Epigenetic Alterations: The Relation Between Occupational Exposure and Biological Effects in Humans

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Abstract Exposome encompass the totality of human environmental exposures, providing a lifelong exposure history and complementing the genome. One of its domains is a specific external environment, which includes occupational exposure. Over the last decades, several publications have shown the higher incidences of exposure-related diseases and its relationship with DNA damage. However, there is a body of evidence that genetic variants cannot fully explain the variability in the risk of chronic diseases initiation and development, leaving a potential role the interaction between environmental and genetic factors. A key phenomenon are epigenetic modifications, heritable changes in gene expression that occur without changes in DNA sequence and play an important role in identifying mechanisms of xenobiotic-induced non-genotoxic carcinogenesis. Recently studies with occupational exposure individuals have shown substantial epigenetic alterations as effect of work-related activity with several xenobiotics, such as benzene, solvent, styrene, heavy metals, and mixtures of chemicals. Exposure to occupational toxicants may contribute to

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S. Jurga, J. Barciszewski (eds.), *The DNA, RNA, and Histone Methylomes*, RNA Technologies, https://doi.org/10.1007/978-3-030-14792-1_11

arising of adverse birth outcomes, neurological and other multifactorial diseases, and increased risk of cancer, and there is evidence that epigenetic aspects intermediate their effects in human health. In the current chapter, we review recently discoveries in the field of occupational exposure, health effects, and the interaction of epigenetic factors for such outcomes. The solid identification of key genetic and/or epigenetic events involved in chemical occupational-related carcinogenesis is a relevant step towards improvement of biomarkers to evaluate exposure, predict biological effects, and prevent adverse health consequences.

Keywords Occupational exposure · Metals · Organic compounds · Complex mixtures · Biomarkers of exposure

1 Introduction

Exposome encompass the totality of human environmental exposures, providing a lifelong exposure history and complementing the genome, being a new paradigm for studying the sum of environmental causes of diseases. Environmental research and public health aspects currently face several challenges such as air and water, as well as industrial pollution, which are particularly of prevalent concern in developing economies (Holland 2017). One of the major domains of exposome is the evaluation of an individual's external environment, which encompasses the increasing in global warming, widespread use of chemicals including pesticides and heavy metals, as much as other endocrinal disruptors, and major changes in nutrition and lifestyle of modern society, such as smoking and drinking habits, hormone-based medicines, high-fat foods and low fibers intake (Faisandier et al. 2011; Holland 2017; Siroux et al. 2016).

Individual's exposome is defined as the total of many exposure factors that comprehend the lifetime of such individual, including exposure to chemicals, radiation, environmental agents, nutritional patterns, stress, among others. Their health behavior, physical activities routine and their microbiome profile are components of the exposome. Specially, occupational exposure is a major issue as regard public health, as the proper identification of hazards and prevention of new threats to health may help in minimizing concerns (Holland 2017). In the past decades, some pathologies have been linked to different occupational exposures, with the main findings for respiratory tract (Gaffney and Christiani 2015), endocrinology diseases (Silins and Högberg 2011), cardiovascular impairments (Fang et al. 2010; Sekhotha et al. 2016), and risk of cancer (Alvanja and Bonner 2012; Charbotel et al. 2014; Fritisch et al. 2015).

Biomarkers are then a powerful tool for occupational health risk assessment. They are generally divided in three main classes for human studies: biomarkers of exposure, of susceptibility and of effect. While the first ones involve measurements of metabolites, mainly compounds and reflects internal and biologically effect dose, the second indicate an often-constitutive ability of an individual to respond to a

given exposure (Schulte and Hauser 2012). Biomarkers of effect compose the majority of occupational studies as, in general, workers are exposed to mixtures of agents. Therefore, those biomarkers help to identify both active components of the mixtures and consequences of specific mixtures exposures. A sub-class of biomarkers of effect is called biomarkers of early disease, which comprehends tests more closely indicative of a plain clinical effect (Silins and Högberg 2011).

Genetics is considered the main player in phenotype, therefore biomarkers of susceptibility represent a substantial knowledge for occupational risk assessment, as they include polymorphisms of specific genes associated with metabolism and detoxification of chemical material in the organism (Schulte and Hauser 2012). Genetic differences in metabolism can have an effect on population level, rather than in individual level, and may result in different effects for a given exposure. However, it is known that DNA sequence alone (i.e., genetic variation) cannot fully explain the observed phenotypic traits. Mutations in several genes are a distinctive feature of cancer cells and support the knowledge that cancer arises through the accumulation of irreversible DNA damage, and act in a 'genotoxic' manner. Despite this, there is a group of carcinogens that induce cancer via non-genotoxic mechanisms. Thus, other determinants of phenotype variation should be considered, and these include epigenetic modifications related to environmental exposure (Ravegnini et al. 2015; Meehan et al. 2018).

Epigenetic mechanisms, such as DNA, RNA and histones modifications, and microRNAs, have been shown to be potential links between the genetic and environmental exposure, which can be determinant to health and disease development. Epigenetics investigates heritable changes in gene expression without modifications in DNA sequence itself and, unlike genetics, they could be reversible. Particularly, epigenetic modifications can alter genome expression and function under exogenous influence (Baccarelli and Bollati 2009; Holland 2017). In the current chapter, the most recently discoveries in the field of occupational exposure health effects and the possible interaction of some epigenetic factors for such outcomes will be reviewed. The solid identification of key genetic and/or epigenetic events involved in chemical occupational-related carcinogenesis is a relevant step towards improvement of biomarkers to evaluate exposure, predict biological effects, and prevent adverse health consequences.

2 Metals

The genetic toxicity aspect of metals has been extensively studied, demonstrating that many common metals in human routine can cause DNA damage (Bal et al. 2011). Lately, its effect on epigenome has been shown through several in vitro and in vivo studies, along with epidemiological research as well.

Table 1 shows the main results observed in different studies as regard epigenetic markers in occupational exposure to metals. Arsenic (As) compounds are important environmental carcinogens that affect DNA methylation status in cell (Cheng et al.

Table 1 Epigenetic alterations in occupational exposure to metals

Exposure	Country	N. of participants	Results[a]	References
Arsenic (As)	China	*Exposed*: 43 As trioxide producers (plant 1), 36 workers who stopped producing 85 days previous (plant 2). *Control*: 24 individuals never exposed	↓ 5 LincRNAs in workers for plant 1. Significant higher base modifications of three exons of *p53* in workers from both plants. Several correlations between different exon base modifications of *p53* and expressions of LincRNAs.	Wen et al. (2016)
Chromium (Cr)	China	*Exposed*: 115 chromate producing facility workers. *Control:* 60 non-exposed local residents	↓ global DNA methylation. RBC-Cr levels negatively associated with global DNA methylation. Folate positively associated with global DNA methylation for both groups.	Wang et al. (2012)
	China	*Exposed*: 29 chrome plating workers. *Control*: 29 non-exposed to Cr matched individuals	methylation of *MT-TF* and *MT-RNR1*. Negatively correlation found for Cr levels and *MT-TF* and *MT-RNR1* gene methylation. CpG sites in *MT-TF* and *MT-RNR1* negatively associated with Cr level.	Linqing et al. (2016)
	China	*Exposed*: 87 blue-collar workers from a chromate factory. *Control*: 30 office workers from the same factory	Cr levels in exposed workers positively correlated with: methylation level of CpG sites in DNA repair genes (*MGMT* and *HOGG1*) and with CpG sites in *RAD51* gene.	Hu et al. (2018)
Lead (Pb)	China	*Exposed*: 103 battery plant workers. *Control:* age- and gender-matched 103 healthy volunteers	↓ methylation of *ALAD* CpG. Individuals with methylated *ALAD* had increased risk of lead poisoning.	Li et al. (2011)
	China	*Exposed*: 53 battery plant workers. *Control*: 57 healthy individuals matched by age and gender, smoking status and alcohol consumption	↓ methylation of *LINE-1*. Lower methylation levels as higher Pb blood levels.	Li et al. (2013)
	China	*Exposed:* 1130 battery factories; top 10% with highest blood lead level (BLL) and bottom 10%	↓ expression of three *miRNAs* in high Pb exposure and high BLL workers: *miR-520c-3p*,	Xu et al. (2017)

