CHAPTER 12. BIOMARKERS OF AIR POLLUTION: DNA AND PROTEIN ADDUCTS

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Biomarkers were introduced in the epidemiology of chronic disease 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. Biomarkers of exposure, such as DNA adducts, are described here, while markers of early damage are considered in a separate chapter.

DNA adducts and exposure to air pollution

Several studies have considered DNA damage as an end-point of the effects of air pollution, especially *bulky* DNA adducts that are related to exposure to aromatic compounds, including polycyclic aromatic hydrocarbons (PAHs).

A systematic review was performed to evaluate whether metabolites of pyrene and DNA adducts are valid markers of low-level environmental (not occupational) exposure to PAHs (Castaño-Vinyals *et al.*, 2004). Thirty five studies with more than 10 subjects were identified that evaluated environmental air pollution with PAHs in relation to metabolites of PAHs, PAH–DNA adducts, or protein adducts. PAH metabolites and, to a lesser extent, PAH–DNA adducts correlated well among the groups with exposure to benzo[*a*]pyrene (B[*a*]P), even at low levels of pollution.

As Table 12.1 and Table 12.2 suggest, studies in different countries have shown that the levels of white blood cell DNA adducts were higher among subjects who were more heavily exposed to air pollutants. This has been observed in different population categories, such as police officers in Italy and Thailand (Peluso *et al.*, 1998; Ruchirawa *et al.*, 2002), residents in highly industrialized areas in Poland (Perera *et al.*, 1992), and bus drivers in Denmark (Nielsen *et al.*, 1996a). In all of these cases, the differences in the levels of adducts between more heavily exposed or less exposed subjects were significant (Table 12.1 and Table 12.2).

More recently, a group of 114 workers exposed to traffic pollution and a random sample of 100 residents were studied in Florence, Italy. Bulky DNA adducts were analysed in peripheral leukocytes donated at enrolment using ^{32}P-postlabelling. Adduct levels were significantly higher for traffic workers among never-smokers ($P = 0.03$) and light current smokers ($P = 0.003$). In both groups, urban residents tended to show higher levels than those living in suburban areas, and a seasonal trend emerged of adduct levels being highest in summer and lowest in winter (Palli *et al.*, 2001).

In a study in Greece, the levels of bulky DNA adducts were measured by ^{32}P-postlabelling in the lymphocytes of 194 nonsmoking students living in the city of Athens and the region of Halkida (Georgiadis *et al.*, 2001). Personal exposures to PAHs were significantly higher among the subjects in Athens. However, the highest adduct levels were observed in a subgroup of students living in Halkida, with a minimal burden of urban air pollution. Among these (but not the remaining subjects), positive correlations were observed between DNA adducts and measured personal exposures to chrysene or B[*a*]P. A much clearer association of adducts with second-hand tobacco smoke was observed.

In Denmark, Sørensen *et al.* (2003) measured personal exposure to particulate matter (PM) < 2.5 μm in diameter and exposure to black smoke in 50 students four times during 1 year and analysed biomarkers of DNA damage. Personal exposure to PM was found to predict the presence of 8-oxo-2′-deoxyguanosine (8-oxo-dG) in lymphocyte DNA, with an 11% increase in 8-oxo-dG per 10 μg/m^3 increase in exposure to PM ($P = 0.007$).

In Europe, a case–control study nested in a large prospective study (European Prospective Investigation into Cancer and Nutrition [EPIC]) has been completed (Peluso *et al.*, 2005). Cases included newly diagnosed lung cancer (n = 115), upper respiratory cancers (pharynx, larynx) (n = 82), bladder cancer (n = 124), and leukaemia (n = 166), and deaths from chronic obstructive pulmonary disease or emphysema (n = 77) that accrued after a median follow-up of 7 years among EPIC former smokers and never-smokers. Leukocyte DNA adducts were analysed blindly using the nuclease P1 modification of the ^{32}P-postlabelling technique. The intensity of adduct patterns was generally stronger in the chromatograms of healthy nonsmokers who developed lung cancer in the following years compared with the other samples. The observed adduct profile has been described previously among subjects environmentally exposed to air pollution. Adducts were associated with a subsequent risk of lung cancer with an odds ratio (OR) of 1.86 (95% confidence interval [CI], 0.88–3.93). The association with lung cancer was stronger in never-smokers (OR, 4.04; 95% CI, 1.06–15.42) and among the younger age groups. After exclusion of the 36 months preceding the onset of lung cancer, the OR was 4.16 (95% CI, 1.24–13.88). In addition, the authors found an association of adduct levels with ozone, suggesting a possible role for photochemical smog in determining DNA damage of nonsmokers in western Europe. This is consistent with the previous investigation in Florence that showed a significant relationship between cumulative exposure to ozone and bulky DNA adducts among nonsmokers

