

REVIEW

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# Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence

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## Abstract

Current evidence supports the notion that environmental exposures are associated with DNA-methylation and expression changes that can impact human health. Our objective was to conduct a systematic review of epidemiologic studies evaluating the association between environmental chemicals with DNA methylation levels in adults. After excluding arsenic, recently evaluated in a systematic review, we identified a total of 17 articles (6 on cadmium, 4 on lead, 2 on mercury, 1 on nickel, 1 on antimony, 1 on tungsten, 5 on persistent organic pollutants and perfluorinated compounds, 1 on bisphenol A, and 3 on polycyclic aromatic hydrocarbons). The selected articles reported quantitative methods to determine DNA methylation including immunocolorimetric assays for total content of genomic DNA methylation, and microarray technologies, methylation-specific quantitative PCR, Luminometric Methylation Assay (LUMA), and bisulfite pyrosequencing for DNA methylation content of genomic sites such as gene promoters, LINE-1, Alu elements, and others. Considering consistency, temporality, strength, dose-response relationship, and biological plausibility, we concluded that the current evidence is not sufficient to provide inference because differences across studies and limited samples sizes make it difficult to compare across studies and to evaluate sources of heterogeneity. Important questions for future research include the need for larger and longitudinal studies, the validation of findings, and the systematic evaluation of the dose-response relationships. Future studies should also consider the evaluation of epigenetic marks recently in the research spotlight such as DNA hydroxymethylation and the role of underlying genetic variants.

**Keywords:** Systematic review, DNA methylation, Environmental chemicals, Cadmium, Lead, Mercury, Metals, Persistent organic pollutants, Bisphenol A, Polycyclic aromatic hydrocarbons

## Review

### Introduction

Beyond lifestyle determinants, the role of environmental chemicals as determinants of DNA methylation has gained considerable attention. Changes in DNA methylation add biological plausibility to the increasingly recognized contribution of environmental chemicals to disease burden [1] as DNA methylation is involved in regulating many cellular processes, including X-chromosome

inactivation, genomic imprinting, chromosome stability, and gene transcription. Environmental chemicals can interfere with the one-carbon and citric acid metabolism pathways, resulting in anomalous DNA-methylation status throughout the genome [2,3]. Environmental chemicals can also directly interact with enzymes involved not only in one-carbon metabolism and citric acid metabolism pathways but also in histone modifications [4-6]. A summary of suggested mechanisms of action of environmental chemicals on DNA methylation machinery is shown in Figure 1. In turn, these epigenetic mechanisms may modify potential toxicity pathways specific to the environmental chemicals in the organism.

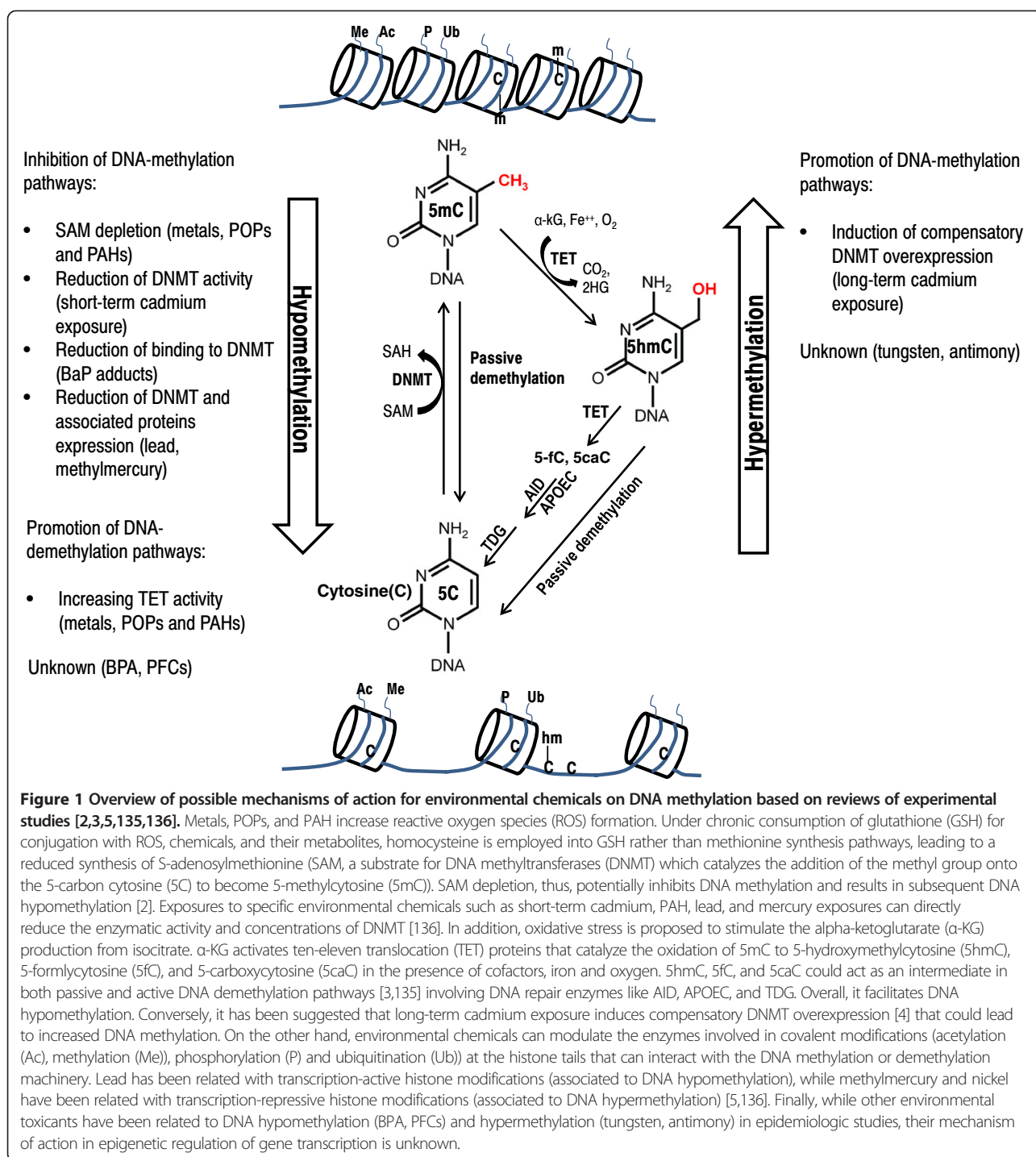
Environmental chemicals have been linked to aberrant changes in epigenetic pathways both in experimental and epidemiological studies. In animal studies, maternal diet

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during pregnancy was associated with the pattern of DNA methylation of specific genes, which resulted in permanent phenotypic changes including body weight and blood pressure levels [7,8]. In humans, populations exposed to famine during the prenatal period showed increased prevalence of cardiometabolic factors and ischemic heart disease mortality [9], with evidence supporting a mediating role of epigenetic mechanisms in disease pathogenesis

[10]. Deleterious effects of epigenetic changes are not restricted to the prenatal period. Monozygotic twins experienced an epigenetic drift in relation to one another with advancing age, time shared together, and behavioral factors such as smoking [11]. There is, however, a need to undertake a systematic appraisal of the epidemiologic evidence evaluating the potential role of environmental chemicals as determinants of DNA methylation in adults.

Our objective was to conduct a systematic review and synthesis of results from epidemiologic studies evaluating the association of environmental chemicals including cadmium, lead, mercury, nickel, persistent organic pollutants (POPs), bisphenol A (BPA), polycyclic aromatic hydrocarbons (PAHs), and phthalates, with DNA methylation levels in adults. We did not include arsenic studies in our search because there is a recently published systematic review published by Bailey *et al.* [12]. Other environmental exposures, which have been related to DNA methylation, such as exposure to tobacco smoke [13-17] and air pollution [18], are out of the focus of the present review, as tobacco smoke and air pollution are mixtures of different types of chemicals rather than individual groups of compounds.

## Methods

### **Search strategy, study selection, and data abstraction**

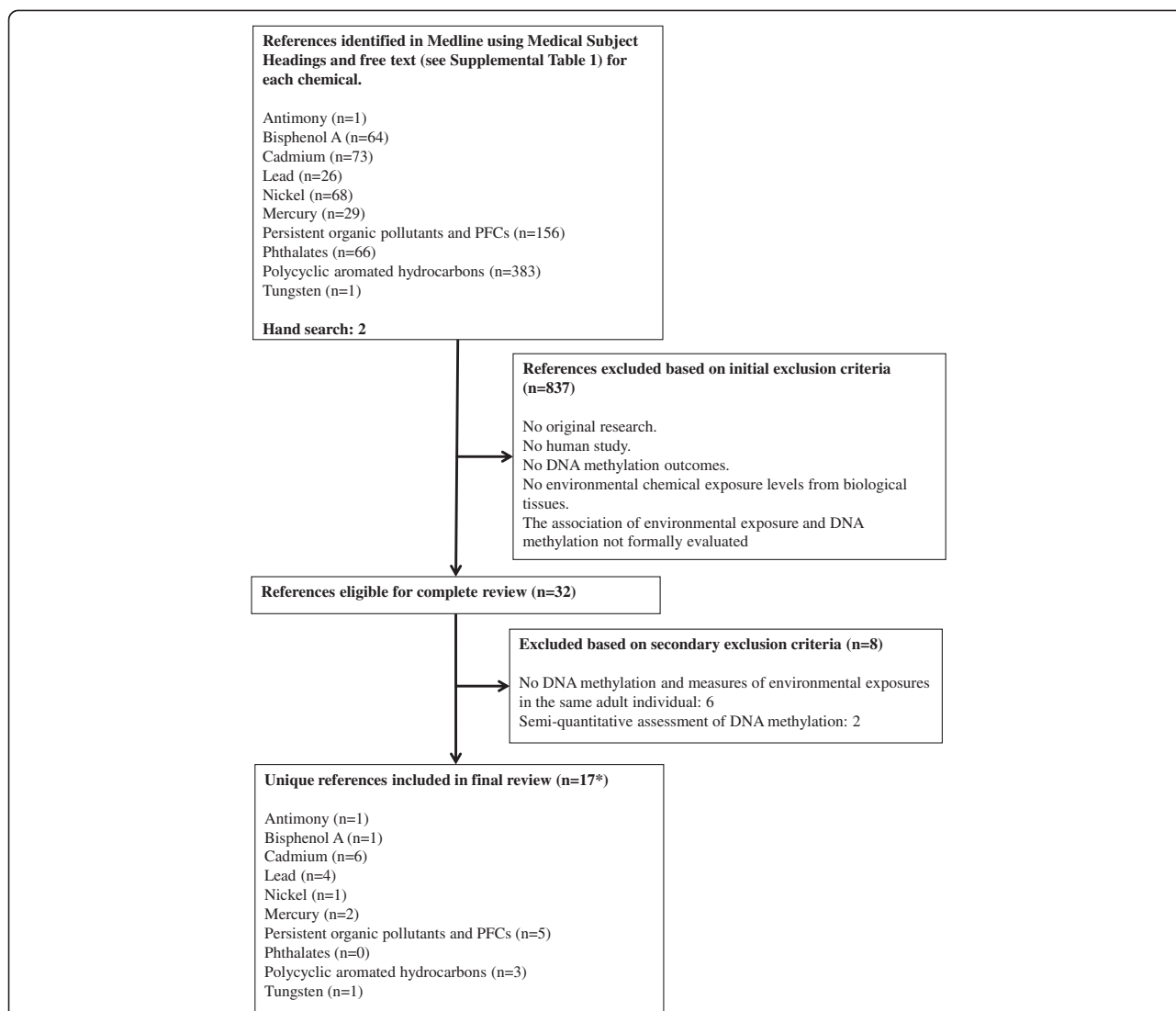
We searched PubMed for relevant studies published through 10 April 2014 using the search strategy described in Additional file 1: Table S1 (Supplemental Material). The search strategy retrieved a total of 867 citations (including duplicates). We included all articles assessing environmental chemical exposures using biomarkers. The search had no language restrictions. We also included two relevant studies published after 10 April 2014 and identified by hand search [19,20]. Two investigators (A.R.H. and C.C.K.) independently reviewed each of all the abstracts and selected 32 papers applying the following study exclusion criteria (Figure 2): a) no original research (that is, reviews, editorials, non-research letters); b) no human study; c) no DNA methylation outcomes; d) no environmental chemical exposure levels measured in biological tissues (for example, environmental measures such as water or air, or distance from a source). In this systematic review, the focus was on the role of environmental chemicals exposure in DNA methylation changes in adults. Therefore, as a second layer of exclusion, we additionally excluded one study focusing on prepubescent girls [21], and five studies that focused on the association of maternal exposure biomarkers and DNA methylation in cord blood or the offspring and did not provide corresponding measures of DNA methylation in the mothers [22-26]. We additionally, excluded two studies with semi-quantitative assessment of DNA methylation [27,28] as the comparison of results with quantitative DNA methylation assessment methods is unclear. Any discrepancies were resolved by consensus, and if necessary, a third reviewer was involved. A native speaker reviewed the full text of any non-English article that could not be included or excluded based on the initial abstract review. We included in the final review 17 papers, some of them measuring multiple environmental toxicants evaluated in unique study populations [19,29,30] (Figure 2). Our review identified no publications investigating the association between phthalates and DNA methylation. After

retrieval of articles from the search, the reference lists of selected articles were checked for other potentially relevant articles, identifying no additional studies. We collected the following data for each study: first author, year of publication, study design, size and population characteristics, exposure assessment and categories for comparison, DNA methylation assessment and endpoint definition, measures of association and 95% confidence interval (CI) or *P* values, and statistical methods including DNA methylation raw data processing methods. For studies modeling exposures both as continuous and as categorical, we reported continuous measures of association due to space constraints in the tables. However, we evaluated flexible dose-response relationships when reported. For polychlorinated biphenyls (PCBs), when multiple congeners were reported, we selected the congener with the weakest, highest, and median association. We also reported all the statistically significant POPs.