(continued)

Table 1 (continued)

Exposure	Country	N. of participants	Results[a]	References
		with lowest BLL defined as high and minimal lead-exposure groups	*miR-211* and *miR-148a,* and ↑ *miR-572* expression.	
Mercury (Hg)	United States	*Exposed:* 41 dentists (36 males, 5 females). *Control*: 90 non-dentists (28 males and 62 females).	*LINE-1* methylation positively correlated with age. Trend of *SEPP1* hypomethylation with increasing Hg hair levels, significant among males, for both groups. Trend remaining when for dentists only.	Goodrich et al. (2013)
Nickel (Ni)	China	*Exposed*: phase 1–30 flash smelting workshop where Ni is processed; phase 2—additional 15 subjects occupationally exposed to Ni. *Control*: phase 1–60 maintenance and office workers; phase 2–15 additional subjects from same place; all frequency-matched by age and smoking habits	↑ H3K4me3 and ↓ H3K9me2. H3K4me3 and H3K9ac were positively and negatively associated with urinary Ni, respectively. H3K4me3, H3K9me2 and H3K9ac histone modifications were relatively stable over time.	Arita et al. (2012)
	China	*Exposed*: 140 nickel-smelting workers divided in seven groups according to age and years of work. *Control*: 140 office workers age-matched	↑ levels of H3K4me3 and ↓ levels of H3K27me3. ↑ H3K4me3 level was the highest in the 30+ service length subgroup. ↓ H3K27me3 levels associated with years of exposure.	Ma et al. (2015)
Nickel (Ni), arsenic (As) and iron (Fe)	Italy	*Exposed*: 63 male steel production plant (pre- and post-exposure in a given week)	↑ H3K4me3 and H3K9ac in association with years of employment; ↑ H3K4me3 increased in association with air levels of Ni, As and Fe; Cumulative exposure to the three agents was positively correlated with H3K4me3 and H3K9ac	Cantone et al. (2011)

[a]Describes results statistically significant (unless stated otherwise) for exposure groups in relation to control or baseline groups (unless stated otherwise)
RBC red blood cells

2012) and are classified in group 1 of IARC (Beyersmann and Hartwig 2008). As has been shown to cause different types of cancer via exposure to contaminated drinking water and/or air pathway (Tchounwou et al. 2003). Oxidative methylation and

reduction reactions of As lead to the generation of methylated metabolites, which are excreted in urine. A Chinese study evaluated As trioxide producers and observed higher levels of urinary As (Wen et al. 2016). The toxicity of As is more frequently related to its trivalent state, the As trioxide, due to its ability to bind thiol groups in various cellular components. The exposed group from Wen et al. (2016) study was composed by workers currently exposed and workers who had stopped producing 85 days previously to the study begins. Regardless, both subgroups showed significant base modifications of exons 5, 6, 7 and 8 of *p53* tumor suppressor gene (Wen et al. 2016). DNA microarray study has found up-regulation of various oncogenes after As exposure, but not for *p53*, while another report show that human lung adenocarcinoma cell lines exposed to As had dose-response hypermethylation of *p53* promoter (reviewed by Cheng et al. 2012). The tumor suppressor p53 plays a key role in the induction of apoptosis and cell cycle arrest in response to a variety of genotoxic stressors, preventing the propagation of damaged cells. Gene-specific methylation of both *p53* and *p16* has been associated with As exposure in different occupational and environmental settings. The p16 is a cyclin-dependent kinase inhibitor that cycle-regulates cells senescence through induction of inflammatory markers (Fischer et al. 2013). Several lines of evidence suggest that As compounds genotoxicity is mediated by increased levels of reactive oxygen species (ROS) and they have also been shown to inhibit DNA repair, mainly thorough nucleotide excision repair (NER) (Beyersmann and Hartwig 2008).

Oxidative stress is a major pathway through which metal compounds can cause DNA damage and epigenetic imbalances in humans. Pentavalent chromium reacts with isolated DNA to produce 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG), the more relevant marker of guanines oxidation. Significantly higher urinary 8-OHdG and increased DNA damage (Hu et al. 2018; Wang et al. 2012), evaluated through Comet assay (Wang et al. 2012) and micronucleus frequency (Hu et al. 2018), were observed in chromate producing facility workers. Altered global methylation of DNA, and methylation of DNA repair genes and specific mtDNA genes were observed in chromate exposed workers in three different studies conducted in China (Hu et al. 2018; Wang et al. 2012; Linqing et al. 2016). Chromium (Cr) has been shown to reduce in-vitro H3 phosphorylation and trimethylation, as well as various acetylation marks in H3 and H4. These changes inhibit RNA polymerase II recruitment and transcription initiation. Thus, epigenetic mechanisms might be a central target of chromium toxicity and inhibition of these mechanisms reduces the capacity of cells to respond to environmental hazards. Long-term exposure to chromium may cause a significant increase in histone deacetylation, which would lead to histone methylation in specific positions involved in gene repression and silencing and to subsequent DNA hypermethylation, which would soon be converted into a complete and efficient state of gene silencing (Schnekenburger et al. 2007). Exposure to different Cr compounds has been extensively related with incidence of respiratory cancer in human and animal models (Beyersmann and Hartwig 2008). In this context, the severity of symptoms of chronic obstructive pulmonary disease (COPD) was correlated with a reduction in histone deacetylase activity (HDAC) in lung cancer and alveolar macrophages (Schottenfeld and Beebe-Dimmer 2006). Increased proliferation kinetics and the

interaction of hydroxyl radicals with DNA increase the likelihood of DNA structural and transcriptional errors. A key enzymatic function of HDAC is the inhibition or modulation of production of proinflammatory cytokines and matrix metalloproteinases by macrophages (O'Sullivan et al. 2010). What is more, regulation of the $p16^{ink4a}$ tumor suppressor gene appears to be a major target of chromium toxicity. Cigarette smoking is a major source of coexposure to chromium and B[a]P, and several studies have reported the association between aberrant p16 methylation and smoking (Jarmalaite et al. 2003; Sun et al. 2015). Hypermethylation of the $p16^{ink4a}$ promoter has also been found in one-third of chromate workers with a history of exposure for 15 years or more who developed lung cancer (Kondo et al. 2006).