Table 12.1 Results on the association between air pollution and DNA adducts in exposed individuals – comparison of means analysis

Reference	Study location	Exposure	Controlled confounders	Group, sample size[a] (total: 1044)	Mean adducts/ 10^8 nucleotides ± SD (unless otherwise stated)	*P*
Perera *et al.* (1992)	Poland	Environmental air pollution	NA	Residents in industrial area, 20 Rural controls, 21	30.4 ± 13.5 11.01 ± 22.6	< 0.05
Hemminki *et al.* (1994)	Stockholm, Sweden	Traffic-related air pollution	Age, smoking	Bus drivers – urban routes, 26 Bus drivers – suburban 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	NS < 0.001 < 0.010
Nielsen *et al.* (1996a)	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 *et al.* (1996b)	Denmark and Greece	Environmental air pollution	Smoking, sex	Students at urban universities, 74 Students at agricultural colleges, 29	Median: 0.205 Median: 0.152	0.02
Yang *et al.* (1996)	Milan, Italy	Traffic-related air pollution	Sex, age, smoking habits	News stand workers in heavy traffic areas, 31 News stand workers in light traffic areas, 22	2.2 ± 1.0 2.2 ± 1.2	0.27
Topinka *et al.* (1997)	Teplice and Prachatice, North and South Bohemia, Czech Republic	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 *et al.* (1997)	Genoa, Italy	Ambient PAH concentrations	NA	Traffic police workers, 94 Urban residents, 52	1.48 ± 1.35 1.01 ± 0.63	0.007

Table 12.1 (continued)

Reference	Study location	Exposure	Controlled confounders	Group, sample size[a] (total: 1044)	Mean adducts/ 10^8 nucleotides ± SD (unless otherwise stated)	*P*
Georgiadis *et al.* (2001)	Greece	Environmental air pollution	Smoking	Students in Athens (highest PAH concentration), 117 Students in Halkida (highest PAH concentration), 77	1.25 ± 1.19 1.54 ± 1.19	< 0.001
Ruchirawa *et al.* (2002)	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 *et al.* (2005)	Germany	PAHs in air (ambient and personal monitoring)	NA	Samples from 16 workers (increased PAH exposure) Samples from 16 workers[a] (reduced PAH exposure)	Range: 0.5–1.19 Range: < 0.5–0.09	< 0.0001
Topinka *et al.* (2007)	Prague, Czech Republic	c-PAH (personal exposure)	Smoking, occupational duration	109 policemen – January (highest exposure) 109 policemen – March	2.08 ± 1.60 1.66 ± 0.65	< 0.0001
Tuntawiroon *et al.* (2007)	Bangkok and Chonburi, Thailand	c-PAH and B[*a*]P	Age and lifestyle (i.e. ETS, transportation, medication, diet, etc.)	Bangkok schoolchildren, 115 Provincial schoolchildren (group matching), 69	0.45 ± 0.03 0.09 ± 0.00	< 0.0001
Ayi-Fanou *et al.* (2011)	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

B[*a*]P, benzo[*a*]pyrene; c-PAH, carcinogenic polycyclic aromatic hydrocarbon; ETS, environmental tobacco smoke; NA, not available; NS, not significant; PAH, polycyclic aromatic hydrocarbon; SD, standard deviation.

[a] The sample sizes reported in the summary tables refer to subjects with measurements available both before and after change in work conditions.

Compiled from Demetriou *et al.* (2012).

