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Review

Epigenetic changes by per- and polyfluoroalkyl substances (PFAS)☆

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ABSTRACT

Increasing studies are examining per- and polyfluoroalkyl substances (PFAS) induced toxicity and resulting health outcomes, including epigenetic modifications (e.g., DNA methylation, histone modification, microRNA expression). We critically reviewed current evidence from human epidemiological, *in vitro*, and animal studies, including mammalian and aquatic model organisms. Epidemiological studies identified the associations between perfluorooctane sulfonate (PFOS) or perfluorooctanoic acid (PFOA) exposure and epigenetic changes in both adult populations and birth cohorts. For *in vitro* studies, various cell types including neuroblasts, preadipocytes, and hepatocytes have been employed to understand epigenetic effects of PFAS. In studies with animal models, effects of early life exposure to PFAS have been examined using rodent models, and aquatic models (e.g., zebrafish) have been more frequently used in recent years. Several studies highlighted oxidative stress as a key mediator between epigenetic modification and health effects. Collectively, previous research clearly suggest involvement of epigenetic mechanisms in PFAS induced toxicity, though these efforts have primarily focused on specific PFASs (i.e. mainly PFOS and PFOA) or endpoints (i.e. cancer). Additional studies are necessary to define specific linkages among epigenetic mechanisms and related biomarkers or phenotypical changes. In addition, future research is also needed for understudied PFAS and complex mixtures. Studies of epigenetic effects elicited by individual PFAS and mixtures are needed within an adverse outcome pathways framework, which will advance an understanding of PFAS risks to public health and the environment, and support efforts to design less hazardous chemicals.

1. Per-and polyfluoroalkyl substances (PFASs) and environmental epigenetics

Per-and polyfluoroalkyl substances (PFASs) are a family of more than 4000 fluorinated substances that are commonly used in consumer products (e.g., non-stick cookware, disposable food packaging), outdoor gear, furniture, carpets, and aqueous film forming foams used for firefighting and training activities (OECD 2018; Sunderland et al., 2019). The most studied PFAS family includes perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs). Among these, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are listed as persistent organic pollutants (POPs) in the Stockholm Convention to restrict (PFOS) or eliminate (PFOA) production and use in order to protect human health and the environment. Additionally, the United States Environmental Protection Agency (USEPA) launched the Stewardship Program and several major companies in the PFAS industry, including 3M, voluntarily agreed to reduce the amount of PFOA and related chemicals in both emissions and products (USEPA, 2018). Despite worldwide efforts to phase out these compounds, many PFASs have still been detected in humans, wildlife, and diverse environmental matrices due to their ubiquitous applications and the stable carbon-fluorine bond (Giesy and Kannan 2001; Hansen et al., 2001; Jian et al., 2018; Schulz et al., 2020), stimulating recent calls to manage PFAS as a chemical class (Kwiatkowski et al., 2020).

Several molecular initiation events and adverse health effects linked to PFAS exposure in humans have been consistently investigated, such as thyroid dysfunction, altered cholesterol levels or metabolic diseases, reproductive toxicity, neurodevelopmental

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Abbreviations

5-hmC	5-hydroxymethylcytosine
5-mC	5-methylcytosine
AOP	adverse outcome pathway
BA	blueberry anthocyanin
BDE-47	2,2′,4,4′-tetrabrominated diphenyl ether
BDNF	brain-derived neurotrophic factor
BPA	bisphenol A
CpGs	cytosine-guanine dinucleotides
DMRs	differentially methylated regions
DNMT	DNA methyltransferase
EDCs	endocrine disrupting chemicals
GD	gestational day
GSTP	glutathione-S-transferase Pi
HAT	histone acetyltransferase
HDAC	histone deacetylase
hMSC	human mesenchymal stem cell
hpf	hours post fertilization
HPLC	high pressure liquid chromatography
IGF2	insulin-like growth factor 2
LINE-1	long interspersed element 1
mEBs	mouse embryoid bodies
mESCs	mouse embryonic stems cells
miRNA	microRNA
PFAS	per-and polyfluoroalkyl substance
PFBS	perfluorobutane sulfonate
PFCA	perfluoroalkyl carboxylic acid or perfluoroalkyl carboxylate
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFSA	perfluoroalkyl sulfonic acid or perfluoroalkanesulfonate
PFUdA	perfluoroundecanoic acid
PND	postnatal day
POPs	persistent organic pollutants
PPAR	peroxisome proliferator activated receptor
ROS	reactive oxygen species
RT-qPCR	real time quantitative polymerase chain reaction
SAM	S-adenosyl-l-methionine
SGBS cell	simpson-Golabi-Behmel syndrome cell
SIRT	Sirtuins
SP1	specificity protein 1
TBT	tributyl chlorotin
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TET	ten-eleven translocation methylcytosine dioxygenase
USEPA	United States Environmental Protection Agency
ZFL cells	zebrafish liver cells