The disruption of DNA repair mechanism seems to be relevant as per toxicity of metal compounds to humans. Similar to Cr, lead (Pb) highly interacts with both NER and base excision repair (BER), being considered a genotoxic agent. The occupational exposure to Pb shows increased in MN frequencies accompanied by influence of polymorphisms in genes involved in DNA repair, such as OGG1 and XRCC4 (García-Lestón et al. 2012). This metal also seems to perturb telomere replication, leading to chromosomal abnormalities, including, telomere loss (Pottier et al. 2013). Nonhomologous end joining (NHEJ) is the predominant form of repair of unprotected DNA ends in mammalian cells and involves proteins that are well characterized, including Ku70, Ku80, DNA-PKcs, LIG4, and XRCC4. NHEJ is also involved in fusion of telomeres as a result of deficiency in TRF2, one of the components of shelterin complex in telomeres (Murnare 2011). Battery factor workers is the largest studied group of individuals occupationally exposed to Pb, for which all the metal levels are significantly higher than control groups (García-Lestón et al. 2012; Li et al. 2011, 2013; Xu et al. 2017). The *ALAD* gene exists in two forms, *ALAD1* and *ALAD2*, and codes for the enzyme ALAD that catalyzes the second step of heme synthesis and may modify lead toxicokinetics and exert impact on individual susceptibility to lead poisoning. Methylated *ALAD* was observed in Chinese battery factory workers and was associated with increased risk of lead poisoning (Li et al. 2011). In other group of workers occupationally exposed to Pb, hypomethylation of *LINE-1* was significantly different from control individuals and inversely associated with Pb levels (Li et al. 2013). Methylation of *LINE-1* helps maintain genomic stability and integrity, while loss of methylation in *LINE-1* may result in higher chances of mitotic recombination (Cheung et al. 2009). Dysregulation of miRNA interferes in translation of their mRNA. In a large study where 1130 battery workers were stratified as per their Pb blood level (BLL), the top 10% of BLL showed lower expression of three miRNAs. Their functional analysis showed a network involved in cellular process, such as apoptosis, phagocytosis and cell proliferation, potentially mediating pathways related to different by Pb exposure (Xu et al. 2017).

Industrial and commercial uses are the main source of exposure to metals for humans. This aspect is not different for nickel (Ni), a transition metal used in industry along with other metals to form alloys to produce jewelry, household equipment, cooking utensils, coins, orthopedic implants, among others. Although Ni is a known carcinogen, it is non-mutagenic or weakly mutagenic in rodent assays (Costa et al. 2005), but its carcinogenicity is thought to be associated with its ability

to exacerbate epigenetic modifications (Arita et al. 2012). Two different studies conducted in China with nickel-smelting workers evidenced nickel's high influence over histone modifications (Arita et al. 2012; Ma et al. 2015). While all histone modifications occur during normal cellular development and processes, dysregulation of the balance of appropriate histone modifications can lead to disease. Histone modifications are of particular interest because histone dynamics play a role in the toxic potential of the chemicals by influencing both transcriptional activity and DNA repair mechanisms (Chappel et al. 2016). Ni induces transcriptional repression of genes involved in homology-dependent DNA double-strand break repair and mismatch repair and Ni accumulation in lung tissues is associated with increased p53 risk mutation in lung cancer patients (Scanlon et al. 2017). Higher levels of H3K4me3 in workers than in the control group were seen in both studies with nickel-smelting occupational exposure, being positively associated with urinary Ni levels (Arita et al. 2012) and with years of exposure to Ni (Ma et al. 2015). Interestingly, an Italian group of male steel production plant, occupationally exposed to nickel, arsenic and iron, had also increased levels of H3K4me3 associated with years of employment and with air levels of the three metals (Cantone et al. 2011). Each of those studies also observed lower levels of H3K9me2 (Arita et al. 2012) and H3K27me3 (Ma et al. 2015), and higher levels of H3K9ac (Cantone et al. 2011) in exposed workers. The redox activity of some nickel compounds is related to histone biding. Furthermore, Ni-induced methylation of H3K9 histones are considered repressing modifications, leading to disruption of transcription factors' access to DNA and silencing of telomeric marker genes (reviewed by Maxwell and Salnikow 2004). The accumulation of the number of subchromosomal regions with allelic imbalance extending to the telomeres is a genomic marker of impaired DNA repair and DNA-damaging agents (Birkbak et al. 2012).

Dental amalgams are a route of exposure to mercury (Hg) by dentists worldwide. Even though, because Hg is also available to humans through consumption of seafood, not many studies are published specific as regard occupational exposure to this metal. Male and female dentists were evaluated in USA and their occupation was predictor of higher Hg blood levels (Goodrich et al. 2013). Although not differences for global DNA methylation were observed, *LINE-1* methylation was positively correlated with age, while a trend of *SEPP1* hypomethylation was observed for male dentists (Goodrich et al. 2013). The *SEPP1* gene is important for Hg toxicokinetics and protection against its toxicity through direct binding, besides its well-established antioxidant activity (Chen et al. 2006). Mercury has been shown to be genotoxic and cause damage to neuronal, cardiovascular and renal systems, besides showing substantial epigenetic modifications in mice offspring (reviewed by Cheng et al. 2012).

3 Organic Compounds

Benzene, toluene, xylene, solvents and carbons remains the environmental chemicals highly used in industrial setting worldwide. They are found in gas stations, in leather products, sports equipment manufacturing, outdoor air, among other workplaces. Professions related to vehicle traffic and petrochemical production are the top ones as regard benzene occupational exposure (Carugno et al. 2012; Byun et al. 2013; Jamebozorgi et al. 2018), as summarized in Table 2. Hypermethylation of the *p15* gene was observed in petrochemical male workers (Carugno et al. 2012; Jamebozorgi et al. 2018), bus drivers, gas station attendants and police officers (Carugno et al. 2012). Benzene exposure has been consistently associated with acute myelogenous leukemia (AML) and although the straight forward mechanism has not been fully understood, aberrant DNA methylation patterns, including global hypomethylation, gene-specific hypermethylation or hypomethylation and loss of imprinting, are commonly observed in AML tissues (Rinsky et al. 2002). Mitochondrial DNA copy number (mtDNAcn) was significantly higher and associated with *LINE-1* hypomethylation in an Italian study comprising individuals working somehow with vehicle traffic (Carugno et al. 2012). Curiously, the aberrant methylation observed for *p15* gene in petrochemical workers was not associated with age or smoking status, neither with DNA damage parameters, such as micronucleus (MN), nucleoplasmic bridges (NPB), and nuclear buds (NBUD) (Jamebozorgi et al. 2018). Additionally, another Italian study did not find any difference on mtDNA methylation between gas-station workers with high- and low-exposure to benzene (Byun et al. 2013).