Table 12.2 Results on the association between air pollution and DNA adducts in exposed individuals – linear regression, logistic regression, and correlation analyses

Reference	Study location	Exposure	Controlled confounders	Effect measure	Sample size (total: 1787)	Subject description	*P*
Binková *et al.* (1995)	Czech Republic	Outdoor air pollution, individual PAH	Age, active and passive smoking, consumption of fried or smoked food, job category	r: 0.541	21	Nonsmoking women working outdoors up to 8 hours – gardeners or postal workers	0.016
Whyatt *et al.* (1998)	Krakow, Poland	Ambient pollution at mother's place of residence	Smoking, dietary PAH, use of coal stoves, home or occupational exposures to PAH and 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 *et al.* (2003)	Copenhagen, Denmark	Personal $PM_{2.5}$	Smoking, diet, season	β: −0.0035	75	Students monitored during four seasons in a year	0.31
Castaño-Vinyals *et al.* (2004)	Review	B[*a*]P (stationary measure)	N/A	r: 0.6	12	Pairs of data	0.038
Peluso *et al.* (2005)	10 European countries	O_3 levels	Age, gender, educational level, country, batch	β: 0.066	564	EPIC cohort subjects	0.0095
Neri *et al.* (2006)	Review	Environmental pollutants (including ETS exposure)	N/A	N/A	178	Newborn to age 17 years Two studies in total, both with statistically significant results	N/A
Pavanello *et al.* (2006)	Northeast Italy	B[*a*]P indoor exposure	Smoking, diet, area of residence, traffic near house, outdoor exposure	β: 0.973	457	Municipal workers (nonsmoking)	0.012
Palli *et al.* (2008)	Florence, Italy	PM_{10} (from heavy traffic stations)	Smoking	r: 0.562	16	Traffic-exposed workers	0.02
Peluso *et al.* (2008)	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 12.2 (continued)

Reference	Study location	Exposure	Controlled confounders	Effect measure	Sample size (total: 1787)	Subject description	*P*
Pavanello *et al.* (2009)	Poland	1-Pyrenol	NA	r: 0.67	92	Coke oven workers and controls	< 0.0001
Pedersen *et al.* (2009)	Copenhagen, Denmark	Residential traffic density	ETS, use of open fireplace, pre-pregnancy weight, folate levels, vitamin B12 levels, maternal education, season of delivery	β: 0.6/0.7	75/69	Women/umbilical cords	< 0.01
García-Suástegui *et al.* (2011)	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 *et al.* (2012)	USA	PAH exposure measured in both air and urine	NA	r: NR	NR	152 participants – prenatal exposure, DNA adducts in cord blood	NS

β, linear regression coefficient: change in DNA adduct levels (adducts/10^8 nucleotides) for every unit change in exposure; B[*a*]P, benzo[*a*]pyrene; ETS, environmental tobacco smoke; NA, not available; N/A, not applicable; NR, not reported; NS, not significant; O_3, ozone; OR, logistic regression odds ratio; PAH, polycyclic aromatic hydrocarbons; $PM_{2.5}$, particulate matter < 5 μm in diameter; PM_{10}, particulate matter < 10 μm in diameter; r, correlation coefficient

Compiled from Demetriou *et al.* (2012).

(Palli *et al.*, 2001). Ozone is a marker of photochemical smog. It is produced by a complex series of reactions involving hydrocarbons and nitrogen dioxide that are emitted primarily during combustion of fossil fuels by industry and transportation activities, and driven by ultraviolet (UV) radiation in sunlight. Ozone may have biological effects directly and/or via free radicals that react with other air pollutants.

The conditions needed for the formation of ozone are generally present in modern large cities, and the average levels at the Earth's surface have more than doubled since pre-industrial times in western Europe. Summer months, characterized by high temperatures, strong UV radiation, long periods of solar radiation, and light winds, provide more favourable conditions for the production of ozone in the troposphere. Indeed, episodes with elevated concentrations of ozone generally occur during periods of warm sunny weather. Transformation reactions that occur in the troposphere during such episodes may induce the formation of highly reactive PAH and nitro-PAH compounds (Finlayson-Pitts and Pitts, 1997). Soot aerosols are the main carriers of PAHs in outdoor air and can react rapidly on the surface with free radicals, such as those produced by ozone photolysis (Finlayson-Pitts and Pitts, 1997). In addition, PAHs such as anthracene, benzo[*a*]anthracene, benzo[*g,h,i*]perylene, B[*a*]P, indeno[1,2,3-*cd*]pyrene, and pyrene may be transformed by UV irradiation, become directly mutagenic, and form highly reactive quinone products. After UV activation, PAHs may produce covalent adducts (e.g. benzo[*a*]anthracene, B[*a*]P, and 1-hydroxypyrene DNA adducts) (Prodi *et al.*, 1984; Dong *et al.*, 2000). UV irradiation has been also shown to synergize with B[*a*]P to enhance significantly the expression levels of the tumour suppressor gene *p53* (Saladi *et al.*, 2003). Recently, an enhancement of the signature of mutations produced by B[*a*]P, i.e. G → T and C → A transversions, has been found after UV irradiation (Besaratinia and Pfeifer, 2003).