problems, kidney disease, immunotoxicity, and various cancers (Braun 2017; Chang et al., 2016; Gore et al., 2015; Kim et al., 2018; Mariussen 2012; Rappazzo et al., 2017; Stanifer et al., 2018; Vieira et al., 2013). Many of these adverse outcomes are related to the fact that PFAS can act as endocrine disrupting chemicals (EDCs). For example, altered thyroid hormone levels due to PFAS exposure during early development can lead to neurotoxic effects, because thyroid hormones are important for brain development and their deficiency during gestation can cause cognitive disorders in offspring (Mariussen 2012). Epidemiological studies also suggest a strong relationship between PFAS exposure and child and adult obesity, gestational diabetes, impaired glucose tolerance, poor semen quality, and breast cancer, all of which are related to the ability of PFAS to mimic fatty acids or to interfere with the endocrine system (Braun 2017).

In recent years, the field of environmental epigenetics has elucidated possible mechanisms explaining the association among diverse EDC exposures and long term health outcomes (Fig. 1) (Baccarelli and Bollati 2009; Ho et al., 2012). Epigenetics is the study of alterations in gene expression that occurs without changing the DNA sequence, and environmental epigenetics focuses specifically on the epigenetic changes resulting from environmental factors such as chemical exposure (Bollati and Baccarelli 2010; Ho et al., 2012). Epigenetic mechanisms are largely classified in three categories: DNA methylation, histone modification, and microRNA (miRNA) expression; however, other mechanistic aspects such as long noncoding RNAs in human disease have also received attention (Bhan and Mandal 2014). DNA methylation, or the addition of a methyl group to the 5' position of the cytosine pyrimidine ring, primarily targets cytosine-guanine dinucleotides (CpGs), which are involved in normal cellular control of gene expression. Aberrant hypermethylation, particularly in a promoter region, is typically associated with decreased gene expression or silence. Modification of histones determines whether DNA wrapped around histones can be transcribed and influences the rate of transcription. miRNAs are small noncoding RNAs that can negatively regulate their target gene expression in a post-transcriptional way. An increasing number of epidemiological, animal, and *in vitro*

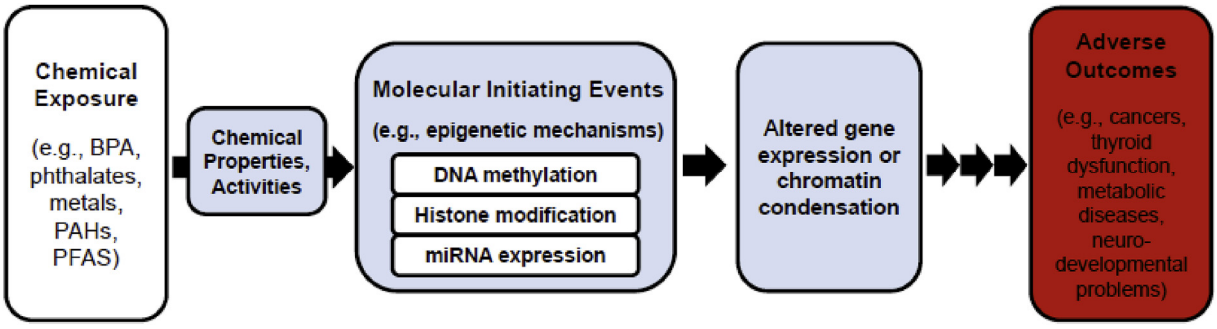

Fig. 1. Potential mechanisms linking chemical exposure to adverse outcomes mediated by epigenetic alterations as molecular initiating events. Epigenetic mechanisms include DNA methylation, histone modification, and miRNA expression.

studies have reported the effects of bisphenol A (BPA), phthalates, metals, and polycyclic aromatic hydrocarbons on epigenetic regulation and its association with health outcomes (Baccarelli and Bollati 2009; Park et al., 2017; Ruiz-Hernandez et al., 2015; Singh and Li 2012). Because induction of epigenetic alteration can be characteristic of carcinogens, studies examining carcinogenicity of PFAS have also been reviewed elsewhere (Temkin et al., 2020).