House builders and decorators represent another group of workers with risk of exposure to benzene in their workplace (Table 2), but mainly to a mixture of benzene, toluene and xylene (BTX). This mixture is used as a solvent and the concentration of its components may vary broadly. In a Chinese study comprehending decorators and painters, BTX levels were significantly higher in those workers compared to control group, but even higher in painters (Sha et al. 2014). The increased levels of BTX were associated with lower mRNA expression of genes involved in genome's methylation pattern maintenance. Cytokinesis-block micronucleus parameters assay did not show differences in this study (Sha et al. 2014). Benzene, toluene and xylene are monocyclic aromatic hydrocarbon compounds and when evaluated separately, it is assumed that toluene and xylene may interact determining benzene toxicity by influencing its toxicokinetics (ATSDR 2004). Exposure to a mix of organic solvents, including chloroform, was also associated with global DNA hypermethylation in a study conducted with 128 pharmaceutical plant workers in Belgium (Godderis et al. 2012). A group of chronic toxic encephalopathy (CTE) patients was also included in this study. Since CTE is a neurobehavioral disorder associated with solvent exposure, authors aimed to explore if DNA methylation patterns could play a role in this disease development and prognostic. Although CTE patients had longer exposure to mix of organic solvents than the pharmaceutical workers, their global DNA methylation patterns were

Table 2 Epigenetic alterations in occupational exposure to organic compounds

Exposure	Country	N. of participants	Results[a]	References
Benzene	Italy	*Exposed*: 153 bus drivers, 78 gas station attendants, 77 police officers, 33 petrochemical workers; *control*: 178 individuals from the same region of study	↑ mtDNAcn for exposed groups; Interquartile range increase in exposure associated with increase in mtDNAcn; mtDNAcn associated with *LINE-1* hypomethylation and *p15* hypermethylation.	Carugno et al. (2012)
	Italy	*Exposed*: 20 gas-station attendants with high exposure to PM_1 ($\geq$7.6 μg/m^3) and 20 with low-exposure to PM_1 ($\leq$3.8 μg/m^3)	No effects on mtDNA methylation (*MT-TF*, *MT-RNR1* and D-loop control region) No association of mtDNA methylation with benzene levels	Byun et al. (2013)
	Iran	*Exposed:* petrochemical male workers (40) exposed to <1 mm of benzene. *Control:* 31 office workers	↑ DNA methylation rate in $p15^{INK4b}$ gene in exposed individuals. No association between methylation and frequency of MN, NPB and NBUD in peripheral blood lymphocytes.	Jamebozorgi et al. (2018)
Toluene	Korea	*Exposed*: 14 short-term (<6.4 year. of exposure) and 14 long-term (>6.4 year. of exposure). *Control*: 14 non-exposed	631 genes upregulated and 263 downregulated in the short-term exposure. 662 genes upregulated and 260 downregulated in the long-term exposure, some overlapping short-term; Cell survival, immune and nerve systems functions associated with upregulated genes.	Hong et al. (2016)
Benzene, toluene and xylene (BTX)	China	*Exposed*: 132 decorators and 129 painters. *Control*: 130 non-exposed workers frequency matched by sex and age	↓ expression of *PARP1, DNMTs* and *MBD2*, and ↓ PARP activity. ↓ *PARP1, DNMT1, DNMT3a, DNMT3b* and *MBD2* mRNA expression was correlated with increased BTX levels.	Sha et al. (2014)
Mixture of organic	Belgium	*Exposed*: 128 pharmaceutical plant workers.	Global DNA hypermethylation	Godderis et al. (2012)

(continued)

Table 2 (continued)

Exposure	Country	N. of participants	Results[a]	References
solvents (mainly chloroform)		*Control*: 41 healthy individuals	associated with solvent exposure *GSTP1* polymorphism significantly associated with global DNA methylation.	
1,6-hexamethylene diisocyanate (HDI)	United States	*Exposed*: 20 automotive spray-painters based on stratified HDI	Two methylated CpG sites from genes LPHN3 and SCARA5 were associated with urine HDI levels and creatinine. Thirty methylated CpG sites from 28 different genes associated with HDI inhalation and skin exposure.	Nylander-French et al. (2014)
Carbon nanotubes	Belgium	*Exposed:* 24 multi-wall carbon nanotubes (MWCNT) workers. *Control*: age-matched 43 office workers from the same company	No differences in global DNA methylation (5-mC), hydroxymethylation (5-hmC) and *LINE-1* methylation between groups. 5-mC and 5-hmC were positively correlated between them. Gene-specific methylation in MWCNT group: DNMT1, HDAC4, NPAT/ATM and SKI.	Ghosh et al. (2017)

[a]Describes results statistically significant (unless stated otherwise) for exposure groups in relation to control or baseline groups (unless stated otherwise)
CBMN cytokinesis-block micronucleus assay, *NBUD* nuclear buds, *NPB* nucleoplasmic bridges

similar to the control group (Godderis et al. 2012). Solvent components may rise the occurrence of ROS and cytotoxicity, which explain the association observed between *GSTP1* polymorphism and global DNA methylation in workers (Godderis et al. 2012).

Table 2 also summarizes results observed for some other solvents. Toluene itself accumulates in tissues, including parts of the brain with high lipid content (Tas et al. 2011). Exposure to toluene has been shown to affect gene expression. A pilot study conducted with individuals short-term and long-term (<6.4 and >6.4 years of exposure) occupationally exposed to this chemical evaluated the variation in gene expression and occurrence of methylation (Hong et al. 2016). Authors found that 26 genes were upregulated and hypomethylated, while 32 genes were downregulated and hypermethylated. The upregulated genes, such as MAPK1, TGFB1, TNFs and

ACHE, were mainly associated with cell survival, nervous and immune systems pathways, suggesting that they can help predict the effects of time-dependent toluene exposure (Hong et al. 2016). A previous study showed that footwear workers exposed to solvent-based adhesives had increased DNA damage than control individuals and workers exposed to water-based adhesives (Heuser et al. 2005). The main solvent used by footwear workers was toluene, a chemical that can induce oxidative stress (Martínez-Alfaro et al. 2010). As much as toluene, 1,6-hexamethylene diisocyanate (HDI), is absorbed by human body through inhalation and skin exposure. This chemical is commonly found in automotive spray dye workplaces. Through system biology approach, Nylander-French et al. (2014) analyzed inter-individual differences for automotive spray-painters based on stratified HDI-exposure levels, as regard CpG DNA methylation interactions with blood and urine markers. Two methylated CpG sites in *LPHN3* and *SCARA5* were associated with urine HDI levels and creatinine (Nylander-French et al. 2014). LPHN3 belongs to a family of proteins that function in both cell adhesion and signal transduction and is a binding partner of ubiquitin, suggesting a role in protein ubiquitination (Boucard et al. 2014). The *SCARA5* gene codes for proteins that work in recognition of host defense by initiating immune system (Jiang et al. 2006). Although *SCARA5* is in chromosome 8 and, along with other genes, is implicated in frequent copy number variation, it has not been related to specific diseases. On the other hand, *LPHN3* gene is reported to be associated with cognitive disabilities (Nylander-French et al. 2014).

Apart from benzene, current knowledge on organic compound toxicity is controversial as regard genotoxicity and human long-term effects. Carbon is one of the major organic compounds that humans are exposed to, in both environmentally and occupationally settings (Table 2). No difference in global DNA methylation, hydroxymethylation and *LINE-1* methylation was observed between a group of multi-wall carbon nanotubes (MWCNT) and a control group (Ghosh et al. 2017). However, the MWCNT group showed gene-specific methylation, such as *DNMT1*, *HDAC4*, *NPAT/ATM* and *SKI* genes. While *DNMT1* plays a role in epigenetic regulation itself, *NPAT/ATM* codes for proteins involved in DNA repair and cell cycle pathways (Blackford and Jackson 2017). The *SKI* gene is considered a potential TGF-β repressor (Zhang et al. 2017). Taken together, such data shows that occupational exposure to carbon do not alter global DNA methylation but modifies gene-specific methylation towards cellular process highly important to genomic stability.

4 Complex Mixtures

The evaluation of occupational exposure to complex mixtures is a challenge itself since such types of exposure have many constituents in common and people can be exposed to more than one of those mixtures at the same period (Manno et al. 2009). Work environments are hardly composed by only one chemical, therefore,

biomarkers of exposure to mixtures can be a strategic tool to understand risks and prevent diseases outburst. Several studies approaching epigenetic effects in individuals occupationally exposed to complex mixtures are shown in Table 3.