Firefighters and oil-well fires

Studies have also been conducted in populations with specific exposures – for example, forest firefighters and the United States troops exposed to oil-well fires in the Persian Gulf. Forest firefighters are exposed to a wide range of carcinogenic PAHs in forest fire smoke. PAH–DNA adducts were measured in 47 California firefighters at two time points, early and late in the 1988 forest fire season. PAH–DNA adduct levels were not associated with cumulative hours of recent firefighting activity. However, firefighters who had consumed charbroiled food within the previous week had elevated PAH–DNA adduct levels, which were related to the frequency of intake of such food. These findings suggest that dietary sources of PAH contribute to levels of PAH–DNA adducts in peripheral white blood cells (Rothman *et al.*, 1993).

In addition, levels of PAH–DNA adducts were determined in a group of United States Army soldiers who were deployed after the Persian Gulf War and were exposed to oil-well fires. The United States Army Environmental Hygiene Agency monitored air and soil for ambient PAHs. PAH–DNA adducts were measured from DNA samples in blood cells of 22 soldiers, and bulky aromatic adducts were measured by ^{32}P-postlabelling in blood cell DNA samples from 20 of the same soldiers. Urinary 1-hydroxypyrene-glucuronide levels were determined in a matched set of samples from 33 soldiers. Contrary to expectations, environmental monitoring showed low levels of ambient PAHs in the areas where these soldiers were working in Kuwait. Both DNA adducts and urinary 1-hydroxypyrene-glucuronide levels were lowest in Kuwait and highest in Germany, where the soldiers returned after the war (Poirier *et al.*, 1998).

DNA adducts in children

Experimental evidence indicates that developing fetuses are more susceptible than adults to the carcinogenic effects of PAHs. To assess fetal versus adult susceptibility to PAHs and secondhand 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 nonsmoking 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 10^8 nucleotides in newborns, with 45% of newborns with detectable adducts, vs 0.22 per 10^8 nucleotides in mothers, with 41% of mothers with detectable adducts). These results indicate an increased susceptibility of the fetus to DNA damage.

Protein adducts

Among newspaper vendors, Pastorelli *et al.* (1996) found a higher level of B[*a*]P–haemoglobin adducts, but the difference between these and levels in less exposed populations was not statistically significant. Richter *et al.* (2001) studied haemoglobin adducts formed by aromatic amines, including 4-aminobiphenyl, in groups of children, and found that children living in the most polluted cities had significantly higher levels of adducts than those living in less polluted cities.

