the influence of duration of exposure, and the persistence of effects.

Target vs. surrogate tissues

Another important consideration is that most biomarker studies available to date use surrogate tissues, such as blood. AAP is more likely to have the largest impact on sites of deposition where doses are highest, such as the upper aerodigestive tract and lung. If DNA and protein adducts are investigated in target tissues, the associations observed are likely to be much stronger, more reliable, and more accurate. Biomarkers that show great potential for the assessment of AAP exposure and respiratory effects are biomarkers in exhaled breath. Such biomarkers include but are not limited to exhaled nitric oxide (FeNO), exhaled breath condensate (EBC) pH, 8-isoprostane, and interleukin 1β (108,109). These exhaled biomarkers of airway oxidative stress and inflammation can provide a more reliable indication of biologically effective dose with respect to respiratory effects than biomarkers in surrogate tissues (109). Biomarkers relevant to other carcinogenicity mechanisms in exhaled breath remain to be identified.

Measurement error and other biases in study design and analysis

Even though the use of biomarkers can improve exposure assessment in future investigations, the studies included in this manuscript point to an overall need for better and more carefully designed studies to assess the carcinogenicity of AAP. The majority of studies reviewed here used measurements from stationaly air pollution monitoring stations or residence/occupation in a heavily polluted city as proxies to personal exposure to AAP. However, future studies should rely more on individual exposures with the use of mobile, individual sensors, as some of the more recent studies have (66,88). In addition, studies focusing on the comparison of means can only account for a limited number of confounding factors (*Table 2*), introducing bias, and thus more sophisticated statistical analyses should be the preferred in future investigations.

Despite the discussed limitations, DNA adducts and DNA methylation are undeniably valuable biomarkers of exposure and early biological effect regarding AAP. A recent review (95) recognized in addition to DNA adducts and DNA methylation, 1-OHP, CAs, micronuclei (MN), and oxidative damage to nucleobases, as valid biomarkers of exposure to air pollution. These biological markers cover the whole spectrum of progression from external exposure to tumour formation. 1-OHP is an excellent marker of internal dose for PAH exposure, and DNA adducts and oxidized nucleobases are markers of the biologically effective dose, whereas MN, CA, and DNA methylation are good markers of early biological effect (95). DNA adducts and DNA methylation have also been suggested to be predictive for the risk of future cancer (56,98,110,111).

Future directions

Application of DNA adducts and DNA methylation as biomarkers of exposure and early biological effect in large prospective studies on AAP has the potential to reduce measurement error and elucidate possible mechanisms of carcinogenesis. In addition, careful consideration of confounders, use of personal air monitors, investigation of different aetiologically relevant time-windows, and use of target tissues where possible can also improve the quality of future studies thus allowing more weight to be placed on their conclusions. Therefore, high quality prospective population studies can strengthen causality assertions and improve understanding, offering possible avenues through which to combat the problem of AAP carcinogenicity.

The genomics era has led to great improvements in the understanding of cancer biology, and together with the development of high-resolution and high-

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throughput technologies interrogating other -omics (such as epigenomics, transcriptomics, proteomics, and metabolomics) they have yielded an unprecedented perspective on cancer omics. These technologies and the emerging knowledge can be used to identify even more biomarkers of AAP exposure and carcinogenicity. Such biomarkers will enable elucidation of new and better understanding of existing carcinogenesis pathways, thus advancing research and addressing the aforementioned gaps in knowledge. Key to such investigations is a multidimensional approach which will help put markers from a specific -omic level into the broader, cellular and molecular context.

Lastly, future studies can be of a transitional nature, aiming to bridge the gap between lab experimentation and population based epidemiology. Validation of *in vitro* results and incorporation of *in vitro* markers in population studies will also strengthen causality inferences, offering multi-level evidence for the carcinogenicity of AAP and the importance of timing, duration, intensity, reversibility of changes and individual susceptibility.

Conclusions

In conclusion, DNA adducts and DNA methylation are important biomarkers that can be used in the investigation of the relationship between AAP and its carcinogenic effects, as they not only improve exposure assessment but also increase our understanding of mechanisms underlying this association. These biomarkers should be used in properly designed future studies of air pollution carcinogenicity. These studies are needed to address current knowledge gaps which would in turn open avenues for prevention, diagnosis, and treatment of cancers and other diseases resulting from air pollution exposure.

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