Brazilians coal miners showed global DNA hypermethylation when compared to a control group (De Souza et al. 2018). Coal is a mixture of several chemicals, mainly inorganic elements and polycyclic aromatic hydrocarbons (PAHs), many of which have mutagenic and carcinogenic effects (Léon et al. 2007). Although there was no correlation of DNA methylation with the other parameters analyzed in the study, authors also observed shorter telomere length for coal miners. DNA methylation regulates and determines transcription, chromatin structure, chromosome integrity, and genomic imprinting. Aberrant DNA methylation can lead to disruption of any or all of these processes and may contribute to carcinogenesis, which is also highly associated with telomeric imbalance. PAHs are known for producing DNA adducts leading to genomic instability, as shown in a mouse model orally exposed to coal tar (Long et al. 2016). Different studies approaching the single components of coal demonstrated their epigenetic alterations in several study models as well (reviewed by De Souza et al. 2018). Polycyclic aromatic hydrocarbons (PAH) are one of the most studied groups of xenobiotics to which people are exposed in their workplaces. Since PAHs are generated in different industries, researchers have high interest in it. Regardless which occupation, all studies with PAHs in Table 3 showed significantly higher levels of PAHs markers in individuals occupationally exposed (Alegría-Torres et al. 2013; Alhamdow et al. 2018; Duan et al. 2013; Pavanello et al. 2009; Yang et al. 2012). Three different studies performed with coke-oven workers observed higher indexes of DNA damage parameters, such as MN and comet cells (Duan et al. 2013; Pavanello et al. 2009; Yang et al. 2012). Methylation of *p53* gene and hypermethylation of 22 CpG sites in *p16* gene were determinant in MN increase (Pavanello et al. 2009; Yang et al. 2012), while hypomethylation of *MGMT* was correlated with higher MN frequency (Duan et al. 2013). Differently from Brazilian coal miners, who are exposed to a mixture of chemicals, Sweden chimney sweeps and creosote-exposed males showed no difference in telomere length when compared to control group (Alhamdow et al. 2018). However, those individuals presented hypomethylation of *AHRR* and *F2RL3* genes, the former only for creosote-exposed ones. A cohort follow-up study pointed hypomethylation of the *F2RL3* gene as a potent predictor of incidence and mortality of lung cancer (Zhang et al. 2015a). *AHRR* hypomethylation status is also considered a predictor of lung cancer risk, in addition to be linked to lymphoblastic anemia (de Smith et al. 2017; Zhang et al. 2016a). Occupational exposure to PAHs alters patterns of global DNA methylation in several markers of this status, such as *MGMT, LINE-1* and *Alu*. It is also interesting that disturbance of methylation provoked by exposure to PAH interferes with other parameters: *Alu* methylation is negatively associated with TNF-α (Alegría-Torres et al. 2013) and positively correlated with MN (Pavanello et al. 2009), *LINE-1* is inversely associated with comet cells and MN frequency (Duan et al. 2013) and positively with MN only (Pavanello et al. 2009). Pavanello et al. (2009) also showed hypomethylation of tumor suppressors genes *p53* and *HIC1*, that synergizes in tumor suppression (Guerardel et al. 2001). The *p53* gene is

Table 3 Epigenetic alterations in occupational exposure to complex mixtures

Exposure	Country	N. of participants	Results[a]	References
Coal	Brazil	*Exposed:* 55 coal miners. *Control:* 27 non-exposed from same region	↑ global DNA methylation. No correlations between global DNA methylation with comet assay, MN, oxidative stress and inorganic elements.	De Souza et al. (2018)
Diesel engine exhaust (DEE)	China	*Exposed*: 117 male DDE-exposed workers from a diesel engine manufacturing plant. *Control*: 112 male non-exposed	↓ methylation of DDR-related genes (*p16, RASSF1A* and *MGMT*). *p16, RASSF1A, MGMT* and *LINE-1* methylation levels negative correlated with CBMN indexes.	Zhang et al. (2016b)
	China	*Exposed:* 20 truck drivers exposed to high traffic-derived elemental carbon (EC; $\geq$16.6 μg/m^3) and 20 with low-exposure to EC ($\leq$16.1 μg/m^3). *Control:* age-matched 20 office workers	No effects on mtDNA methylation (MT-TF, MT-RNR1 and D-loop control region).	Byun et al. (2013)
Hair dye and hair waving products	Sweden	*Exposed*: 295 hairdressers. *Control*: 92 non-hairdressers	↓ frequency of *CDKN2A* methylation	Li et al. (2016)
Particulate matter (PM)	Italy	*Exposed*: 63 steel production plant workers (baseline: first day of a workweek before shift starts X postexposure: fourth day of the week)	↑ *miR-222* and *miR-21* expression in postexposure samples. No correlation of miRNA expression with any personal and demographic characteristics. *miR-222* expression positively associated with lead levels. *miR-146a* expression negatively associated with lead and cadmium.	Bollati et al. (2010)
	Italy	*Exposed*: 63 steel production plant workers (baseline: first day of a workweek before shift	↓ *hTERT* expression in post-exposure but not dose-dependent with PM. No differences for CpG	Dioni et al. (2011)

(continued)

Table 3 (continued)

Exposure	Country	N. of participants	Results[a]	References
		starts X postexposure: fourth day of the week)	sites in *hTERT* promoter.	
	Italy	*Exposed*: 40 steel workers exposed to PM_1 (20 high-, 20 low-exposure)	↑ methylation of *MT-TF* and *MT-RNR1* in the 20-high exposure group.	Byun et al. (2013)
	United States	*Exposed*: 38 male boilermaker welders in high-exposure welding day and low-exposure welding day (pre-shift and post-shift)	$PM_{2.5}$ was associated with hypermethylation of *iNOS* promoter gene. Years of work were associated with *iNOS* hypermethylation.	Kile et al. (2013)
Polycyclic aromatic hydrocarbons (PAH)	Mexico	*Exposed*: 39 male brick manufacturers (pre- and post-exposure in a given week)	1-hydroxypyrene (1-OHP) urine concentration negatively associated with *IL-12* and *p53* DNA methylation; negative association trend observed for *TNF-alpha* and *Alu* methylation	Alegría-Torres et al. (2013)
	Sweden	*Exposed*: 151 chimney sweeps and 19 creosote-exposed male workers. *Controls*: 152 healthy men	↓ methylation of *AHRR* CpG sites. ↓ methylation of *F2RL3* in creosote-exposed.	Alhamdow et al. (2018)
	China	*Exposed*: 82 coke-oven workers. *Control*: 62 unexposed workers age and gender matched	↓ methylation of *LINE-1* and *MGMT*. *LINE-1*, *MGMT* and its hot CpG site-specific methylation negatively correlated with 1-OHP. *LINE-1* methylation inversely associated with comet cells and micronucleus frequency. ↑ MN in *MGMT* hypomethylation individuals	Duan et al. (2013)
	Poland	*Exposed*: 49 non-smoking coke-oven workers. *Controls*: 43 non-exposed, matched by gender, age and smoke status	↑ methylation of *LINE-1* and *Alu* sequences. ↓ methylation of *p53* and *H1C1*.	Pavanello et al. (2009)

(continued)

Table 3 (continued)