To assess study quality, we adapted the criteria used by Longnecker *et al.* for observational studies (Supplemental Material, Additional file 2: Table S2) [31]. We followed the criteria proposed by the 2004 US Surgeon General Report on the health consequences of smoking [32], which include the evaluation of consistency, temporality, strength, dose-response relationship, and biological plausibility including confounding. As a result, the evidence for each environmental chemical and DNA methylation was classified into four groups as modified from the Surgeon General Report [32]: sufficient evidence, suggestive but not sufficient evidence, insufficient evidence to infer a relationship, and suggestive of no relationship. We organized the presentation of the results by environmental chemical.

### **Current perspectives and results**

**Cadmium and DNA methylation** Cadmium exposure from tobacco smoke, air pollution, occupation, and diet (leafy and root vegetables, grains, and offal) is widespread in general populations [33]. In the US, cadmium exposure has substantially decreased during the last decades, in part related to reductions in smoking [34]. Cadmium exposure, however, remains an important concern, because even at the currently reduced levels of exposure, cadmium has been related to cardiovascular, bone, and kidney disease in studies of the US National Health and Nutrition Examination Survey (NHANES) 1999 to 2008 data [35-41]. In epidemiologic studies, cadmium concentrations in blood and urine are established biomarkers of cadmium exposure and internal dose [33,42]. Both biomarkers can reflect cumulative exposure, although blood cadmium also reflects short-term fluctuations in exposure [33,42]. Experimental *ex vivo* evidence showed that cadmium was an effective, noncompetitive inhibitor of *M.SssI* DNA-methyltransferase (DNMT) (a bacterial DNMT that recognizes the same sequence as mammalian's DNMTs) [4]. In rat liver cells, short-term cadmium



**Figure 2 Flow diagram of the study selection process.** Summary of inclusion and exclusion criteria used in this systematic review of studies investigating the association between environmental chemicals and DNA methylation levels, 10 April 2014. \*17 references include the following studies with multiple environmental toxicants evaluated in unique study populations: Hanna *et al.* (2012) [29] examined in SMART population urine cadmium, blood lead and mercury, and serum BPA. Tajuddin *et al.* (2013) [30] examined in EPICURO population toenail cadmium, nickel, and lead. Tellez-Plaza *et al.* (2014) [19] examined in the SHS populations urine tungsten, antimony, and cadmium. Abbreviations: BPA, bisphenol A; PCF, perfluorinated compounds.

exposure induced DNA global hypomethylation [4]. Prolonged exposure, however, resulted in global DNA hypermethylation [4,43-45]. In general, most *in vitro* and *in vivo* studies showed increased gene-specific DNA methylation after exposure to cadmium [46-52].

We identified six publications investigating the association between cadmium and DNA methylation (Table 1). These studies were conducted in the US [19,29,53], Argentina [54], Spain [30], and China [55]. Cadmium exposure was measured in urine only [19,29], blood only [53], both in urine and blood [54,55], and in toenail [30]. Global DNA methylation was assessed by pyrosequencing of LINE-1 elements (a surrogate marker of global DNA methylation) in three

studies [29,30,54] and by an ELISA-like method (measurement of percent 5-methylcytosine [5-mC] in DNA sample) in one study [19]. CpG site-specific DNA methylation was measured in candidate genes by pyrosequencing in one study [55] and in an exploratory genome-wide manner using microarray technologies in two studies [53,54].

In general, studies mostly showed a trend towards positive associations of cadmium exposure and DNA methylation. In a study population from Argentina (N=200), however, blood cadmium was positively associated with DNA methylation in LINE-1 elements, but the association with urine cadmium was inverse [54]. Among five studies evaluating global or candidate gene methylation, three studies reported

**Table 1 Studies of cadmium exposure biomarkers and DNA methylation outcomes (6 studies available)**

First author, year	Design	Population	Size	Men (%) <sup>a</sup>	Age Range (yr) <sup>a</sup>	Exposure assessment	Exposure categories	DNA methylation Assessment	DNA methylation endpoint	Association	95% Confidence Interval or p-value	Adjustment Factors
Hanna, 2012 [29]	CS	U.S. (Study of Metals and Assisted Reproductive Technologies [SMART])	42	0	Mean 36 (28 to 44)	Urine by DRC- ICPMS	Above and below the median	Whole blood	1,505 CpG sites percent methylation	A trend towards hypermethylation if difference score >  30  (p < 0.05) No significant region <sup>b</sup> .		Normalization. QC reported. BEE NR. CH partially addressed. Data unadjusted. MCC NR.
						Median = 0.38 µg/L		Site specific Illumina GoldenGate and bisulfite pyrosequencing of significant regions <sup>b</sup>				
								Global by bisulfite pyrosequencing of LINE-1		Approximately 0.2 % increase in median DNAm	p = 0.39	
Hossain, 2012 [54]	CS	Andean plateau, Northern Argentina	202	0	Median 34 (18-64)		Per log-unit increase	Whole blood	Average percent methylation	Difference		QC reported. CH not addressed. Only 4 participants were smokers. Regression models adjusted for age, coca chewing, and arsenic in urine. Cadmium concentrations corrected to the mean specific gravity of urine.
						Blood by DRC-ICPMS (Median = 0.36 µg/L)		Site specific MLH1		0.19	-0.53, 0.91	
								CDKN2A		0.24	-0.29, 0.77	
								Global LINE-1		0.45	-0.23, 1.12	
						Urine by DRC-ICPMS (Median = 0.23 µg/L)		Site specific MLH1		-0.073	-0.50, 0.36	
								CDKN2A		-0.11	-0.42, 0.21	
								Global LINE-1		-0.42	-0.82, -0.025	
Zhang, 2013 [55]	CS	Southern China	81	39.5	53.9 (IQR 48.0-59.0)	Graphite Furnace-AAS	Per log-unit increase	Whole blood	Average percent methylation	Difference		QC reported. CH not addressed. Regression models adjusted for age, sex, BMI, smoking, alcohol drinking, albumin, B2M, eGFR, N-acetyl-b-d glucosaminidase (NAG).
						Blood (Median = 2.62 µg/L)		Site specific by bisulfite pyrosequencing in: RASAL1		0.49	0.21, 0.77	
								KLOTHO		1.18	0.54, 1.83	
						Urine by (Median = 5.20 µg/g creatinine)		RASAL1		0.88	0.57, 1.20	
								KLOTHO		1.55	0.75, 2.35	

**Table 1 Studies of cadmium exposure biomarkers and DNA methylation outcomes (6 studies available) (Continued)**

Tajuddin, 2013 [30]	CS	Spain (EPICURO study)	659	89	66	Toenail by ICPMS (Median = 0.01 µg/g)	Per 1 µg/g increase	Blood granulocytes Global by bisulfite pyrosequencing in LINE-1	Average percent methylation	Difference 0.1	-0.3, 0.6	QC reported. CH addressed. Adjusted for age, sex, study region, and smoking status
Sanders, 2014 [53]	Nested sub-CO	Durham county, US (CEHI study)	17	0	Maternal age: 28 (19–42)	Blood Median = 0.2 µg/L	Above and below the median	Blood leukocytes Site specific MBD2b/ MBD3L1 enrichment in Affymetrix Human Promoter 1.0R array	Average percent methylation in 16 421 CpG islands	General pattern toward increased methylation with increased cadmium in 92 significant <sup>c</sup> genes		Normalization. BEE NR. CH addressed. No adjustment conducted, but evaluation of participant characteristics by cadmium and DNAm levels, with no significant differences reported. FDR corrected q-value provided. SNP-related clustering of DNA methylation not evaluated.
										TWSG1 = 1.79	0.0007	
										USP30 = 1.70	0.0023	
										FAM83H = 1.52	0.0052	
										PPP2R5B = 1.56	0.0060	
										PRKCG = 1.44	0.0068	
Tellez-Plaza, 2014 [19]	CS	13 American Indian communities, US (SHS)	48	31.3	55 ± 7.3	Urine by ICPMS Median = 0.87 µg/g	Above and below the median in 1989-1991	Global by ELISA-like commercial kit Blood leukocytes in 1989–1991 Whole blood in 1997–1999	Logit-transformed percent methylation relative to cytosine genomic content	Odds ratio 1.75	0.96, 3.20	QC reported. Models adjusted for age, sex, smoking status, BMI and, in prospective analyses only, log-transformed total count of white blood cells and percent of neutrophils.
	CO									1.03	0.50, 2.11	

AAS: atomic absorption spectrometry; BEE: batch effects evaluation; BMI: body mass index; CC: case-control; CH: Cell heterogeneity; CI: confidence interval; CO: cohort; CS: cross-sectional; DNAm, DNA methylation; FDR: false discovery rate; MCC: multiple comparison correction; NR: not reported; LOD: limit of detection; QC: quality control.

<sup>a</sup>Sociodemographic data available in the article, not necessarily in the subsample without missing values in DNA methylation or exposures.

<sup>b</sup>Significance was defined as a difference score > |13| (p < 0.05) and >10% absolute difference between the means for each group.

<sup>c</sup>Significance defined as a minimum absolute change of 30% comparing exposure groups and a p-value < 0.05.



significant or marginally significant associations with cadmium biomarkers [19,54,55]. In US American Indians, the multi-adjusted odds ratio of percent 5-mC comparing participants with urine cadmium levels above and below 0.87  $\mu\text{g/g}$  was 1.75 (95% CI 0.96, 3.20) [19]. In the Argentinian population, the difference in percent DNA methylation in LINE-1 elements per log-unit increase in urine cadmium was -0.42% (95% CI -0.82, -0.025) [54]. In a population from Southern China ( $N = 81$ ) [55], the difference in average percent methylation in RASAL1 and KLOTHO genes per log-unit increase in urine cadmium was, respectively, 0.88% (95% CI 0.57, 1.20) and 1.55% (95% CI 0.75, 2.35). Both epigenome-wide association studies [29,53] evaluated general patterns in the association of DNA methylation in specific CpG sites and cadmium biomarkers in CpG sites with an effect size considered relevant, consistently finding a trend towards increased methylation with elevated cadmium exposure. In the Study of Metals and Assisted Reproductive Technologies (SMART) study, conducted in US women undergoing ovarian stimulation [29], no sites were considered significant. In the CEHI study, conducted in US mother-newborn pairs, percent increase in DNA methylation in the top five associated CpG sites ranged from 44% to 79% [53]. None of the genome-wide studies reported statistically significant regions after controlling for a false discovery rate, although the study sample sizes were relatively small [29,53]. Confounding by sex, age, and smoking status was generally addressed, with exceptions [29]. Only two studies [19,30] addressed the potential confounding effect of tissue cell heterogeneity.