Dose–response relationship

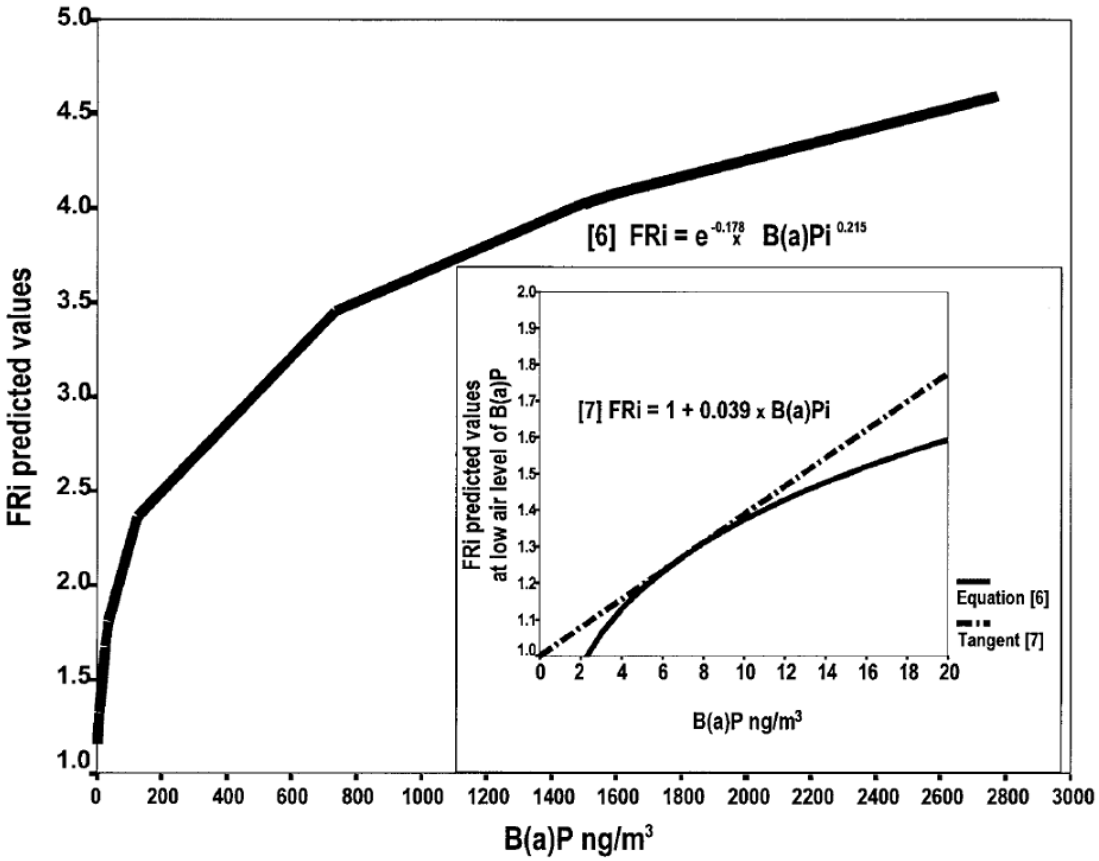
Lewtas *et al.* (1997) observed that human populations exposed to PAH via air pollution exhibit a nonlinear relationship between levels of exposure and white blood cell-DNA adducts. Among highly exposed subjects, the level of DNA adducts per unit of exposure was significantly lower than those measured after environmental exposures. The observation was confirmed in a meta-analysis of the epidemiological studies (Peluso *et al.*, 2001) (Figure 12.1). 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 (Lutz, 1990; Garte *et al.*, 1997).

Conclusions

On the basis of recent large cohort studies in the USA and Europe, there are reasonable grounds for concern that air pollution may increase the risk of lung cancer, especially in combination with other known risk factors such as voluntary and involuntary smoking and occupational exposures. Although there are examples of biomarkers contributing to the understanding of the health effects of air pollution, there are still many aspects that need clarification, such as the reliability of the markers (e.g. *bulky* DNA adducts have a considerable degree of variation by batch and between laboratories) (Peluso *et al.*, 2005).

Production of DNA damage primarily reflects carcinogenic exposures, but is also regulated by inherited and acquired susceptibilities. Age, gender, body mass index, physical exercise, consumption of charcoal-broiled food, consumption of fresh fruit and vegetables, and seasonal variations have also been reported to influence the formation of aromatic DNA adducts. DNA adduct levels have been found to be dependent on polymorphisms in metabolic genes (i.e. the *CYP1A1*, *MspI*, and *GSTM1* null genotypes) (Shields *et al.*,1993; Ryberg *et al.*, 1997; Pastorelli *et al.*, 1998; Butkiewicz *et al.*, 2000; Rojas *et al.*, 2000; Teixeira *et al.*, 2002; Georgiadis *et al.*, 2005). DNA damage may be repaired, but the ability of

Fig 12.1 Dose–response relationship between frequency ratios and external concentrations of benzo[*a*]pyrene (B[*a*]P) in work environments in a meta-analysis of occupational exposure to air pollution.