Exposure	Country	N. of participants	Results[a]	References
	China	*Exposed:* 69 male coke-oven workers. *Control:* 59 male non-exposed workers	↑ $p16^{INKa}$ expression. Hypermethylation of 22 CpG sites in $p16^{INKa}$. Hypermethylated CpG sites positively correlated with 1-OHP and CBMN parameters	Yang et al. (2012)
Pesticides	Brazil	*Exposed*: 137 male soybean farmers. *Control:* 83 male non-farmers	↑ global DNA methylation. Positive correlation between MN and global DNA methylation	Benedetti et al. (2018)
	Mexico	*Exposed:* 127 urban pesticide sprayers (moderate- and high-exposure). *Control*: 63 non-exposed	↓ *LINE-1* methylation in exposed group, but higher for high-exposure compared to moderate-exposure. ↓ CpG1 *LINE-1* methylation in both exposed groups compared to control. ↓ CpG2 and CpG3 *LINE-1* methylation in moderate-exposed group compared to control. *LINE-1* methylation associated with alcohol consumption in high-exposure group	Benitez-Trinidad et al. (2018)
	Brazil	*Exposed*: 56 tobacco farmers. *Control*: 74 unexposed individuals from the same region	↓ global methylation ↑ *p16* methylation associated with shortest telomeres.	Kahl et al. (2018a)
	Brazil	*Exposed*: 40 tobacco farmers. *Control*: 40 individuals non-exposed matched by gender and age	↓ global methylation	Kahl et al. (2018b)
	Netherlands	*Exposed:* 108 low-exposure and 61 high- exposure. *Control:* 1392 non-exposed subjects	↑ DNA methylation in 4 CpGs for women in high-exposure group and ↓ DNA methylation in one CpG. High pesticide exposure individuals showed differential	van der Plaat et al. (2018)

(continued)

Table 3 (continued)

Exposure	Country	N. of participants	Results[a]	References
			DNA methylation of 31 CpG sites annotated to 29 genes; 20 of those found in subjects with airway obstruction; Seven of the 31 CpG sites were associated with modified gene expression levels	
Volatile organic compounds (VOC)	Mexico	*Exposed*: 40 shoe factory workers (LS) and 36 gas station attendants (GS). *Control*: 66 university employees	↑ promoter methylation in *TOP2A* (compare to control group), *SOD1* and *TNF-alpha* (compare to both control and GS group) genes in LS group. Correlation between *GSTP1* promoter methylation and iNOS and COX-2 methylation in LS group. Both LS and GS groups had ethylbenzene levels correlated with *TOP2A* methylation	Jiménez-Garza et al. (2018)
	Korea	*Exposed:* 128 workers from dockyards. *Control:* 41 unexposed individuals from different working areas	Identification of deregulated miRNAs: 467 for toluene, 221 for xylene and 695 ethylbenzene exposures	Song and Ryu (2015)
Welding fumes/respirable dust		*Exposed*: 101 men welders. *Control*: 127 non-welders men	No significant difference of *APC* methylation in the fully model.	Li et al. (2015)

[a]Describes results statistically significant (unless stated otherwise) for exposure groups in relation to control or baseline groups (unless stated otherwise)
CBMN cytokinesis-block micronucleus assay, *MN* micronucleus

the best characterized B[*a*]P (a marker of PAH exposure) mutagenic target and, together with tumor suppressor *p16*, is frequently epigenetically altered in smoking PAH-associated lung cancer (Risch and Plass 2008).

Epidemiological approaches have consistently linked both short- and long-term exposure to particulate matter (PM) with increased morbidity and mortality (Anderson et al. 2012). The two major mechanisms by which PMs act on human body are through increased inflammation and oxidative stress. A study conducted with boilermakers welders demonstrated $PM_{2.5}$ levels associated with hypermethylation of promoter region of *iNOS* gene (Kile et al. 2013), which is involved in production of nitric

oxide and plays an important role in cardiovascular health (Tsutsui et al. 2010). Authors highlight that increased in *iNOS* DNA methylation may have been produced by systemic inflammation from inhaling fine particulate (Kile et al. 2013). Two Italian studies evaluated the same group of production plant steel workers but looking into different aspects of epigenetics and genotoxicity (Bollati et al. 2010; Dioni et al. 2011). There was increase in expression for *miR-222* and *miR-21* (Bollati et al. 2010), increase in telomere length and decrease in *hTERT* expression (Dioni et al. 2011) in the post-exposure period. Methylation of CpG-rich sequences of the hTERT promoter is involved in hTERT expression (Guilleret et al. 2002). Telomere length was also positively associated with PM_{10} and PM_1 levels (Dioni et al. 2011). Telomeres shorten in each cell division due the end-replication problem and are, therefore, markers of cellular senescence (O'Sullivan and Karlseder 2010). The end-replication problem can be overcome, and telomeres can be maintained, by telomerase: a core enzyme composed by a reverse transcriptase catalytic component (hTERT) and an RNA component (hTR). Telomerase is suppressed to undetectable levels in human somatic cells but can be reactivated in majority of cancer cells to counteract telomere shortening (Reddel 2014) and is positively regulated by hTERT. The decreased *hTERT* expression would not explain the increase in telomere length also observed in this group (Dioni et al. 2011). However, there is a high possibility that those individuals are activating the alternative telomere lengthening (ALT), based on homologous recombinant that overcomes telomere trimming, and is present in 15% of cancers (Reddel 2014). Interestingly, PM is a major toxic component of air pollution that has been associated in epidemiological investigations with increased mortality from several outcomes, including lung cancer (Brook et al. 2004). In concordance on how ALT may be triggering the telomere length elongation on those individuals, a study demonstrated that the lung carcinoma cell line SK-LU-1 is telomerase negative and presents ALT (Bryan et al. 1997).

Diesel engine exhaust (DEE) is a mixture of several chemicals, among which are elemental carbon, PM and PAHs. Recently, DEE was reevaluated by IARC and included as a known carcinogen for humans (Benbrahim-Tallaa et al. 2012; Zhang et al. 2016b). For exposed populations, the genotoxic effects of DEE are determined not only by DNA damage induced, but also by DNA damage response (DDR). No effects of elemental carbon were observed in mtDNA methylation parameters in 20 truck drivers in China, regardless being exposed to high or low levels of it (Byun et al. 2013). On the other hand, 117 Chinese DDE-exposed workers from a diesel engine manufacturing plant showed exacerbated levels of $PM_{2.5}$, elemental carbon and six urinary PAHs, when compared to 112 control individuals (Zhang et al. 2016b). A previous work with same group of DDE-exposed individuals demonstrated higher levels of CBMN parameters, indicating DNA damage related to the exposure (Zhang et al. 2015b). Afterwards, authors observed a negative correlation of CBMN parameters and methylation of *p16, LINE-1, MGMT* and *RASSF1A* genes. While *LINE-1* represents global DNA methylation of repetitive elements, the other three genes are DDR-related ones, and were found hypomethylated in this study (Zhang et al. 2016b). The hypomethylation of those genes can lead to their increased expression levels and consequent activation of DDR. Hypomethylation is also

typically associated with higher transcriptional levels, which leads to cell-cycle arrest that facilitates DNA damage repair in the case of *p16* and *RASSF1A* and directly strengthen DDR in the case of *MGMT*, mitigating genomic instability.