**Lead and DNA methylation** Lead in the environment has decreased over the last decades when regulations banning the use of lead in gasoline, paint, and solders were implemented [56,57]. The general population is exposed through ambient air, alcohol consumption, and tobacco smoke [58,59]. Patella and tibia lead are biomarkers of cumulative lead exposure and body burden, while blood lead is a biomarker of recent exposure including endogenous exposure from bone [60]. Patella lead is biologically more active than tibia lead [61], having a role in internal exposure dose from redistribution of accumulated lead in the body. Studies have shown associations between low-exposure to lead and increased risk of neurocognitive outcomes, high blood pressure, chronic kidney disease, hyperuricemia, gout, cardiovascular disease, cancer, and other health effects [60,62,63]. In *in vivo* and *in vitro* studies, lead exposure was associated with changes in DNA methylation and expression of specific genes [64-67], although experimental studies evaluating the molecular mechanisms of lead-induced changes in DNA methylation are needed.

We identified four publications investigating the association between lead and DNA methylation (Table 2).

These studies were conducted in the US [29,68], China [69], and Spain [30]. Lead exposure was measured in blood [29,68,69], patella and tibia [68], or toenail [30]. Global DNA methylation was assessed by quantitative pyrosequencing of LINE-1 or Alu elements (Alu is another surrogate marker for global DNA methylation) in three studies [29,30,68] and by methylation specific real-time PCR in one study [69]. CpG site-specific DNA methylation was measured in an exploratory genome-wide manner using microarray technologies in one study [29], with validation of significant regions by quantitative pyrosequencing.

In general, all the studies reported a trend towards inverse associations of lead exposure and global DNA methylation. Two studies reported statistically significant associations of DNA methylation with lead biomarkers [19,55]. In a Chinese population ( $N = 110$ ), participants showed 86.3%, 78.6%, and 73.9% average LINE-1 methylation in blood lead groups including <100, 100 to 200, and >200  $\mu\text{g/L}$ , respectively ( $P$  trend <0.001). In 678 men from the US Normative Aging Study, the absolute difference in average LINE-1 methylation percentage was -0.25% (95% CI -0.44, -0.05) per an interquartile range change (19  $\mu\text{g/g}$ ) in patella lead concentrations [68]. Blood and tibia lead biomarkers, however, did not show statistically significant associations with LINE-1 methylation in this study population, although the direction of the association was similar as compared to patella. The authors interpreted that the redistribution of accumulated lead from bone over time is associated with DNA methylation in circulating leukocytes. In the only epigenome-wide association study ( $N = 24$ ) [53], a CpG site in the *COLIA2* gene showed decreased DNA methylation with elevated blood lead exposure under the established significance threshold. In pyrosequencing validation, this site showed a 38% decrease in average percent methylation ( $P$  value = 0.004) comparing individuals above and below 0.73  $\mu\text{g/dL}$  of blood lead concentrations. Among CpG sites with an effect size considered relevant by the authors, a general trend towards hypomethylation with increasing blood lead levels was observed. There were not reported statistically significant regions after controlling for a false discovery rate [29]. Two (out of four) studies addressed potential confounding by sex, age, smoking status, and tissue cell heterogeneity in DNA methylation status [30,68]. While one of the studies was a cohort study with repeated measurements of lead biomarkers and DNA methylation [68], all the studies reported cross-sectional associations.

#### **Mercury and other metals and DNA methylation**

Mercury is a highly reactive metal with unknown physiological activity, which is persistent in the food chain [70]. While the main source of inorganic mercury is occupation (dentistry, mining, artisans manipulating

**Table 2 Studies of lead exposure biomarkers and DNA methylation outcomes (4 studies available)**

First author, year	Design	Population	Size	Men (%) <sup>a</sup>	Age Range (yr) <sup>a</sup>	Exposure assessment	Exposure categories	DNA methylation Assessment	DNA methylation endpoint	Association	95% Confidence Interval or p-value	Data pre-processing and adjustment factors
Hanna, 2012 [29]	CS	U.S. (Study of Metals and Assisted Reproductive Technologies [SMART])	24	0	Mean 36 (28 to 44)	Blood by DRC-inductively coupled plasma mass spectrometry  Median = 0.73 µg/dL	Above and below the median	Whole blood DNA  Site specific Illumina GoldenGate and bisulfite pyrosequencing of significant regions <sup>b</sup>	1,505 CpG sites percent methylation	A trend towards hypomethylation if difference score >  30  (P < 0.05)  COL1A2 38% decrease in mean DNA m r = - 0.45;  Approximately 0.1% increase in median DNAm	P = 0.004 P = 0.03 P = 0.76	Normalization. QC reported. BEE NR. CH partially addressed. Data unadjusted. MCC NR.
Tajuddin, 2013 [30]	CS	Spain (EPICURO study)	659	89	66	Toenail by ICPMS (Median = 0.40 µg/g)	Per 1 µg/g increase	Granulocyte DNA  Global by Quantitative pyrosequencing in LINE-1	Average % methylation	Difference  -0.06	-0.1, 0.02	QC reported. CH addressed. Adjusted for age, sex, study region, and smoking status
Li, 2013 [69]	CS	Wuxi region, China	110	91	mean = 39.45 (range 20-55)	Blood by AAS	<100 µg/L 100-200 µg/L >200 µg/L	Peripheral leukocytes  Global LINE-1 by methylation-specific real-time PCR	Average % methylation	86.3%, 78.6%, 73.9%	P < 0.001	No QC reported. CH addressed and adjustments not reported.
Wright, 2010 [68]	CO	US, Normative Aging Study	679	100	72.4			Buffy coat  Global by quantitative pyrosequencing	Average % methylation	Difference		QC reported. Models adjusted for age, BMI, percent lymphocytes, education, smoking pack-years, and blood lead levels.
						Tibia	Per IQR (15 µg/g) increase	LINE-1 Alu		-0.07 0.02	-0.29, 0.14 -0.10, 0.13	
						Patella	Per IQR (19 µg/g) increase	LINE-1 Alu		-0.25 -0.03	-0.44, -0.05 -0.14, 0.08	
						Blood	Per IQR (2 g/dL) increase	LINE-1 Alu		0.04 0.03	-0.10, 0.19 -0.05, 0.10	

AAS, atomic absorption spectrometry; BEE: batch effects evaluation; CH: Cell heterogeneity; DNAm, DNA methylation; IQR, interquartile range; LOD: limit of detection; MCC: multiple comparison correction; NR: not reported; QC: quality control.

<sup>a</sup>Sociodemographic data available in the article, not necessarily in the subsample without missing values in DNA methylation or exposures.

<sup>b</sup>Significance was defined as a difference score > |13| (p < 0.05) and >10% absolute difference between the means for each group.



mercury-containing materials) and dental amalgams, the general population is mainly exposed to organic mercury through consumption of fish (specially large predatory fish) and in a lesser degree shellfish and other marine animals [70]. Blood and hair mercury reflects exposure to methylmercury. Urine mercury, however, mainly reflects exposure to inorganic mercury [70]. Methylmercury is especially toxic for the neurologic system, especially during infancy [71]. Both methylmercury and inorganic mercury have immunotoxic effects, although the immunotoxicity is higher for inorganic mercury [71]. Other mercury-related health outcomes include cardiovascular disease, cancer, alterations of the reproductive system, and kidney disease [71-74]. There is evidence from experimental studies that mercury can change DNA methylation patterns. In rat embryonic neural stem cells and prenatally exposed adult rats, methylmercury reduced neural cell proliferation and was associated with global DNA hypomethylation [75]. In mouse stem cells, mercury exposure induced aberrant DNA methylation at specific gene loci [76]. The molecular mechanisms for potential epigenetic effects of mercury, however, are unknown.

Other nonessential metals are also of concern because they have been related to diverse health outcome in human studies. Tungsten has been related to cancer mortality [77], lung cancer, respiratory alterations, electrocardiograph abnormalities, and sudden death [78], and with prevalent cardiovascular disease and peripheral arterial disease [38,79]. Antimony was associated with peripheral arterial disease [38]. Nickel is an established carcinogen in occupational settings (respiratory cancers), especially insoluble nickel subsulfide and nickel oxide [80]. Other chronic health effects associated to nickel include rhinitis, sinusitis, nasal septum perforations, asthma, skin allergies, and reproductive effects [80]. However, experimental evidence indicating a potential role in altering DNA methylation for these metals is scarce, except for nickel. *In vitro* studies treatment with nickel resulted in both promoter hypermethylation and increased global DNA methylation [81,82]. Nickel may also influence DNA methylation by deregulating epigenetic enzymes involved in post-translational histone modifications [83,84].

For mercury, we identified two publications investigating the association between mercury and DNA methylation (Table 3). Both studies were conducted in the USA [29,85]. Mercury exposure was measured in blood [29] or urine and hair [85]. For other metals, we only identified one publication investigating the association of DNA methylation with toenail nickel in a population from Spain [30] and urine tungsten and antimony in US American Indians [19]. Among all the retrieved studies evaluating mercury and other metals, global DNA methylation was assessed by pyrosequencing of LINE-1 elements in three studies [29,30,85] and by and ELISA-like method in one study

[19]. Site-specific DNA methylation was measured in candidate genes by pyrosequencing in one study [85] and in an exploratory genome-wide manner using microarray technologies in one study [29]. In 659 participants from the Spanish Bladder Cancer Study (EPICURO) [55], the difference in average percent methylation in LINE-1 elements per 1  $\mu\text{g/g}$  increase in toenail nickel was 0.02% (95% CI 0.005, 0.03). In the only study reporting both cross-sectional and prospective associations, conducted in US American Indians [19], the odds ratio of global DNA methylation after 10 years of follow-up was 2.15 (95% CI 1.15, 4.01) comparing participants with baseline urine antimony levels above and below 0.27  $\mu\text{g/g}$ . The cross-sectional association, however, was not statistically significant [19]. In one epigenome-wide association study in the SMART study population ( $N = 43$ ) [53], only two CpG sites in the *GSTM1* gene showed increased DNA methylation with elevated blood mercury exposure under the established significance threshold of minimum absolute change of 10% and a  $P$  value  $< 0.05$ . In pyrosequencing validation, CpG sites in this gene showed a 39% increase in average % methylation ( $P$  value = 0.04) comparing individuals above and below 2.88  $\mu\text{g/L}$  of blood mercury concentrations. In this study, no statistically significant positions were reported after controlling for a false discovery rate [29]. The nickel, antimony, and tungsten [19,30], but not mercury [29,85], studies reported fully adjusted models including sex, age, and smoking status. For mercury, since the major source of exposure in humans is methylmercury from seafood consumption [86], adjustments for nutrients (for example, selenium, magnesium, n-3 fatty acids), lifestyle (seafood as a proxy for healthy diet), and other toxicants (POPs) in seafood should be considered. Only nickel, antimony, and tungsten studies [19,30] addressed the potential confounding effect of tissue cell heterogeneity.

**Persistent organic pollutants and other endocrine disruptors and DNA methylation** POPs are industrial chemicals that persist in the environment for decades even after production has been stopped [87]. The most well known are dioxins, PCBs, and polybrominated diphenyl ethers (PBDEs). Human exposure begins prenatally as many POPs can cross the placenta [88]. After birth, exposure occurs through breast milk [88] and also through inhalation (dust), ingestion (dairy and animal products), and skin contact [88,89]. POPs are lipophilic and accumulate in the adipose tissue. The potential effects of POPs include skin rashes to endocrine disruption, developmental delays, metabolic syndrome and diabetes, and cancer, depending on the type of compound and exposure [88].