Here, we critically reviewed the available literature on the epigenetic alterations by PFAS from human epidemiological studies, and experimentation using *in vitro*, mammalian, and aquatic species models. Literature searches (e.g., with PubMed, Google Scholar) were performed with various combinations of keywords (mainly "compound name", "epigenetic", or specific mechanism such as "DNA methylation") between April and October 2020. Twenty four PFAS, selected based on US Environmental Protection Agency Method 537, were examined during our searches, which did not include emerging PFAS (e.g., phosphinic acids) and PFAS alternatives (e.g., GenX).

2. Epigenetic effects of PFASs

2.1. Human epidemiological studies

In recent years, an increasing number of human epidemiological studies have examined associations among PFAS exposure and epigenetic changes in adults or mother-infant pairs in the USA, Europe, and Asia, where PFOS and PFOA have been predominantly studied (Table 1). However, most research has focused on DNA methylation, which indicates a research gap for other epigenetic mechanisms. For example, global or gene specific methylation analysis was widespread in previous studies (Guerrero-Preston et al., 2010; Kobayashi et al., 2017; Leter et al., 2014; Liu et al., 2018; Watkins et al., 2014), while other more recent efforts performed genome-wide analysis using Illumina Infinium platforms (Kingsley et al., 2017; Leung et al., 2018; Miura et al., 2018; van den Dungen et al., 2017a) as omics technology such as DNA sequencing has advanced. Moreover, it should be noted that very limited types of genes or elements (e.g., LINE-1) related to global methylation or imprinting have been examined in non-genome wide analysis. To understand the mechanism(s) of action in human diseases, more specific approaches with a robust research hypothesis and/or omics techniques are necessary in the future. One study of miRNA expression alteration found an inverse association between PFOA exposure and elevated miR-26 b and miR-199a-3p levels in the blood of Chinese adults; herein, a significant inverse association of PFOA with decreased high-density lipoprotein cholesterol was only observed in occupationally exposed workers (Wang et al., 2012). It is known that miR-26 b functions as a tumor suppressor while miR-199a-3p regulates cell proliferation and survival (Shatseva et al., 2011; Tsai et al., 2016).

Among the studied PFAS thus far, significant epigenetic alterations have only been reported for PFOS and PFOA in both adult and birth cohort studies (Table 1). In addition to most toxicological work focusing on PFOS and PFOA to date, this may imply the strong effect of these compounds on the epigenetic modification process, or may have merely resulted from the relatively higher contamination levels of PFOS and PFOA in humans. For example, in one study of 685 American adults, the geometric means of PFOS and PFOA concentrations for blood samples collected at enrollment in the study (2005–2006) and at follow-up (2010) were 14.1 (PFOS) and 57.9 ng/mL (PFOA), respectively, whereas the mean concentrations of perfluorononanoic acid (PFNA) was 1.4 and perfluorohexane sulfonate (PFHxS) was 2.6 ng/mL (Watkins et al., 2014). In the Watkins et al. (2014) study, PFOS exposure was significantly associated with increased methylation of long interspersed element 1 (LINE-1) in American adults (Watkins et al., 2014). A repetitive element, LINE-1 is one of the most commonly used surrogate markers of global DNA methylation, along with Alu (Yang et al., 2004). The methylation of LINE-1 is also related to genome instability, and has been used as a biological indicator for cancers, cardiovascular diseases, and neurodegenerative diseases (Kazazian Jr and Goodier 2002; Pogribny and Beland 2009). While methylation levels of LINE-1 or Alu were commonly analyzed via a bisulfite pyrosequencing assay to predict global methylation levels (Kobayashi et al., 2017; Leter et al., 2014; Liu et al., 2018; Watkins et al., 2014), several studies measured 5-methylcytosine (5-mC) levels through enzyme-linked immunosorbent assay (ELISA)-like methods (Guerrero-Preston et al., 2010) or flow cytometric immunodetection on a cell-by-cell basis (Leter et al., 2014). In another study of 34 Dutch men aging from 40 to 70 years old, PFOS exposure was associated with differentially methylated regions (DMRs) of multiple genes, including cancer or immune function related genes, similar to the observations for other POPs such as dioxins and polychlorobiphenyls (van den Dungen et al., 2017a).