A study performed with samples (benign and malignant biopsies, blood and saliva) from individuals with impalpable lesions of breast cancer also observed higher methylation of *CDKN2A* gene (Delmonico et al. 2015), that encodes for $p16^{INK4a}$ protein. Among the malignant samples, ATM, a serine/threonine kinase that is recruited and activated by DNA double-strand breaks leading to DDR, was the most hypermethylated in lesions, followed by $p16^{INK4a}$ in blood and saliva samples (Delmonico et al. 2015). In this aspect, *CDKN2A* is a key factor in cell cycle regulation and its hypermethylation has been found associated with bladder cancer. Working as hairdressers has been associated with increased bladder cancer risk, particularly due to aromatic amines in hair dyes and oxidative hair dying, waving and bleaching products (Bolt and Golka 2007; IARC 2010). Hairdressers showed shorter telomere length in comparison with non-hairdressers in a study performed in 2016 in Sweden (Li et al. 2016). Hair waving was associated with less frequent *CDKN2A* methylation and with the shortest telomeres observed in studied group. Authors highlight that the methylation patterns found were not expected as per the literature states. Nevertheless, the high content of oxidative chemicals in the hairdressers' work environment may lead to complex and controversial results (Li et al. 2016).

Welding fumes have been categorized as possible human carcinogen into Group 2B (IARC 1990) and studies show increased risk for lung cancer in welders. Several million people worldwide are occupationally exposed to welding fumes (Antonini 2003), which means exposure to combustion-derived products, such as metal oxide particles (Leonard et al. 2010). A group of 101 Sweden male welders were recently evaluated as per oxidative stress, telomere length and DNA methylation patterns in relation to their occupational exposure (Li et al. 2015). Because telomere length is highly affected by oxidative stress, mainly 8-OHdG generation, authors measured telomere length in studied population through qPCR. They did not observe any significant difference between control and exposed groups, and similar result was found for 8-OHdG measurement through liquid chromatography (Li et al. 2015). However, every working year was associated with shorter telomeres and hypermethylation of *MGMT* in time-dependent manner, indicating a possible cumulative effect of welding fumes. Among the five investigated genes, only *APC* had higher methylation frequency in welders. The tumor suppressor *APC* gene regulates the Wnt signaling pathway, which plays an important role in cell growth regulation (Sparks et al. 1998) and has been found hypermethylated in serum and plasma of lung cancer patients (Li et al. 2015).

Human health effects ranging from neurotoxicity to cancer have been reported in cases of chronic exposure to volatile organic compounds (VOC). Exposure to VOC occurs through both environmental (motor vehicle exhaust) and industrial (solvents) sources (Masiol et al. 2014) Due to its composition, including mainly but not only benzene, toluene, ethylbenzene and xylene, VOCs are compounds hard to evaluate as per occupational exposure. Increased levels of six VOCs were found in shoe factory workers (LS group) and of two VOCs in gas station attendants (GS group) in

a recent Mexican study (Jiménez-Garza et al. 2018). In both LS and GS groups, authors observed hypermethylation of *TOP2A* promoter, a gene that encodes for TOP2A, an enzyme that catalyzes the breaking and rejoining of DNA strands, playing a critical role in DNA replication, recombination, chromosome separation and condensation, and gene transcription (Nitiss 2009). The LS group, when compared to GS and control groups, also showed *SOD1* and *TNF-α* promoter hypermethylation, demonstrating an effect of oxidative stress and inflammation. In agreement, the *GSTP1* promoter methylation frequency was correlated with both *iNOS* and *COX-2* methylation (Jiménez-Garza et al. 2018). *TNF-α, iNOS* and *COX-2* are genes associated with inflammation parameters, while *GSTP1* and *SOD1* are linked to oxidative stress response. Cellular events for cancer development have been characterized by ongoing oxidative stress that may lead to inflammation (Imbesi et al. 2013). Taken together that promoter methylation correlates negatively with gene expression, several miRNAs were identified as deregulated in another group of VOC-exposed workers. In 128 dockyards employees, over 450 and 220 miRNAs were deregulated for toluene and xylene, respectively (Song and Ryu 2015). Interestingly, the highest number of deregulated miRNAs, 695, was found for ethylbenzene among dockyards workers (Song and Ryu 2015), while in the Mexican study, both LS and GS groups had ethylbenzene levels strongly correlated with *TOP2A* methylation status (Jiménez-Garza et al. 2018).

Pesticides are one of the most xenobiotics concerns worldwide. General population can be exposed to low concentrations of those chemicals through contamination of air, water and food, while high exposures are associated with occupational exposure, such as production, packaging and application of these compounds. A majority of 39 out of 46 studies reported positive findings as regard pesticide occupational exposure and CBMN parameters (Bolognesi and Holland 2016). Three different Brazilian studies evaluated epigenetic parameters in farmworkers in soybean and tobacco fields. Soybean farmers showed increased global DNA methylation, positively correlated with MN frequency (Benedetti et al. 2018). In this same work, authors observed higher levels of DNA damage and cell death parameters through buccal micronucleus Cytome assay (BMCyt) and Comet assay, also associated with oxidized guanines (Benedetti et al. 2018). On the other hand, tobacco farmers, who are exposed to pesticides and nicotine (natural pesticide), showed global DNA hypomethylation in two studies (Kahl et al. 2018a, b), showing that the epigenetic mechanism is different for farmers working in those two crops. While acute effects of pesticides are widely known, chronic effects are still largely under speculation. For some pesticides, mechanisms such as endocrine disruption are hypothesized. Additionally, it had been suggested that health effects observed are related to specific mutagenic effects of particular pesticides (Mostafalou and Abdollahi 2017), which may explain differences between soybean and tobacco farmers. Moreover, Brazilian tobacco farmers had shorter telomeres and increased DNA damage (Kahl et al. 2018a, b), which was associated with years of exposure (Kahl et al. 2018a). Hypermethylation of tumor suppressor *p16* was positively correlated with the shortest telomeres among tobacco farmers (Kahl et al. 2018a). $p16^{INK4a}$ contributes to the p53-independent response to telomere damage (Jacobs

and De Lange 2004), suggesting that in tobacco farmers, the hypermethylation of *p16* in shortest telomeres may be a response to pesticide-induced oxidative stress (Mostafalou and Abdollahi 2017).

Global dose-dependent DNA hypermethylation was observed in Indian adults with chronic arsenic exposure, but this effect was modified by folate (Pilsner et al. 2007). Interestingly, the *MTHFR C677T* polymorphism, the most common gene in folate metabolism, influenced in both DNA damage and telomere length in tobacco farmers (Kahl et al. 2018b), suggesting that DNA methylation is dependent of methyl availability, interfering with genomic stability. Global DNA methylation in oncogenes or genes that favors apoptosis resistance argue in the addition of methionine, choline, folate and vitamin B12 as methyl donors to both prevent and limit cancer aggressiveness (Hervouet et al. 2013). Hypomethylation of *LINE-1* repetitive element was observed in a group of urban pesticide sprayers, showing differences for gender, as men had higher *LINE-1* methylation when compared to women. The exposed group was also evaluated as per level of exposure, as high- and moderate-exposure. The high-exposure subgroup had exacerbated *LINE-1* hypomethylation when compared to moderate-exposure (Benitez-Trinidad et al. 2018). Similarly, high-exposure to pesticides in a Netherland study showed differential DNA methylation patterns for 31 CpG sites annotated for 29 genes; 20 of those found in subjects with airway obstruction (van der Plaat et al. 2018), which is a common human chronic disease associated with pesticide exposure (Mostafalou and Abdollahi 2017). Those data suggest that levels of exposure are related to human response to pesticide exposure, including epigenetic modifications. Tobacco and soybean farmers were not divided according to levels of exposure, mainly because they are all exposed in long-term periods (Benedetti et al. 2018; Kahl et al. 2018a, b). Occupational long-term exposure to pesticides is associated with birth defects, reproductive problems, diabetes, respiratory diseases, amyotrophic lateral sclerosis, cognitive impairments, and cancer (Mostafalou and Abdollahi 2017). Growing progress has been made in the recognition of epigenetic modifications in pesticide-exposure approach leading to chronic diseases.