Perfluorinated compounds (PFC) including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are fluorocarbons with at least one additional atom or

**Table 3 Studies of mercury and other non-essential metals exposure biomarkers and DNA methylation outcomes (4 studies available)**

First author, year	Design	Population	Size	Men (%) <sup>a</sup>	Age Range (yr) <sup>a</sup>	Exposure assessment	Exposure categories	DNA methylation Assessment	DNA methylation endpoint	Association	95% Confidence Interval or p-value	Data pre-processing and adjustment factors
<b>Mercury</b>												
Hanna, 2012 [29]	CS	U.S. (Study of Metals and Assisted Reproductive Technologies [SMART])	43	0	Mean 36 (28 to 44)	Whole blood by DRC-ICPMS Median = 2.88 µg/L	Above and below the median	Whole blood DNA Site specific Illumina GoldenGate and bisulfite pyrosequencing of significant regions <sup>b</sup> Global by bisulfite pyrosequencing of LINE-1	1,505 CpG sites % methylation	A trend towards hypermethylation if difference score >  30  (p < 0.05) GSTM1 39% increase <i>r</i> pearson = 0.17 ~0.2% decrease in median DNAm	p = 0.04 p = 0.27 p = 0.42	Normalization. QC reported. BEE NR. CH partially addressed. Data unadjusted. MCC NR.
Goodrich, 2013 [85]	CS	US (Michigan Dental Association members)	131	49	55.8 ± 11.6	Total levels by direct Mercury Analyzer Spot urine (Mean = 0.7 µg/L) Hair (Mean = 0.37 µg/g)	Per log-unit increase	Buccal mucosa Quantitative pyrosequencing Site specific DNMT1 SEPW1 SEPP1 Global LINE-1 Site specific DNMT1 SEPW1 SEPP1 Global LINE-1	Average % methylation	Difference     -0.03 0.06 2.38  0.37  -0.13 -0.01 -2.02  0.12	-0.32, 0.26 -0.12, 0.24 -1.23, 5.99  -0.75, 1.49  -0.40, 0.14 -0.19, 0.17 -5.55, 1.51  -0.96, 1.20	QC reported. Assessment of CH NR. Regression models adjusted for age and BMI.
<b>Other non-essential metals</b>												
Tajuddin, 2013 [30]	CS	Spain (EPICURO study)	659	89	66	Nickel Toenail by ICPMS (Median = 0.47 µg/g)	Per 1 µg/g increase	Granulocyte DNA Global by Quantitative pyrosequencing in LINE-1	Average % methylation	Difference  0.02	0.03, 0.005	QC reported. CH addressed. Adjusted for age, sex, study region, and smoking status

**Table 3 Studies of mercury and other non-essential metals exposure biomarkers and DNA methylation outcomes (4 studies available) (Continued)**

Study	Design	Population	n	Mean	SD	Measurement	Exposure	Method	Outcome	OR	CI	Notes
Tellez-Plaza, 2014 [19]	CS, CO	13 American Indian communities, US (SHS)	48	31.3	55 ± 7.3	Urine by ICPMS	Above and below the median at baseline	Global Methylamp Methylated DNA quantification kit (Epigentek)	Logit-transformed % methylation relative to cytosine genomic content			QC reported. Models adjusted for age, sex, smoking status, BMI and, in prospective analyses only, log-transformed total count of white blood cells and percent of neutrophils.
						Antimony (Median = 0.27 µg/g)		Blood leukocytes in 1989–1991		1.24	0.71, 2.15	
								Whole blood in 1997–1999		2.15	1.15, 4.01	
						Tungsten (Median = 0.13 µg/g)		Blood leukocytes in 1989–1991		1.46	0.85, 2.52	
								Whole blood in 1997–1999		0.93	0.46, 1.86	

BEE: batch effects evaluation; BMI: body mass index; CDT, Comparative Toxicogenomics Database; CC: case-control; CH: Cell heterogeneity; CI: confidence interval; CO: cohort; CS: cross-sectional; NR: not reported LOD: limit of detection; QC: quality control.

<sup>a</sup>Sociodemographic data available in the article, not necessarily in the subsample without missing values in DNA methylation or exposures.

<sup>b</sup>Significance was defined as a difference score > |13| (p < 0.05) and >10% absolute difference between the means for each group.

functional group and are included in the most recent list of POPs regulated by The Stockholm Convention [88]. For consistency with The Stockholm Convention and as previously done in other systematic reviews [90], we included PFCs in our search strategy for POPs. Drinking water is the primary route of PFCs exposure in some populations [91], but exposure sources are not well understood. While PFCs are persistent in the environment and in the body (half-life in humans is 3 to 5 years depending on the compound), they are not metabolized in humans and they are not lipophilic [91]. Animal data indicate that PFCs can cause several types of tumors and neonatal death and may have toxic effects on the immune, liver, and endocrine systems. Data on the human health effects include reported positive associations with cholesterol levels, hepatic enzymes, and adverse reproductive outcomes [91].

BPA is a compound with a shorter half-life compared to POPs, but it is frequently grouped together with POPs given its ubiquity and endocrine disruptor functions [88]. While humans are exposed through the placenta and ingestion (canned food), BPA is also present in dust and ambient air [88,92].

There are some studies evaluating the effect of POPs and other endocrine disruptors on DNA methylation in experimental settings. Exposure to dichlorodiphenyltrichloroethane (DDT) induced hypomethylation of CpG islands in *Sst*, *Gal*, *Arf1*, *Ttr*, *Mx1*, and *Grifin* genes in the hypothalamus of young male rats [93]. Rats treated *in utero* and postnatally with organochlorine pesticides and PCBs also showed decreased methylation of CpG sites in the promoter of the tumor suppressor gene *p16 (INK4a)* compared to controls [94]. Perfluorooctanoic acid induced gene promoter hypermethylation of *GSTP1* in human liver L02 cells [95]. Maternal BPA exposure disrupted genomic imprinting in the mouse embryos and placenta [96]. In rats, maternal exposure to BPA modified methylation of the metastable loci *Avy* and *CapbIAP* [97].

We identified four epidemiologic studies investigating the association between POPs [20,98-100], and one publication investigating PFCs [101] and BPA [29], respectively, with DNA methylation in adults (Table 4). These studies were conducted in the USA [29,101], South Korea [98], Sweden [99], Denmark [100], and Japan [20]. In studies assessing POPs, exposure was measured in plasma [100] or serum [20,98,99]. BPA was measured in serum [29] and PFCs were measured in blood [101]. Global DNA methylation was assessed by quantitative pyrosequencing of LINE-1 or Alu elements in four studies [29,98,100,101] and by Luminometric Methylation Assay (LUMA) in two studies [20,99]. CpG site-specific DNA methylation was measured in an exploratory genome-wide manner using microarray technologies in one study [29]. For most POPs, studies evaluating DNA methylation globally showed a trend

towards hypomethylation with increasing levels of exposure [20,98,100]. In studies measuring DNA methylation in LINE-1 elements, no statistically significant association was observed. The two studies measuring DNA methylation in Alu elements [98,100] showed consistent statistically significant inverse associations with oxychlorodane, p,p'-DDE and DDT. Increasing PCB183, heptachlor epoxide, trans-nonachlorodane, and PBDE47 in a study population from Korea ( $N = 86$ ) and PCB 156, 99, and 105,  $\beta$ -HCH,  $\alpha$ -chlordane, mirex, sum of PCBs, and sum of POPs in a study population from Denmark ( $N = 70$ ) was significantly associated with lower DNA methylation in Alu elements. Consistently, in a population of Japanese women ( $N = 399$ ), serum POPs were inversely associated with the global DNA methylation level measured by LUMA [20]. In an elder population from Sweden ( $N = 519$ ) [99], however, increasing total and non-ortho toxic equivalency (TEQ) levels, PCB126, and p,p'-DDE concentrations was significantly associated with increasing global DNA methylation levels also measured by LUMA ( $P < 0.05$ ) [99]. For PFCs, in a study population from the US ( $N = 671$ ) [101], a 12 ng/mL increase in PFOS levels was associated a difference of 20% (95% CI 0.09 to 0.32) in average 5-mC levels. Other PFCs did not show statistically significant associations. In one epigenome-wide association study in the SMART study population ( $N = 35$ ) [29], only one CpG site in the *TSP50* gene promoter showed increased DNA methylation with elevated BPA exposure under the established significance threshold of minimum absolute change of 10% by BPA levels and a  $P$  value  $< 0.05$ . In pyrosequencing validation, a region in this gene showed a 26% decrease in average percent methylation ( $P$  value = 0.005) comparing individuals above and below 2.39  $\mu\text{g/L}$  of serum unconjugated BPA concentrations. In this study, no statistically significant regions were reported after controlling for a false discovery rate [29].

All studies tested at least five POPs, but only one study [100] reported addressing multiple testing due to the elevated number of compounds. Most studies addressed potential confounding by sex, age, and smoking status [20,98,99,101]. One study did not adjust for sex, although the proportion of women was low [100]. One study presented unadjusted results [29]. POPs are highly lipophilic and their serum concentrations are closely related to serum lipid levels. Therefore, it is common practice to correct POP levels by lipid levels (that is, divide POP concentrations by total lipid concentrations). Alternatively, some authors argue that lipid correction may be problematic under certain assumptions [102]. In addition to lipid correction, it is advisable to conduct sensitivity analyses to evaluate robustness of findings using different approaches of handling lipid adjustment, such as conducting separate adjustment for total lipid levels with lipid-uncorrected POPs in regression settings. All retrieved studies evaluating POPs

**Table 4 Studies of persistent organic pollutants (POPs) and other endocrine disruptors biomarkers and DNA methylation outcomes (6 studies available)**

First author, year	Design	Population	Size	Men (%) <sup>a</sup>	Age Range (yr) <sup>a</sup>	Exposure assessment	Exposure categories	DNA methylation Assessment	DNA methylation endpoint	Chemical(s) (if PCBs, highest, lowest & median association and /or statistically significant)	Association	95% Confidence Interval or p-value	Adjustment Factors	
<b>Persistent Organic Pollutants</b>														
Rusiecki, 2008 [100]	CS	Greenland, Denmark (AMAP)	70	87	19 to 67	Plasma by GC  PCB 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183 and 187, p,p'-DDT, p,p'-DDE, β-HCH, Hexachlorobenzene, Chlordane, Cis-chlordane, Oxychlordane, α-Chlordane, Mirex, Toxaphene, ΣPCBs, ΣPOPs	Per log-transformed ng/g lipid increase	Peripheral leukocyte  Global by quantitative pyrosequencing in:  LINE-1	Average % methylation		PCB 118 PCB 128 PCB 156, 170	Difference  -0.73 -0.01 -0.48	P = 0.12 P = 0.99 P = 0.26 and 0.15	QC reported. BEE or CH assessment NR. Models adjusted for age and smoking,
								Alu		PCB 156 PCB 52 PCB 99, 105 p,p'-DDT p,p'-DDE β-HCH Oxychlordane α-Chlordane Mirex ΣPCBs ΣPOPs	-0.66 -0.12 -0.51 -0.26 -0.38 -0.48 -0.32 -0.75 -0.27 -0.56 -0.48	P < 0.01 P = 0.36 P < 0.01 both P = 0.01 P = 0.01 <0.01 <0.01 P = 0.05 P = 0.01 <0.01		
Kim, 2010 [98]	CS	Ulsjin county, South Korea.	86	39.5	56.2 ± 7.0	Serum POPs by GC-HRMS  PCB 74, 99, 105, 118, 138, 146, 153, 156, 157, 164, 167, 172, 177, 178, 180, 183 and 187, β-HCH, HCB, Heptachlor epoxide, Oxychlordane, trans-Nonachlor, p,p'-DDE, p,p'-DDD, p,p'-DDT, Mirex, BDE47, BDE99	Per ng/g lipid increase	Whole blood.  Global by quantitative pyrosequencing in:  LINE-1	Average % methylation	PCB 157 PCB 146 PCB 105, 118, 156, 172, 180	Pearson correlation  -0.14 -0.02 -0.07	p ≥ 0.05 p ≥ 0.05 p ≥ 0.05	QC reported. BEE or CH assessment NR. Models adjusted for age, sex, BMI, cigarette smoking, and alcohol drinking	

**Table 4 Studies of persistent organic pollutants (POPs) and other endocrine disruptors biomarkers and DNA methylation outcomes (6 studies available)**  
(Continued)