The inset shows an extrapolated dose–response curve at low exposure doses, assuming a linear dose–response relation, for B[*a*]P levels between 0 and 4.5 ng/m^3, the lowest value in the database. FRi: frequency ratio for the *i*th study. Source: Peluso *et al.* (2001); reproduced with permission from Oxford University Press.

a person to remove aromatic DNA adducts may vary from individual to individual.

In conclusion, DNA and protein adducts seem to be valuable markers of exposure to air pollutants in spite of errors in measurement. Since DNA adducts express genetic and acquired susceptibility, they can usefully complement other measures of exposure in research on risks for cancer.

Addendum (2012 update) [1]

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Numerous studies have considered DNA and protein adducts as biomarkers of exposure to genotoxic carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), present in environmental air pollution.

The association between air pollution and DNA adducts was investigated in 26 studies.

[1] See Preface for explanation of updating process.

These are cross-sectional and case–control studies, some nested within prospective cohorts. Some studies (Perera *et al.*, 1992; Hemminki *et al.*, 1994; Nielsen *et al.*, 1996b, 1996b; Yang *et al.*, 1996; Topinka *et al.*, 1997; Georgiadis *et al.*, 2001; Merlo *et al.*, 1997; Ruchirawa *et al.*, 2002; Marczynski *et al.*, 2005; Topinka *et al.*, 2007; Tuntawiroon *et al.*, 2007; Ayi-Fanou *et al.*, 2011) compared the mean DNA adduct levels in individuals with estimated high or low external exposures (Table 12.1), while others (Binková *et al.*, 1995; Whyatt *et al.*, 1998; Sørensen *et al.*, 2003; Castaño-Vinyals *et al.*, 2004; Peluso *et al.*, 2005; Neri *et al.*, 2006; Pavanello *et al.*, 2006; Palli *et al.*, 2008; Peluso *et al.*, 2008; Pavanello *et al.*, 2009; Pedersen *et al.*, 2009; García-Suástegui *et al.*, 2011; Herbstman *et al.*, 2012) carried out correlation and regression analyses on all subjects (Table 12.2). As illustrated in Figure 12.2, most studies (including two reviews) found 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, among others, residents in an industrial area and rural controls in Poland (Perera *et al.*, 1992), bus and taxi drivers in Stockholm (Hemminki *et al.*, 1994), students in Denmark and in Greece (Nielsen *et al.*, 1996b), bus drivers in Copenhagen (Nielsen *et al.*, 1996a), policemen in Genoa (Merlo *et al.*, 1997), policemen in Bangkok (Ruchirawa *et al.*, 2002), and schoolchildren in Thailand (Tuntawiroon *et al.*, 2007), as well as street vendors, taxi drivers, gasoline salesmen, and roadside residents in Benin (Ayi-Fanou *et al.*, 2011). Fetal exposures were associated with DNA adducts in newborns (Topinka *et al.*, 1997; Whyatt *et al.*, 1998; Pedersen *et al.*, 2009). Only two studies did not find an association (Yang *et al.*, 1996; Sørensen *et al.*, 2003). One study found that students in a rural area had higher DNA adduct levels than students in Athens, the most heavily polluted city; however, exposure to second-hand smoke (SHS) explains this paradoxical observation (Georgiadis *et al.*, 2001).

Protein adducts and exposure to air pollution

Far fewer studies (Hemminki *et al.*, 1994; Pastorelli *et al.*, 1996; Nielsen *et al.*, 1996c; Richter *et al.*, 2001) have examined the relationship between exposure to air pollution and protein adducts, and their results are inconsistent (Table 12.3). Only one study (Richter *et al.*, 2001) shows a statistically significant association between exposure to air pollution and protein adducts, while another shows a significant association between exposure to diesel exhaust and protein adducts (Nielsen *et al.*, 1996c). The other two studies show either no significant association (Pastorelli *et al.*, 1996) or significant association only between certain subject groups (Hemminki *et al.*, 1994).