5 Discussion and Conclusion

Faisandier et al. (2011) establish that, in occupational field, the exposome is considered the network of occupational health problems, sharing components of the set of occupational exposure. This view helps building basis for coherent discussion towards the development of networks for monitoring occupational exposure situations, including its varied origins, several effects and biomarkers of exposure usefulness (Faisandier et al. 2011). Adverse health effects due to exposure have been estimated for up to 75% of global noncommunicable diseases (NCDs). Environmental exposures are ranked as top risk factors for chronic disease mortality (WHO 2010). "Environmental epigenetics" is a term that refers to how environmental exposures affect epigenetic changes (Reamon-Buettner et al. 2008). Life

experiences, habits, and the environment shape who individuals are by virtue of their impact on epigenome and health. A great example is identical twins that, although they share the same genome and are superficially phenotypically similar, they are unique individuals with definable differences. These differences result from distinct gene expression influenced by epigenetic factors. Behavior, nutrition, and exposure to toxins and pollutants are among the lifestyle factors known to be associated with epigenetic modifications (Tiffon 2018).

Environmental toxicants can alter epigenetic regulatory processes, and mediate specific mechanisms of toxicity and responses. Growing evidence suggests that at least fifteen environmental chemicals may lead directly to diseases via epigenetic mechanism-regulated gene expression changes (Hou et al. 2012). Environmental and occupational factors induce epigenetic alterations that can contribute to the onset of NCDs, of which cancer is the most prevalent. Because these epigenetic changes are small, potentially cumulative, and may develop in long-term periods, there is a difficulty in establishing a direct relationship of cause-effect among occupational factors, epigenetic changes and diseases arising (Baccarelli and Bollati 2009; Ravegnini et al. 2015). On the other hand, literature also suggests that epigenetic modifications play a major role in human complexes diseases, particularly cancer. Carcinogenicity is now considered to develop as an epigenetic disease the same as it is considering a genetic disease. There is massive understanding of the contribution of epigenetic events in the initiation, promotion and progression of different types of cancers, mainly through silencing of tumors suppressor genes and/or activation of proto-oncogenes (Jones and Baylin 2002). Importantly, DNA methylation and apoptosis resistance are characteristics of cancer cells. Proteins related to apoptosis are considered to counteract the oncogenic Wnt signaling pathway and the inactivation of this gene may increase cancer development and progression (Hervouet et al. 2013). Many cancers are characterized by global DNA hypomethylation, previously associated with chromosomal instability and, paradoxically, with both gene-specific hypo- and hypermethylation (Esteller 2008). Knowledge on heritable changes in gene expression that result from epigenetic events is of increasing relevance in the development of strategies for prevention, early diagnosis and treatment of cancer. In addition, non-genotoxic carcinogens are a group of chemical agents that are known to cause tumors without directly damaging DNA. Evidences suggest that these compounds can lead to prominent epigenomic alterations in tissues that are targets for carcinogenesis as a result of exposure (Koturbash et al. 2011).

In animal studies, several chemicals have been reported to induce transgenerational phenotypic effects (Baccarelli and Bollati 2009; Chen et al. 2016) and humans studies have also shown that males can pass their traits acquired during lifetime as regard changes in dietary intake, chemical exposure, stress, or trauma, onto their offspring (Chen et al. 2016). Transgenerational transmission of chemically-induced epigenetic modifications have been considered the probable mechanism for these effects (Baccarelli and Bollati 2009; Tran and Miyake 2017). In fact, both paternal and maternal exposure to environmental xenobiotics during gametogenesis or gestation has been shown to be responsible for the offspring's epigenome. In some cases, the potential for persistent transgenerational modification of the epigenome may also

inform on parental germ cell exposures. Exposure to toxicants during fetal life and exposure of germ cells, possibly at a specific developmental stage, can induce heritable epigenetic changes. Epigenetic mechanisms can underlie the effects of in utero and early life exposures on adult health, as these fetal exposure to epigenetically-active chemicals can lead to health effects later in life, even independently of environmental and/or occupational risk factors in adulthood (Baccarelli and Bollati 2009; Tran and Miyake 2017).

Epigenetic parameters have been currently reported as a robust tool for studying carcinogenesis of occupational settings (Ziech et al. 2010). Therefore, they represent a class of biomarkers with great potential in the identification of exposure status, damage response, and/or disease state. The incorporation of such parameters in chemical safety assessments still depends on characterization of the epigenetic alterations induced by xenobiotics (Holland 2017; Koturbash et al. 2011). Epigenetic modifications are relatively stable and influenced by environment. Exposure to different classes of xenobiotics, such as metals, organic compounds and complexes mixtures, may lead to epigenome alterations, as seen in this chapter. Experimental, clinical and epidemiological studies have increased the current knowledge of mechanisms of action by which such chemical compounds can modify gene expression. Taken together, the evidence outlined in this chapter demonstrate that epigenome can be regulated by systematic factors, i.e., in response to environmental changes (Hou et al. 2012).

Both genetic and epigenetic responses of organisms to environmental factors, including chemical exposures, influence adaptation, susceptibility to toxicity and biodiversity. In model organisms, it is established that epigenetic alterations, including changes to the methylome, can create a memory of the received signal. Thus, it is proposed that epigenetic "foot-printing" of organisms could identify classes of chemical contaminants to which they have been exposed throughout their lifetime. However, a better understanding is necessary to decide which epigenetic alterations are most informative, which can take to an effective use of epigenetic endpoints as markers of exposure. Specifically, additional studies are needed to characterize the relationship between epigenetic alterations and toxicity phenotypes, and the epigenetic-specific dose-response (Baccarelli and Bollati 2009; Hou et al. 2012) and how, ultimately, toxicant exposure affects the composition and differentiation status of cell types in a given tissue (Meehan et al. 2018).

Overall, studies of aberrant DNA methylation represented the most commonly studied epigenetic feature, followed by changes in the expression of noncoding RNAs, and finally histone modifications. Knowledge on heritable changes in gene expression that result from epigenetic events is of increasing relevance in the development of strategies for prevention, early diagnosis and treatment of cancer. Today, epigenetics is one of the most exciting fields of biological sciences, as it is involved in occupational and environmental exposures related programming and transgenerational effects. Risks arising from some NCDs, like pneumoconiosis, cancers and allergies, are predictable and preventable. Because most of epigenetic changes are also reversible, there is growing field for developing personalized preventive medicine (Hou et al. 2012; Meehan et al. 2018). Preventive strategies,

such as exposure reduction, and pharmacological, dietary and/or lifestyle interventions may take an important part in future epigenomic research. Progress in these areas will require development and adaptation of new technologies, as much as interdisciplinary research, including toxicology, bioinformatics, epigenomics and data generation (Holland 2017; Hou et al. 2012; Meehan et al. 2018). Consequently, preventive action could lead to decreasing disease morbidity and mortality arising from many occupational-related diseases that are of major public concern.

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