Study	Country	Location	N	Age	Exposure	Biomarker	Outcome	Methylation	Methylation		Assessment		
									β	CI			
Lind, 2013 [99]	CS	Uppsala, Sweden (PIVUS study)	519	52	70	Serum by HRGC-HRMS PCB 74, 99, 105, 118, 126, 138, 153, 156, 157, 169, 170, 180, 189, 194, 206 and 209 Octachlorodibenzo-p-dioxin, HCB, TNC, p,p'-DDE, BDE47	Per log-transformed ng/g lipid increase	Leukocytes Global methylation by LUMA	LUMA methylation index <sup>b</sup>	Alu	-0.23	p < 0.05	QC NR. CH assessment NR. Same age. Models adjusted for sex and smoking status.
										PCB 183	-0.23	p < 0.05	
										PCB 167	-0.05	p ≥ 0.05	
										PCB 177, 178	-0.14	p ≥ 0.05	
										Heptachlor epoxide,	-0.23	<0.05	
										Oxychlorodane,	-0.28	<0.05	
										trans-nonachlorodane,	-0.28	<0.05	
										p,p'-DDE,	-0.29	<0.01	
										p,p'-DDT,	-0.22	<0.05	
										BDE47	-0.25	<0.05	
Itoh, 2014 [20]	CS	Japan	399	0	53.9 ±10.2	Serum by GC-HRMS PCB 17, 28, 52/69, 48/47, 74, 66, 90/101, 99, 118, 114, 105, 146, 153, 164/163, 138, 128/162, 167, 156, 182/187, 183, 177, 180, 170, 189, 202, 198/199, 196, 203, 194, 208, 206 and 209, p,p'-DDE, o,p'-DDT, p,p'-DDT, trans-Nonachlor, cis-Nonachlor, Oxychlorodane, β-HCH, HCB, Mirex	Per increase in 1 quartile categories (as an ordinal variable)	Peripheral leukocytes Global methylation by LUMA	1 - (LUMA methylation index <sup>b</sup> )	Difference			QC NR. CH assessment NR. Models adjusted for age, BMI, smoking status and alcohol drinking. Lipid-corrected values.
										Total PCB TEQ	-1.67	-3.17, -0.16	
										Non-ortho PCB TEQ	-1.76	-3.26, -0.26	
										Mono-ortho PCB TEQ	0.11	-1.37, 1.60	
										PCB 169	-3.27	-6.92, 0.37	
										PCB 206	-0.16	-3.71, 3.38	
										PCB 189	-0.56	-3.10, 1.97	
										Octachlorodibenzo-p-dioxin,	-3.19	-5.98, -0.39	
										p,p'-DDE	-2.87	-4.74, -1.00	
										PCB196	-0.009	-0.38, 0.36	
PCB74	-0.64	-1.08, -0.20											
PCB28 and 66	-0.23	-0.59, 0.12											
PCB17	-0.43	-0.78, -0.08											
PCB52/69	-0.33	-0.67, -0.0007											
PCB114	-0.46	-0.88, -0.05											
PCB183	-0.45	-0.82, -0.07											
p,p'-DDE,	-0.77	-1.12, -0.42											
o,p'-DDT,	-0.75	-1.11, -0.40											
p,p'-DDT,	-0.83	-1.17, -0.49											
trans-Nonachlor,	-0.44	-0.84, -0.04											
Oxychlorodane,	-0.53	-0.90, -0.15											





only conducted analyses with lipid-corrected concentrations. Both standardization of summary POP measurements (TEQ versus measured values or sum of POPs functional subgroups) and adjustment for lipid levels are ongoing challenges that require consensus in order to facilitate data comparison and meta-analysis. No study reported evaluation of the potential confounding effect of tissue cell heterogeneity.

**Polycyclic aromatic hydrocarbons and DNA methylation** PAHs are widespread environmental contaminants from incomplete combustion of organic materials such as fossil fuels, which are comprised of two or more fused benzene rings arranged in various configurations [103]. PAH metabolites in human urine, including 1-hydroxypyrene (1-OHP), 1-hydroxypyrene-O-glucuronide, 3-hydroxybenzo[a]pyrene, 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene, and a other hydroxylated PAHs, can be used as biomarkers of internal dose to assess recent exposure to PAHs [104]. Development of biomarkers of exposure to PAHs and related compounds includes detection of protein and DNA adducts, which can be interpreted as indicators of effective dose [105]. The occurrence of PAHs in ambient air, food, drinking water, tobacco smoke, automobile exhausts, dust, and contaminated air from occupational settings [106,107] is an increasing concern for general populations given their carcinogenicity and other reported potential health effects including allergy, asthma, cardiovascular, and respiratory diseases [108]. The causative mechanisms of PAH-related health effects on the molecular level are not completely understood, and epigenetic mechanisms may be involved. Benzo[a]pyrene (BaP) has been reported to disrupt DNA methylation patterns in experimental models [109,110]. In breast cancer cell lines, BaP treatment was related to hypomethylation events at a number of repeat elements [109]. BaP induced a 12% decrease in total 5-mC content of cellular DNA of BALB/3 T3 mouse cells [110]. BaP exposure to zebrafish embryos significantly decreased global DNA methylation by 44.8% [111]. Binding of BaP adducts to DNA decreased methylation by reducing binding and activity of DNMTs [112,113]. Interestingly, experimental evidence suggests that PAH-DNA adduct formation may preferentially target methylated genomic regions [114-117] that may interfere their DNA methylation status. As a result, the interpretation of BPDE-adducts as indicators of effective dose in studies of DNA methylation is not clear.

We identified three publications investigating the association between PAHs and DNA methylation (Table 5). These studies were conducted in Mexico [118], Poland [119], and China [120]. PAH exposure was measured in urine as 1-hydroxypyrene [118,120] or 1-pyrenol [119] and in peripheral blood leukocytes as anti-

B[a]PDE-DNA adducts [119]. Global DNA methylation was assessed by quantitative pyrosequencing of LINE-1 and Alu elements in two studies [118,119]. CpG site-specific DNA methylation was measured in candidate genes by quantitative pyrosequencing in two studies [118,119] and by methylation-specific quantitative PCR in one study [120]. In the Polish study population ( $N = 92$ ) [119], increasing levels of blood and urine exposure biomarkers were associated with increasing DNA methylation in LINE-1 and Alu elements (all  $P$  values  $< 0.004$ ). In contrast, in the Mexican study population ( $N = 39$ ), urine 1-hydroxypyrene was inversely associated with LINE-1 and Alu elements [118]. The associations, however, were not statistically significant. The two studies evaluating DNA methylation in candidate regions by quantitative pyrosequencing showed consistent directions in the associations with increasing exposure biomarkers levels in genes *p53* and *IL-6* [118,119]. The associations, however, were statistically significant only in the Polish study (absolute difference in average percent 5-mC per unit increase in urine exposure biomarker was  $-1.58\%$  ( $P < 0.001$ ) in *p53* and  $1.06\%$  ( $P = 0.012$ ) *IL-6* genes) [119]. In the Mexican population, the difference in average percent 5-mC was  $-1.57\%$  (95% CI  $-2.9\%$ ,  $-0.23\%$ ) for a genomic region in *IL-12* [118]. In the Chinese study population ( $N = 128$ ), the *p16<sup>INK4a</sup>* promoter methylation measured by methylation-specific quantitative PCR [120] showed a positive correlation with urine 1-hydroxypyrene (Spearman  $r = 0.45$ ,  $P < 0.001$ ), which was not consistent with the nonsignificant results from the Polish study [119]. Only one study addressed potential confounding including sex, age, and smoking status [118]. No study reported evaluation of potential confounding effect of tissue cell heterogeneity.

#### **General discussion and needs for future epidemiologic research**

Epidemiologic evidence from distinct study populations suggests a trend for an association between increasing cadmium exposure with increased DNA methylation and a trend for an association between increasing lead and POP exposures with decreased DNA methylation, although additional studies are needed to confirm those trends. For other environmental chemicals, the low number of studies did not allow to recognize patterns in their associations with measures of DNA methylation. The epidemiologic associations were mostly in agreement with experimental evidence, although additional work is needed to better understand the relevance of the dose levels and routes of administration used in experimental studies in the context of human exposure. While the limited number of studies and the heterogeneity in DNA methylation markers limit the conclusion of this review,

**Table 5 Studies of PAH exposure biomarkers and DNA methylation outcomes (3 studies available)**

First author, year	Design	Population	Size	Men (%) <sup>a</sup>	Age Range (yr) <sup>a</sup>	Exposure assessment	Exposure categories	DNA methylation Assessment	DNA methylation endpoint	Association	95% Confidence Interval or p-value	Data pre-processing and adjustment factors																	
Alegria-Torres, 2013 [118]	CS	San Luis Potosi, Mexico. Occupational population	39	100	42.5 (16 to 75)	Urine 1-Hydroxypyrene by HPLC  (Mean=0.18 µg/g creatinine)	Per µg/g increase	Peripheral leukocytes Quantitative Pyrosequencing Specific Interleukin 12 p53 TNF-α IFN-γ IL-6 Global LINE-1 Alu	Average % methylation	Difference	-1.57 -2.9, -0.23 -2.7 -5.46, 0.06 -3.9 -8.28, 0.48 -0.43 -16.45, 15.59 0.22 -9.19, 9.63 -0.49 -4.74, 3.76 -0.55 -1.25, 0.16	QC or CH assessment NR. Models adjusted for smoking status, usual alcohol drinking, current medication, age, and average number of cigarettes smoked.																	
													Pavanello, 2009 [119]	CS	Poland	92	100	37 (20-59)	Urine 1-pyrenol by HPLC-F  Peripheral blood lymphocytes	Per µmol/mol creatinine increase	Peripheral blood lymphocytes Quantitative pyrosequencing Specific p53 p16 HIC1 IL-6 Global LINE-1 Alu	Average % methylation	Difference	-1.58 P < 0.001 -0.01 P = 0.736 -0.57 P = 0.059 1.06 P = 0.012 0.72 P = 0.01 0.13 P = 0.004	QC reported. CH assessment or adjustment for potential confounders NR. All participants were non-current smokers				
																										Anti-BPDE-DNA by HPLC-F analysis of BP-tetrol-I-1	Per adducts /10 <sup>8</sup> nucleotides increase	Specific p53 p16 HIC1 IL-6 Global LINE-1 Alu	-1.04 P < 0.001 -0.02 P = 0.314 -0.31 P = 0.142 0.57 P = 0.043 0.63 P < 0.001 0.10 P < 0.001

**Table 5 Studies of PAH exposure biomarkers and DNA methylation outcomes (3 studies available) (Continued)**

Yang, 2012 [120]	CS	Anshan City, Liaoning, China	128	100	42.07	Urine	Log transformed μg/L	Peripheral blood lymphocytes	% methylation	QC or CH NR. Unadjusted for potential confounders.
						1-Hydroxypyrene		Specific by methylation specific quantitative PCR		
						(Overall mean=6.56)		p16 <sup>INK4a</sup>	r spearman = 0.450	<0.001

AAS, atomic absorption spectrometry; BaP, benzo[a]pyrene; CH: cell heterogeneity; HPLC, high-performance liquid chromatography; HPLC-F, high-performance liquid chromatography-fluorescence; IQR, interquartile range; LOD, limit of detection; NR: not reported; QC: quality control.

<sup>a</sup>Sociodemographic data available in the article, not necessarily in the subsample without missing values in DNA methylation or exposures.

the evidence accrued so far supports the importance of environmental exposures in modulating the epigenome.

A limitation of the review was the substantial heterogeneity in the assessment methods of DNA methylation, especially for studies reporting global DNA methylation, which challenged the comparability across studies. For instance, LINE-1 and Alu repetitive elements have been classically used as a surrogate marker for global DNA methylation because they are abundant, hypermethylated, regions in the genome (more than one third of DNA methylation in these repetitive elements) [121]. LINE-1 and Alu elements, however, could be regulated by specific mechanisms and respond specifically to cellular stressors [122]. Other studies assessed DNA methylation globally by estimating the percentage of methylated DNA over the total number of genomic cytosines [19] or the LUMA methylation index [20,99] that goes from 0 (fully methylated DNA) to 1 (fully demethylated DNA). Among studies reporting absolute differences in global DNA methylation, the strength of the statistically significant associations ranged between a difference (absolute value) in DNA methylation percent of 0.25 in LINE-1 per IQR (19  $\mu\text{g/g}$ ) of patella lead [68] to 0.75 in Alu per log ng/g lipid increase of  $\alpha$ -Chlordane concentrations [100]. Among studies reporting differences in the relative scale, the corresponding associations ranged from a relative change of 14% comparing tertiles 3 to 1 of lead [69] to 75% comparing participants above and below the median cadmium levels [19]. Some of the retrieved studies reported dose-responses using flexible approaches (that is, quantile categories or nonparametric splines) and mostly showed fairly monotonic relationships of DNA methylation with cadmium [55], lead [68,69], and POPs [20,98-100], which add further significance to the findings.