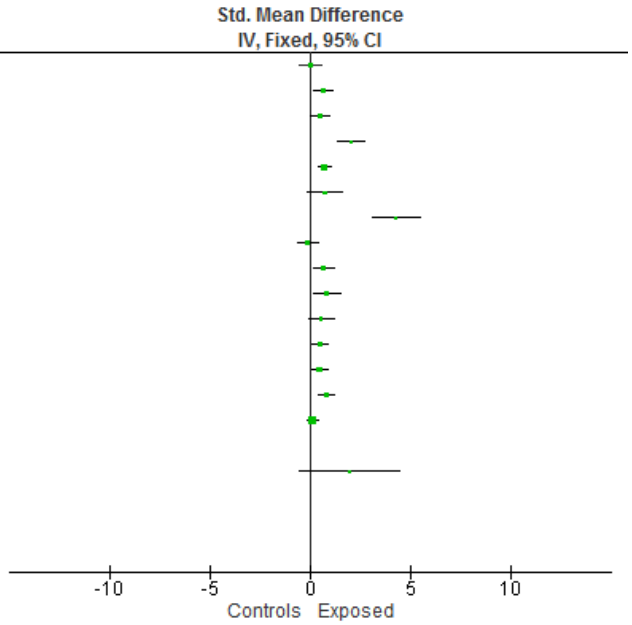
Critical issues in evaluating the relationship between air pollution and biomarkers

Confounding

Of the studies on DNA adducts, only 14 adjusted for various potential confounders and only 7 of those 14 adjusted for PAHs in diet, indicating lack of adequate adjustment for confounding. Similarly, adjustment for confounders was minimal in the studies on protein adducts. Considering that PAHs in diet, smoking, and exposure to SHS are factors that have great impact on DNA adduct and protein formation, these exposures need to be accounted for when investigating the association between exposure to air pollution and DNA adducts.

Fig 12.2 Standardized mean difference forest plot of studies on DNA adducts reporting difference in means.

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total[0]	Control Mean	Control SD	Control Total[0]	Weight ☆	Std. Mean Difference 95% CI
Autrup 1999	1.74	4.69	29	1.54	4.29	20	4.3%	0.04 [-0.53, 0.61]
Buthbumrung 2008 - in WBC	0.25	0.13	40	0.08	0.34	32	6.1%	0.68 [0.20, 1.16]
Buthbumrung 2008 - urine	2.16	1.84	43	1.32	1.24	32	6.5%	0.52 [0.05, 0.98]
Calderon-Garciduenas 1999	602	195	86	210	122	12	3.1%	2.07 [1.40, 2.74]
Chuang 2003	0.33	0.2	95	0.2	0.14	75	14.4%	0.73 [0.42, 1.05]
Fanou 2006	2.05	1.25	35	1.11	0.82	6	1.8%	0.77 [-0.12, 1.65]
Harri 2005 - MNBC	4.84	0.17	19	4.11	0.16	18	0.9%	4.32 [3.10, 5.55]
Harri 2005 - urine	1.52	0.44	29	1.56	0.61	36	5.9%	-0.07 [-0.56, 0.42]
Lai 2005	13.3	7.1	47	8.4	6.2	24	5.5%	0.71 [0.21, 1.22]
Novotna 2007 - January	2.91	1.84	54	1.36	1.53	11	3.2%	0.85 [0.19, 1.52]
Novotna 2007 - March	2.12	1.62	54	1.22	0.96	11	3.3%	0.58 [-0.08, 1.24]
Rossner 2007 - Season A	7.59	2.25	50	6.29	2.59	50	8.8%	0.53 [0.13, 0.93]
Rossner 2007 - Season B	6.73	2.48	50	5.51	2.36	50	8.9%	0.50 [0.10, 0.90]
Rossner 2007 - Season C	5.67	2.5	50	3.82	1.73	50	8.4%	0.85 [0.44, 1.26]
Singh 2007 - MNBC	33	30.1	98	29.2	21.2	105	18.6%	0.15 [-0.13, 0.42]
Staessen 2001	0.57	0	42	0.44	0	100		Not estimable
Suzuki 1995	9.9	2.5	3	4.22	2	3	0.2%	2.01 [-0.51, 4.52]
Svecova 2009	14.6	0	495	15.2	0	399		Not estimable



Source: Demetriou *et al.* (2012); reproduced with permission from BMJ Publishing Group Ltd.
☆ Weight was derived using the inverse of the variance in a fixed effects model. Forest plots are presented for clarity in data presentation; however, formal meta-analysis was not performed due to the heterogeneity of the studies included in the review.
[0] Total refers to total sample size in the experimental (exposed) and control groups.