Overall, the temporality of the reported associations cannot be evaluated in this systematic review given the low number of prospective studies. Among the four studies with originally prospective designs [19,55,68,101], all of them reported cross-sectional analyses with samples for DNA methylation and exposure status determination collected at the same time point. Only two of the prospective studies [19,101] included repeated measurements and additionally reported prospective associations of baseline exposures with DNA methylation in samples collected at follow-up visits. For cadmium, the cross-sectional association with global DNA methylation was statistically significant, whereas the prospective association after 10-year follow-up was not [19]. For PFCs, the associations with DNA methylation measured at the end of follow-up were reported not to be different either using biomarkers from samples collected at enrollment, at the end of follow-up, or the average of both [101]. The relevant type of exposure (short term versus long term), latency time, and persistence of the potential epigenetic effects of individual environmental chemicals in human populations, however, are

unknown and may differ by compound. Future longitudinal studies with sufficient repeated measurements over time, which can enable the evaluation of trends and trajectories of DNA methylation by environmental exposures levels, are needed.

A major challenge in the evaluation of the association between environmental chemicals and DNA methylation was the heterogeneity of adjustment for potential confounders. For instance, residual confounding by smoking is a typical concern in epidemiologic studies assessing potential environmental chemical-epigenetic effects, because tobacco smoke is a major source of chemicals, including cadmium, lead, and PAHs, and others [32] that can have potential epigenetic effects. Most, but not all [29,53,69,85], of the retrieved articles assessed potential confounding by smoking. Sex and age are important sociodemographic factors that must be also considered as potential confounders, since they have also been related to differences in DNA methylation [123,124]. Only three studies did not address confounding by both sex and age [29,69,119]. In addition to adjustment in regression models, an alternative strategy to evaluate residual confounding is to perform separate analyses in subgroups of interest, for instance sex or smoking. For cadmium, one study in Argentinean women [54] the study population was mostly made of never smokers. For PAH, one study reported levels of DNA methylation separately for smokers and non-smokers with no statistically significant differences [120], and another study reported that all participants were not current smokers [119]. For POPs, two studies evaluated findings in smoking status subgroups [99,100], with no significant differences in the estimated associations. Four studies stratified by sex [85,99-101], reporting similar results in men and women, except a study of mercury in dental professional [85] that found a significant association between hair mercury and *SEPP1* hypomethylation only among males. Eight study populations were made only of adult men or women [20,29,53,54,68,118-120]. In addition to sex and smoking, four articles additionally performed subgroup analysis by candidate polymorphisms [29,30,54,99] mostly in genes from one-carbon metabolism and exposure-related pathways (that is, polymorphisms in the Ah receptor for POPs). In addition to candidate genes, there is mounting evidence now supporting a role of the genetic variation in *cis* in determining DNA methylation status [124,125]. For site-specific methylation, thus, it is advisable to evaluate whether the observed associations may be attributed to nearby polymorphisms, which may be unbalanced by exposure levels by chance. Only one study reported evaluation of SNP-related clustering of DNA methylation [53]. Another study incorporated into the analysis genotypes from SNPs known to determine DNA methylation in the significant regions of interest [29].

Artifactual variation from DNA isolation and processing and methylation assessment methods and tissue-specific nature of DNA methylation profiles are other sources of potential biases. It is well established now that differential tissue-type cell heterogeneity [123,126] and, for large studies and studies using 'omics' technologies for DNA methylation assessment, evaluation and correction of potential batch effects [127,128] and background correction and normalization methods [128,129] are compelling issues that must be addressed and adequately reported. Only five studies are reported addressing tissue-specific cell heterogeneity [19,30,53,68,99]. None of the two studies using microarrays technologies reported evaluating potential batch effects [29,53]. Moreover, in the specific case of microarray technologies, given the large numbers of statistical tests conducted, it is usually required to correct for multiple comparisons. The only microarray-based study reporting methods to address multiple comparisons found no significant associations after controlling of the false positive rate, something expected given the small sample size of that study ( $n = 17$ ) [53]. A total of three [54,69,120] studies attempted to validate significant regions either by using alternative DNA methylation assays to assess the consistency of results and/or by conducting functional assays in experimental models. While there is evidence supporting that arsenic-related methylation changes are associated with changes in gene expression [12,130], for other environmental chemicals, the available epidemiologic evidence is limited. Only one of the reviewed studies for cadmium [54] had available genome-wide gene expression measurements. However, the association of changes in DNA methylation with gene expression was not directly evaluated for the cadmium-related epigenetic regions. Epidemiologic studies that include assessments of environmental chemicals and coupled DNA methylation and gene expression data are needed.

An emerging issue relates to the inability of sodium bisulfite conversion, which is the commonly used method for determination of 5-mC at single-base resolution, to distinguish 5-mC from its oxidative derivative 5-hydroxymethylcytosine (5-hmC) [131]. It has been reported that 5-hmC is enriched in intergenic regions, including LINE-1 elements and gene body regions [132-134]. In studies evaluating the association of DNA methylation and environmental chemicals using bisulfite conversion based methods for DNA methylation assessment, residual measurement error by 5-hmC content is, thus, possible. In one of the reviewed papers [19], which measured global DNA methylation and hydroxymethylation in human blood samples (using antibodies specific for 5-mC and 5-hmC with no cross-specificity), there was a positive and statistically significant correlation between both epigenetic marks. Moreover, the direction of the association of both epigenetic markers with diverse determinants, including some metals, was mostly consistent. In

addition, there is increasing evidence that 5-hmC could also play a role in epigenetic regulation of gene expression and be associated with disease susceptibility [135,136]. The health implications of the relationship between DNA methylation and hydroxymethylation in differentiated tissues are currently unknown. Advanced technology for high-throughput parallel sequencing on 5-mC and 5-hmC profiling across the genome may help to understand the role of DNA hydroxymethylation and its determinants in health.

Finally, the role of pre-natal exposure to environmental chemicals as a determinant of DNA methylation was out of the scope of this systematic review. Given the relevance of potential heritability of DNA methylation changes and post-birth effects of maternal environmental exposures, we briefly summarize here the epidemiologic evidence that reported results on the association of maternal exposure biomarkers and DNA methylation in cord blood and was excluded as a result of secondary exclusion criteria. We identified two studies focusing on cadmium [26,53] and POPs [22,24], respectively, and one study investigating lead [25] and PAHs [23], respectively. These studies overall support an association of pre-natal exposure to environmental chemicals with epigenetic markers in the offspring, but specific systematic reviews are needed.

## Conclusions

Increasing evidence supports the role of environmental chemicals in DNA methylation changes. For cadmium, lead, and POPs, the evidence could be classified as 'suggestive but insufficient' considering some consistency and evidence of a dose-response relationship across studies, biological plausibility from experimental findings, and adjustment of confounding in epidemiologic studies. However, we finally concluded that for all the environmental chemicals evaluated, including cadmium, lead, and POPs, the current evidence is 'insufficient' to support causality given the heterogeneity among epidemiologic studies in potential for residual confounding of the associations, differences in DNA methylation assessment methods and, random error, especially because of the limited sample sizes. Important questions include the need for larger and longitudinal studies with repeated measures, validation and replication of findings, the relevance of epigenetic markers recently gaining attention such as DNA hydroxymethylation, the systematic evaluation of the dose-response relationships, and the investigation of the role of genetic variation. An emerging area of research is the role of joint exposures in changing DNA methylation, although statistical methods to comprehensively tackle mixtures of compounds are needed. As large cohorts with available measurements of environmental chemicals and genome-wide DNA methylation data become



increasingly available, collaborative meta-analyses will enable to disentangle the role of environmental chemicals as determinants of DNA methylation and, also, to test the hypothesis that genomic DNA methylation may mediate chemical-related health effects.

## Additional files

**Additional file 1: Table S1.** PubMed search strategies for environmental chemicals and DNA methylation.

**Additional file 2: Table S2.** Study quality criteria (17 studies included in the current review).

## Abbreviations

1-OHP: 1-hydroxypyrene; 5-hmC: 5-hydroxymethylcytosine; 5-mC: 5-methylcytosine; AAS: atomic absorption spectrometry; BaP: benzo[a]pyrene; BDE: polybrominated diphenyl ether; BEE: batch effect evaluation; BMI: body mass index; BPA: bisphenol A; CC: case-control; CDT: Comparative Toxicogenomics Database; CH: cell heterogeneity; CI: confidence interval; CO: cohort; CS: cross-sectional; DDE: dichlorodiphenyldichloroethylene; DDT: dichlorodiphenyl trichloroethane; DNAm: DNA methylation; GC: gas chromatography; HPLC: high-performance liquid chromatography; HPLC-F: high-performance liquid chromatography-fluorescence; HRGC-HRMS: high-resolution chromatography coupled to high-resolution mass spectrometry; HRMS: high-resolution mass spectrometry; IQR: interquartile range; LUMA: Luminometric Methylation Assay; MCC: multiple comparison correction; NR: not reported; PAH: polycyclic aromatic hydrocarbons; PBDEs: polybrominated diphenyl ether; PCBs: polychlorinated biphenyls; PFC: perfluorinated compounds; PFOA: perfluorooctanoic acid; PFOS: perfluorooctanesulfonic acid; POPs: persistent organic pollutants; TEQ: toxic equivalency.

## Competing interests

The authors declared that they have no competing interests.

## Authors' contributions

All authors conceptualized the review. ARH, CCK, MTP, and ANA developed the search strategy. ARH and CCK reviewed all the retrieved abstracts. ANA and MTP acted as third reviewers in case of inconsistent articles selection by ARH and CCK. ARH, WYT, and MTP drafted and edited the figure. ARH and MTP drafted the data extraction tables. CCK and ANA assisted in editing data extraction tables. All the authors interpreted the data extraction tables. ARH and MTP wrote the initial draft of the manuscript. CCK, ANA, PRG, WYT, JR, and JMO assisted in writing the manuscript. All authors read and approved the final manuscript.

## Acknowledgements

Maria Tellez-Plaza was supported by the Strategic Action for Research in Health sciences (CP12/03080), which is an initiative from Carlos III Health Institute Madrid and the Spanish Ministry of Economy and Competitiveness and co-funded with European Funds for Regional Development (FEDER). Ana Navas-Acien was supported by grants R01ES021367 and R01ES025216 from the National Institute of Environmental Health Sciences.

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Received: 5 November 2014 Accepted: 9 February 2015

Published online: 29 April 2015

## References

- Pruss-Ustun A, Vickers C, Haefliger P, Bertollini R. Knowns and unknowns on burden of disease due to chemicals: a systematic review. *Environ Health*. 2011;10:9.
- Lee DH, Jacobs Jr DR, Porta M. Hypothesis: a unifying mechanism for nutrition and chemicals as lifelong modulators of DNA hypomethylation. *Environ Health Perspect*. 2009;117:1799–802.
- Chia N, Wang L, Lu X, Senut MC, Brenner C, Ruden DM. Hypothesis: environmental regulation of 5-hydroxymethylcytosine by oxidative stress. *Epigenetics*. 2011;6:853–6.
- Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP. Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp Cell Res*. 2003;286:355–65.
- Chervona Y, Costa M. The control of histone methylation and gene expression by oxidative stress, hypoxia, and metals. *Free Radic Biol Med*. 2012;53:1041–7.
- Dai H, Wang Z. Histone modification patterns and their responses to environment. *Curr Envir Health Rpt*. 2014;1:11–21.
- Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr*. 2007;27:363–88.
- Bogdarina I, Welham S, King PJ, Burns SP, Clark AJ. Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. *Circ Res*. 2007;100:520–6.
- Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A*. 2008;105:17046–9.
- Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet*. 2009;18:4046–53.
- Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A*. 2005;102:10604–9.
- Bailey KA, Fry RC. Arsenic-associated changes to the epigenome: What are the functional consequences? *Curr Envir Health Rpt*. 2014;1:22–34.
- Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SJ, et al. Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. *Hum Mol Genet*. 2012;21:3073–82.
- Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, et al. 450 K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect*. 2012;120:1425–31.
- Shenker NS, Polidoro S, van Veldhoven K, Sacerdote C, Ricceri F, Birrell MA, et al. Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking. *Hum Mol Genet*. 2013;22:843–51.
- Zeilinger S, Kuhnel B, Klopp N, Baurecht H, Kleinschmidt A, Gieger C, et al. Tobacco smoking leads to extensive genome-wide changes in DNA methylation. *PLoS One*. 2013;8:e63812.
- Sun YV, Smith AK, Conneely KN, Chang Q, Li W, Lazarus A, et al. Epigenomic association analysis identifies smoking-related DNA methylation sites in African Americans. *Hum Genet*. 2013;132:1027–37.
- Breton CV, Marutani AN. Air pollution and epigenetics: recent findings. *Curr Envir Health Rpt*. 2014;1:35–45.
- Tellez-Plaza M, Tang WY, Shang Y, Umans JG, Francesconi KA, Goessler W, et al. Association of global DNA methylation and global DNA hydroxymethylation with metals and other exposures in human blood DNA samples. *Environ Health Perspect*. 2014;122:946–54.

20. Itoh H, Iwasaki M, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, et al. Association between serum organochlorines and global methylation level of leukocyte DNA among Japanese women: a cross-sectional study. *Sci Total Environ*. 2014;490:603–9.
21. Kim JH, Rozek LS, Soliman AS, Sartor MA, Hablas A, Seifeldin IA, et al. Bisphenol A-associated epigenomic changes in prepubescent girls: a cross-sectional study in Gharbiah, Egypt. *Environ Health Perspect*. 2013;121:33.
22. Huen K, Yousefi P, Bradman A, Yan L, Harley KG, Kogut K, et al. Effects of age, sex, and persistent organic pollutants on DNA methylation in children. *Environ Mol Mutagen*. 2014;55:209–22.
23. Herbstman JB, Tang D, Zhu D, Qu L, Sjodin A, Li Z, et al. Prenatal exposure to polycyclic aromatic hydrocarbons, benzo[a]pyrene-DNA adducts, and genomic DNA methylation in cord blood. *Environ Health Perspect*. 2012;120:733–8.
24. Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Illi-Gargas C, Lebron C, Witter FR, et al. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. *Epigenetics*. 2010;5:539–46.
25. Pilsner JR, Hu H, Ettinger A, Sanchez BN, Wright RO, Cantonwine D, et al. Influence of prenatal lead exposure on genomic methylation of cord blood DNA. *Environ Health Perspect*. 2009;117:1466–71.
26. Kippler M, Engstrom K, Mlakar SJ, Bottai M, Ahmed S, Hossain MB, et al. Sex-specific effects of early life cadmium exposure on DNA methylation and implications for birth weight. *Epigenetics*. 2013;8:494–503.
27. Kovatsi L, Georgiou E, Ioannou A, Haitoglou C, Tzimagiorgis G, Tsoukali H, et al. p16 promoter methylation in Pb2+ -exposed individuals. *Clin Toxicol (Phila)*. 2010;48:124–8.
28. Li C, Xu M, Wang S, Yang X, Zhou S, Zhang J, et al. Lead exposure suppressed ALAD transcription by increasing methylation level of the promoter CpG islands. *Toxicol Lett*. 2011;203:48–53.
29. Hanna CW, Bloom MS, Robinson WP, Kim D, Parsons PJ, Vom Saal FS, et al. DNA methylation changes in whole blood is associated with exposure to the environmental contaminants, mercury, lead, cadmium and bisphenol A, in women undergoing ovarian stimulation for IVF. *Hum Reprod*. 2012;27:1401–10.
30. Tajuddin SM, Amaral AF, Fernandez AF, Rodriguez-Rodero S, Rodriguez RM, Moore LE, et al. Genetic and non-genetic predictors of LINE-1 methylation in leukocyte DNA. *Environ Health Perspect*. 2013;121:650–6.
31. Longnecker MP, Berlin JA, Orza MJ, Chalmers TC. A meta-analysis of alcohol consumption in relation to risk of breast cancer. *JAMA*. 1988;260:652–6.
32. U.S. Department of Health and Human Services. A report of the surgeon general: How tobacco smoke causes disease. The biology and behavioral basis for smoking-attributable diseases. 2010. Rocksville, Maryland. <http://www.surgeongeneral.gov/library/tobaccosmoke/report/index.html>. Accessed 14 Jan 2015.
33. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for cadmium. 2012. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=48&tid=15>. Accessed 14 Jan 2015.
34. Tellez-Plaza M, Navas-Acien A, Caldwell KL, Menke A, Muntner P, Guallar E. Reduction in cadmium exposure in the United States population, 1988–2008: the contribution of declining smoking rates. *Environ Health Perspect*. 2012;120:204–9.
35. Egwuogu H, Shendell DG, Okosun IS, Goodfellow L. The effect of urinary cadmium on cardiovascular fitness as measured by VO2 max in white, black and Mexican Americans. *Environ Res*. 2009;109:292–300.
36. Gallagher CM, Kovach JS, Meliker JR. Urinary cadmium and osteoporosis in U.S. Women > or = 50 years of age: NHANES 1988–1994 and 1999–2004. *Environ Health Perspect*. 2008;116:1338–43.
37. Navas-Acien A, Selvin E, Sharrett AR, Calderon-Aranda E, Silbergeld E, Guallar E. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation*. 2004;109:3196–201.
38. Navas-Acien A, Silbergeld EK, Sharrett R, Calderon-Aranda E, Selvin E, Guallar E. Metals in urine and peripheral arterial disease. *Environ Health Perspect*. 2005;113:164–9.
39. Navas-Acien A, Tellez-Plaza M, Guallar E, Muntner P, Silbergeld E, Jaar B, et al. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. *Am J Epidemiol*. 2009;170:1156–64.
40. Peters JL, Perlstein TS, Perry MJ, McNeely E, Weuve J. Cadmium exposure in association with history of stroke and heart failure. *Environ Res*. 2010;110:199–206.
41. Tellez-Plaza M, Navas-Acien A, Menke A, Crainiceanu CM, Pastor-Barriuso R, Guallar E. Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population. *Environ Health Perspect*. 2012;120:1017–22.
42. Jarup L, Rogenfelt A, Elinder CG, Nogawa K, Kjellstrom T. Biological half-time of cadmium in the blood of workers after cessation of exposure. *Scand J Work Environ Health*. 1983;9:327–31.
43. Benbrahim-Tallaa L, Waterland RA, Dill AL, Webber MM, Waalkes MP. Tumor suppressor gene inactivation during cadmium-induced malignant transformation of human prostate cells correlates with overexpression of de novo DNA methyltransferase. *Environ Health Perspect*. 2007;115:1454–9.
44. Jiang G, Xu L, Song S, Zhu C, Wu Q, Zhang L, et al. Effects of long-term low-dose cadmium exposure on genomic DNA methylation in human embryo lung fibroblast cells. *Toxicology*. 2008;244:49–55.
45. Poirier LA, Vlasova TI. The prospective role of abnormal methyl metabolism in cadmium toxicity. *Environ Health Perspect*. 2002;110 Suppl 5:793–5.
46. Yuan D, Ye S, Pan Y, Bao Y, Chen H, Shao C. Long-term cadmium exposure leads to the enhancement of lymphocyte proliferation via down-regulating p16 by DNA hypermethylation. *Mutat Res*. 2013;757:125–31.
47. Inglot P, Lewinska A, Potocki L, Oklejewicz B, Tabecka-Lonczynska A, Koziorowski M, et al. Cadmium-induced changes in genomic DNA-methylation status increase aneuploidy events in a pig Robertsonian translocation model. *Mutat Res*. 2012;747:182–9.
48. Castillo P, Ibanez F, Guajardo A, Llanos MN, Ronco AM. Impact of cadmium exposure during pregnancy on hepatic glucocorticoid receptor methylation and expression in rat fetus. *PLoS One*. 2012;7:e44139.
49. Wang B, Li Y, Tan Y, Miao X, Liu XD, Shao C, et al. Low-dose Cd induces hepatic gene hypermethylation, along with the persistent reduction of cell death and increase of cell proliferation in rats and mice. *PLoS One*. 2012;7:e33853.
50. Zhou ZH, Lei YX, Wang CX. Analysis of aberrant methylation in DNA repair genes during malignant transformation of human bronchial epithelial cells induced by cadmium. *Toxicol Sci*. 2012;125:412–7.
51. Fujishiro H, Okugaki S, Yasumitsu S, Nomoto S, Himeno S. Involvement of DNA hypermethylation in down-regulation of the zinc transporter ZIP8 in cadmium-resistant metallothionein-null cells. *Toxicol Appl Pharmacol*. 2009;241:195–201.
52. Majumder S, Ghoshal K, Li Z, Bo Y, Jacob ST. Silencing of metallothionein-I gene in mouse lymphosarcoma cells by methylation. *Oncogene*. 1999;18:6287–95.
53. Sanders AP, Smeester L, Rojas D, Debusscher T, Wu MC, Wright FA, et al. Cadmium exposure and the epigenome: exposure-associated patterns of DNA methylation in leukocytes from mother-baby pairs. *Epigenetics*. 2014;9:212–21.
54. Hossain MB, Vahter M, Concha G, Broberg K. Low-level environmental cadmium exposure is associated with DNA hypomethylation in Argentinean women. *Environ Health Perspect*. 2012;120:879–84.
55. Zhang C, Liang Y, Lei L, Zhu G, Chen X, Jin T, et al. Hypermethylations of *RASAL1* and *KLOTHO* is associated with renal dysfunction in a Chinese population environmentally exposed to cadmium. *Toxicol Appl Pharmacol*. 2013;271:78–85.
56. Smith DR, Flegal AR. Lead in the biosphere: recent trends. *Ambio*. 1995;24:21–3.
57. Muntner P, Menke A, DeSalvo KB, Rabito FA, Batuman V. Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. *Arch Intern Med*. 2005;165:2155–61.
58. Hense HW, Filipiak B, Novak L, Stoeppeler M. Nonoccupational determinants of blood lead concentrations in a general population. *Int J Epidemiol*. 1992;21:753–62.
59. Apostolou A, Garcia-Esquinas E, Fadowski JJ, McLain P, Weaver VM, Navas-Acien A. Secondhand tobacco smoke: a source of lead exposure in US children and adolescents. *Am J Public Health*. 2012;102:714–22.
60. ATSDR. Toxicological profile for lead. 2007. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=96&tid=22>. Accessed 14 Jan 2015.
61. Hu H, Shih R, Rothenberg S, Schwartz BS. The epidemiology of lead toxicity in adults: measuring dose and consideration of other methodologic issues. *Environ Health Perspect*. 2007;115:455–62.
62. Menke A, Muntner P, Batuman V, Silbergeld EK, Guallar E. Blood lead below 0.48 micromol/L (10 microg/dL) and mortality among US adults. *Circulation*. 2006;114:1388–94.
63. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease—a systematic review. *Environ Health Perspect*. 2007;115:472–82.
64. Bihagi SW, Huang H, Wu J, Zawia NH. Infant exposure to lead (Pb) and epigenetic modifications in the aging primate brain: implications for Alzheimer's disease. *J Alzheimers Dis*. 2011;27:819–33.
65. Bihagi SW, Zawia NH. Alzheimer's disease biomarkers and epigenetic intermediates following exposure to Pb in vitro. *Curr Alzheimer Res*. 2012;9:555–62.
66. Dosunmu R, Alshwal H, Zawia NH. Genome-wide expression and methylation profiling in the aged rodent brain due to early-life Pb exposure and its relevance to aging. *Mech Ageing Dev*. 2012;133:435–43.