Table 12.3 Results on the association between air pollution and protein adducts in exposed individuals

Reference	Study location	Exposure	Controlled confounders	Outcome measure	Group, sample size (total: 1044)	Protein adduct levels	*P*
Hemminki *et al.* (1994)	Stockholm, Sweden	Traffic-related air pollution	Age, smoking	Plasma protein (albumin) adducts	Bus drivers – urban routes, 26	Mean: 232 fmol/mg Ab (SD:108)	NS
					Bus drivers – suburban routes, 23	Mean:120 fmol/mg Ab (SD:102)	NS
					Taxi drivers – mixed routes, 19	Mean: 318 fmol/mg Ab (SD:180)	< 0.001
					Controls, 22	Mean: 119 fmol/mg Ab (SD:109)	
Pastorelli *et al.* (1996)	Italy	Traffic-related air pollution	Smoking	Benzo[*a*]pyrene–haemoglobin adducts	Newspaper vendors – high exposure, 30	Median: 0.3 fmol/mg Hb Range: ≤ 0.1–3.3	0.09
					Newspaper vendors – low exposure, 23	Median: ≤ 0.1 fmol/mg Hb Range: ≤ 0.1–0.41	
Nielsen *et al.* (1996a)	Copenhagen, Denmark	Diesel exhaust exposed workers	Smoking, sex	Haemoglobin adducts	Bus garage workers and mechanics, 10	Median: 33.3 fmol/mg Hb Range: 25.4–58.8	0.0038
					Bus company administrative staff, 12	Median: 22.1 fmol/mg Hb Range: 8.0–37.0	
Richter *et al.* (2001)	Germany	Environmental air pollution		4-ABP-haemoglobin adducts	Children from Munich, 34	Mean: 30.7 pg/g Hb	< 0.001
					Children from Eichstatt and Augsburg, 64 and 126	Mean: 20.7 pg/g Hb	

Ab, albumin; ABP, aminobiphenyl; Hb, haemoglobin; NS, not significant; SD, standard deviation. Compiled from Demetriou *et al.* (2012).

Reversibility of changes and individual susceptibilities

A second issue is the plasticity and reversibility of protein and DNA adduct changes. Protein adducts cannot be repaired and thus better reflect exposure, whereas DNA adducts can be eliminated by DNA repair mechanisms and are therefore more transient indicators of external exposure. 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 (i.e. the *CYP1A1*, *MspI*, and *GSTM1* null genotypes) (Shields *et al.*, 1993; Ryberg *et al.*, 1997; Pastorelli *et al.*, 1998), which may determine an individual's ability to remove DNA adducts.

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 air pollution. Studies show that developing fetuses are more susceptible than adults to the carcinogenic effects of PAHs (Topinka *et al.*, 1997; Pedersen *et al.*, 2009). 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 (Barouki *et al.*, 2012). Studies also show that exposure to PAHs and DNA adduct formation are not linearly associated (Lewtas *et al.*, 1997). Instead, as shown in Figure 12.1, among highly exposed subjects the level of DNA adducts per unit of exposure was significantly lower than those at lower exposures (Peluso *et al.*, 2001). There is little evidence in the literature about the impact of duration of exposure on the formation of protein and DNA adducts.

Target versus surrogate tissues

Another important consideration is that most studies available to date use surrogate tissues, such as blood. Air pollution 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.

Conclusions

Despite these considerations, DNA adducts are undeniably a valuable biomarker of exposure to air pollution. A recent review (Demetriou *et al.*, 2012) recognized DNA adducts, along with 1-hydroxypyrene (1-OHP), chromosomal aberrations (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, 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. DNA adducts have also been suggested to be predictive for the risk of future cancer (Peluso *et al.*, 2005; Veglia *et al.*, 2008). This multilevel evidence adds to the plausibility of a causal association between exposure to ambient air pollution and lung cancer.

In conclusion, biomarkers, including DNA adducts, are without question a valuable tool in the investigation of the relationship between air pollution and cancer since they not only improve exposure assessment but also increase our understanding of mechanisms underlying this association.

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