67. Senut MC, Sen A, Cingolani P, Shaik A, Land SJ, Ruden DM. Lead exposure disrupts global DNA methylation in human embryonic stem cells and alters their neuronal differentiation. *Toxicol Sci.* 2014;139:142–61.
68. Wright RO, Schwartz J, Wright RJ, Bollati V, Tarantini L, Park SK, et al. Biomarkers of lead exposure and DNA methylation within retrotransposons. *Environ Health Perspect.* 2010;118:790–5.
69. Li C, Yang X, Xu M, Zhang J, Sun N. Epigenetic marker (LINE-1 promoter) methylation level was associated with occupational lead exposure. *Clin Toxicol (Phila).* 2013;51:225–9.
70. ATSDR. Toxicological profile for mercury. 1999. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=115&tid=24>. Accessed 14 Jan 2015.
71. Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, et al. Evidence on the human health effects of low-level methylmercury exposure. *Environ Health Perspect.* 2012;120:799–806.
72. Hong YS, Kim YM, Lee KE. Methylmercury exposure and health effects. *J Prev Med Public Health.* 2012;45:353–63.
73. Park JD, Zheng W. Human exposure and health effects of inorganic and elemental mercury. *J Prev Med Public Health.* 2012;45:344–52.
74. Roman HA, Walsh TL, Coull BA, Dewailly E, Guallar E, Hattis D, et al. Evaluation of the cardiovascular effects of methylmercury exposures: current evidence supports development of a dose-response function for regulatory benefits analysis. *Environ Health Perspect.* 2011;119:607–14.
75. Bose R, Onishchenko N, Edoff K, Janson Lang AM, Ceccatelli S. Inherited effects of low-dose exposure to methylmercury in neural stem cells. *Toxicol Sci.* 2012;130:383–90.
76. Arai Y, Ohgane J, Yagi S, Ito R, Iwasaki Y, Saito K, et al. Epigenetic assessment of environmental chemicals detected in maternal peripheral and cord blood samples. *J Reprod Dev.* 2011;57:507–17.
77. Moulin JJ, Wild P, Romazini S, Lasfargues G, Peltier A, Bozec C, et al. Lung cancer risk in hard-metal workers. *Am J Epidemiol.* 1998;148:241–8.
78. ATSDR. Toxicological profile for Tungsten. 2005. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=806&tid=157>. Accessed 14 Jan 2015.
79. Agarwal S, Zaman T, Tuzcu EM, Kapadia SR. Heavy metals and cardiovascular disease: results from the National Health and Nutrition Examination Survey (NHANES) 1999–2006. *Angiol.* 2011;62:422–429.
80. Klein CB, Costa M, Nickel. In Nordberg GF, Fowler BF, Nordberg M and Friberg L editors. *Handbook on the toxicology of metals*. Amsterdam: Elsevier; 2007: 743–758.
81. Lee YW, Klein CB, Kargacin B, Salnikow K, Kitahara J, Dowjat K, et al. Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol Cell Biol.* 1995;15:2547–57.
82. Lee YW, Broday L, Costa M. Effects of nickel on DNA methyltransferase activity and genomic DNA methylation levels. *Mutat Res.* 1998;415:213–8.
83. Chervona Y, Arita A, Costa M. Carcinogenic metals and the epigenome: understanding the effect of nickel, arsenic, and chromium. *Metallomics.* 2012;4:619–27.
84. Yan Y, Kluz T, Zhang P, Chen HB, Costa M. Analysis of specific lysine histone H3 and H4 acetylation and methylation status in clones of cells with a gene silenced by nickel exposure. *Toxicol Appl Pharmacol.* 2003;190:272–7.
85. Goodrich JM, Basu N, Franzblau A, Dolinoy DC. Mercury biomarkers and DNA methylation among Michigan dental professionals. *Environ Mol Mutagen.* 2013;54:195–203.
86. Selin NE, Sunderland EM, Knightes CD, Mason RP. Sources of mercury exposure for U.S. seafood consumers: implications for policy. *Environ Health Perspect.* 2010;118:137–43.
87. United States Environmental Protection Agency. Persistent organic pollutants: A global issue, a global response. 2014. <http://www2.epa.gov/international-cooperation/persistent-organic-pollutants-global-issue-global-response>. Accessed 14 Jan 2015.
88. Haffner D, Schecter A. Persistent organic pollutants (POPs): a primer for practicing clinicians. *Curr Envir Health Rpt.* 2014;1:123–31.
89. Schecter A, Pavuk M, Papko O, Ryan JJ, Birnbaum L, Rosen R. Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environ Health Perspect.* 2003;111:1723–9.
90. Kuo C, Moon K, Thayer KA, Navas-Acien A. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. *Curr Diab Rep.* 2013;13:831–49.
91. Steenland K, Fletcher T, Savitz DA. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). *Environ Health Perspect.* 2010;118:1100–8.
92. ATSDR. Toxicological Profile for Phenol. 2008. <http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=148&tid=27>. Accessed 14 Jan 2015.
93. Shutoh Y, Takeda M, Ohtsuka R, Haishima A, Yamaguchi S, Fujie H, et al. Low dose effects of dichlorodiphenyltrichloroethane (DDT) on gene transcription and DNA methylation in the hypothalamus of young male rats: implication of hormesis-like effects. *J Toxicol Sci.* 2009;34:469–82.
94. Desaulniers D, Xiao GH, Lian H, Feng YL, Zhu J, Nakai J, et al. Effects of mixtures of polychlorinated biphenyls, methylmercury, and organochlorine pesticides on hepatic DNA methylation in prepubertal female Sprague-Dawley rats. *Int J Toxicol.* 2009;28:294–307.
95. Tian M, Peng S, Martin FL, Zhang J, Liu L, Wang Z, et al. Perfluorooctanoic acid induces gene promoter hypermethylation of glutathione-S-transferase Pi in human liver L02 cells. *Toxicology.* 2012;296:48–55.
96. Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol A exposure disrupts genomic imprinting in the mouse. *PLoS Genet.* 2013;9:e1003401.
97. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc Natl Acad Sci U S A.* 2007;104:13056–61.
98. Kim KY, Kim DS, Lee SK, Lee IK, Kang JH, Chang YS, et al. Association of low-dose exposure to persistent organic pollutants with global DNA hypomethylation in healthy Koreans. *Environ Health Perspect.* 2010;118:370–4.
99. Lind L, Penell J, Luttrupp K, Nordfors L, Syvanen AC, Axelsson T, et al. Global DNA hypermethylation is associated with high serum levels of persistent organic pollutants in an elderly population. *Environ Int.* 2013;59:456–61.
100. Rusiecki JA, Baccarelli A, Bollati V, Tarantini L, Moore LE, Bonefeld-Jorgensen EC. Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit. *Environ Health Perspect.* 2008;116:1547–52.
101. Watkins DJ, Wellenius GA, Butler RA, Bartell SM, Fletcher T, Kelsey KT. Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation. *Environ Int.* 2014;63:71–6.
102. Schisterman EF, Whitcomb BW, Louis GM, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. *Environ Health Perspect.* 2005;113:853–7.
103. ATSDR. Toxicological profile for polycyclic aromatic hydrocarbons. 1995. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=122&tid=25>. Accessed 14 Jan 2015.
104. Strickland P, Kang D, Sithisarankul P. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. *Environ Health Perspect.* 1996;104 Suppl 5:927–32.
105. Talaska G, Underwood P, Maier A, Lewtas J, Rothman N, Jaeger M. Polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs and related environmental compounds: biological markers of exposure and effects. *Environ Health Perspect.* 1996;104 Suppl 5:901–6.
106. Diggs DL, Huderson AC, Harris KL, Myers JN, Banks LD, Rekhadevi PV, et al. Polycyclic aromatic hydrocarbons and digestive tract cancers: a perspective. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev.* 2011;29:324–57.
107. Whitehead TP, Metayer C, Petreas M, Does M, Buffler PA, Rappaport SM. Polycyclic aromatic hydrocarbons in residential dust: sources of variability. *Environ Health Perspect.* 2013;121:543–50.
108. Shiraiwa M, Selzle K, Poschl U. Hazardous components and health effects of atmospheric aerosol particles: reactive oxygen species, soot, polycyclic aromatic compounds and allergenic proteins. *Free Radic Res.* 2012;46:927–39.
109. Sadikovic B, Rodenhiser DI. Benzopyrene exposure disrupts DNA methylation and growth dynamics in breast cancer cells. *Toxicol Appl Pharmacol.* 2006;216:458–68.
110. Wilson VL, Jones PA. Inhibition of DNA methylation by chemical carcinogens in vitro. *Cell.* 1983;32:239–46.
111. Fang X, Thornton C, Scheffler BE, Willett KL. Benzo[a]pyrene decreases global and gene specific DNA methylation during zebrafish development. *Environ Toxicol Pharmacol.* 2013;36:40–50.
112. Drahovsky D, Morris NR. Mammalian DNA methylase: binding and methylation site selection. *Hoppe Seylers Z Physiol Chem.* 1972;353:700–1.
113. Subach OM, Maltseva DV, Shastry A, Kolbanovskiy A, Klimasauskas S, Geacintov NE, et al. The stereochemistry of benzo[a]pyrene-2'-deoxyguanosine adducts affects DNA methylation by Sss1 and Hha1 DNA methyltransferases. *Febs j.* 2007;274:2121–34.
114. Chen JX, Zheng Y, West M, Tang MS. Carcinogens preferentially bind at methylated CpG in the p53 mutational hot spots. *Cancer Res.* 1998;58:2070–5.
115. Denissenko MF, Chen JX, Tang MS, Pfeifer GP. Cytosine methylation determines hot spots of DNA damage in the human P53 gene. *Proc Natl Acad Sci U S A.* 1997;94:3893–8.

116. Tretyakova N, Matter B, Jones R, Shallop A. Formation of benzo[a]pyrene diol epoxide-DNA adducts at specific guanines within K-ras and p53 gene sequences: stable isotope-labeling mass spectrometry approach. *Biochemistry*. 2002;41:9535–44.
117. Weisenberger DJ, Romano LJ. Cytosine methylation in a CpG sequence leads to enhanced reactivity with Benzo[a]pyrene diol epoxide that correlates with a conformational change. *J Biol Chem*. 1999;274:23948–55.
118. Alegria-Torres JA, Barretta F, Batres-Esquivel LE, Carrizales-Yanez L, Perez-Maldonado IN, Baccarelli A, et al. Epigenetic markers of exposure to polycyclic aromatic hydrocarbons in Mexican brickmakers: a pilot study. *Chemosphere*. 2013;91:475–80.
119. Pavanello S, Bollati V, Pesatori AC, Kapka L, Bolognesi C, Bertazzi PA, et al. Global and gene-specific promoter methylation changes are related to anti-B[a]PDE-DNA adduct levels and influence micronuclei levels in polycyclic aromatic hydrocarbon-exposed individuals. *Int J Cancer*. 2009;125:1692–7.
120. Yang P, Ma J, Zhang B, Duan H, He Z, Zeng J, et al. CpG site-specific hypermethylation of p16INK4alpha in peripheral blood lymphocytes of PAH-exposed workers. *Cancer Epidemiol Biomarkers Prev*. 2012;21:182–90.
121. Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Res*. 2004;32:e38.
122. Li TH, Schmid CW. Differential stress induction of individual Alu loci: implications for transcription and retrotransposition. *Gene*. 2001;276:135–41.
123. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol*. 2014;15:R31.
124. Shah S, McRae AF, Marioni RE, Harris SE, Gibson J, Henders AK, et al. Genetic and environmental exposures constrain epigenetic drift over the human life course. *Genome Res*. 2014;24:1725–33.
125. Kerkel K, Spadola A, Yuan E, Kosek J, Jiang L, Hod E, et al. Genomic surveys by methylation-sensitive SNP analysis identify sequence-dependent allele-specific DNA methylation. *Nat Genet*. 2008;40:904–8.
126. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13:86.
127. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, Geman D, Baggerly K, Irizarry RA. Tackling the widespread and critical impact of batch effects in high-throughput data. *Nat Rev Genet*. 2010;11:733–739.
128. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014;30:1363–9.
129. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*. 2003;19:185–93.
130. Rojas D, Rager JE, Smeester L, Bailey KA, Drobná Z, Rubio-Andrade M, et al. Prenatal arsenic exposure and the epigenome: identifying sites of 5-methylcytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes. *Toxicol Sci*. 2015;143(1):97–106.
131. Huang Y, Pastor WA, Shen Y, Tahiliani M, Liu DR, Rao A. The behaviour of 5-hydroxymethylcytosine in bisulfite sequencing. *PLoS One*. 2010;5:e8888.
132. Booth MJ, Branco MR, Ficiz G, Oxley D, Krueger F, Reik W, et al. Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. *Science*. 2012;336:934–7.
133. Stewart SK, Morris TJ, Guilhamon P, Bulstrode H, Bachman M, Balasubramanian S, Beck S: oxBS-450K: A method for analysing hydroxymethylation using 450K BeadChips. *Methods* 2014. doi:10.1016/j.ymeth.2014.08.009.
134. Nazor KL, Peterson SE, Boland MJ, Bibikova M, Klotzle B, Yu M, et al. Application of a low cost array-based technique - TAB-Array - for quantifying and mapping both 5mC and 5hmC at single base resolution in human pluripotent stem cells. *Genomics*. 2014;104:358–67.
135. Dao T, Cheng RYS, Revelo MP, Mitzner W, Tang WT. Hydroxymethylation as a novel environmental biosensor. *Curr Environ Health Rep*. 2014;1:1–10.
136. Cheng TF, Choudhuri S, Muldoon-Jacobs K. Epigenetic targets of some toxicologically relevant metals: a review of the literature. *J Appl Toxicol*. 2012;32:643–53.

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