As in adult studies, several significant associations of PFOS and PFOA have been reported in studies of birth cohorts. PFOA exposure was inversely associated with global DNA methylation (Guerrero-Preston et al., 2010), methylation of 7 CpG sites across three genes (Kingsley et al., 2017), and methylation of imprinted gene *insulin-like growth factor 2* (*IGF2*) in a Japanese study (Kobayashi et al., 2017). In a genome-wide analysis, exposure to a combination of PFOS and PFOA resulted in multiple DMRs and differentially methylated positions (DMPs) (Miura et al., 2018). Another birth cohort study found that PFOS was linked to increased methylation of 10,598 CpG sites, though only in males, and the significant CpG sites were associated with genes related to cancer (Leung et al., 2018). The authors found that the top common function affected by PFOS exposure was nervous system development and function (Leung et al., 2018). PFOS was also significantly associated with hypomethylation of Alu, a global DNA methylation indicator (Liu et al., 2018). It appears that the direction of association of PFOS is likely to depend on the sample matrix or country. For example, serum PFOS was positively associated with LINE-1 methylation of blood from the USA (Watkins et al., 2014), but not significantly associated with the methylation level measured in semen in European countries (Leter et al., 2014) or in cord blood from Japan (Kobayashi et al., 2017).

Though these previous studies suggest possible association between PFAS exposure and epigenetic changes in humans, only limited PFAS measurements were used for analysis and significant associations were generally found for PFOS and PFOA at higher exposure levels. Even in studies examining multiple PFAS (Leter et al., 2014; Liu et al., 2018; Watkins et al., 2014), multicollinearity or multiple testing issues were rarely considered in statistical analyses, which may lead to false positive results. As an example of the effort to avoid this scenario, our group recently applied a multiple-POP model using principal component analysis in order to examine potential associations among exposure to multiple POPs and promoter methylation of placental genes in Korean mother-child pairs (Kim et al., 2019). Along with various epigenetic markers, similar potential relationships with other PFAS should be investigated using this multipollutant approach.

2.2. In vitro studies

Various cell types originating from humans, mice, and zebrafish have been used to examine the *in vitro* effects of PFAS (Table 2). These cells include neuroblasts, hepatocytes, preadipocyte fibroblasts, trophoblasts, and embryonic stem cells (Bastos Sales et al., 2013; Blanc et al., 2019; Guo et al., 2017; Li et al., 2015; Ma et al.,

Table 1
Previous human epidemiological studies examining the effects of PFAS exposure on epigenetic alterations.

Country	Subjects	PFAS exposure		Epigenetic studies				Reference
		Matrix	Compound(s)	Matrix	Epigenetic mechanism	Method	Major findings	
Adult Study								
USA	671 adults	Blood	PFHxS, PFOS, PFOA, PFNA,	Blood leukocytes	Global DNA methylation (LINE-1)	Bisulfite pyrosequencing	PFOS increased LINE-1 methylation	Watkins et al. (2014)
Netherlands	34 adult males	Blood	PFOS	Blood	Genome-wide DNA methylation	Illumina Infinium Methylation	PFOS resulted in 29 DMPs and 38 DMRs	van den Dungen et al. (2017a)
Greenland, Poland, and Ukraine	262 adult males	Blood	PFHxS, PFOS, PFOA, PFNA	Semen	Global DNA methylation (Alu, LINE-1, Satα repeats)	Bisulfite pyrosequencing	No significant association.	Leter et al. (2014)
					Global DNA methylation	Flow cytometric immunodetection		
China	10 occupational workers and 10 residents	Serum	PFOA	Serum	miRNA expression	miRNA microarray and PCR	PFOA increased miR-26 b and miR-199a-3p levels	Wang et al. (2012)
Birth Cohort								
USA	30 mother-infant pairs	Cord Blood[a]	PFOS, PFOA	Cord Blood[a]	Global DNA methylation	ELISA-like method (commercial kit)	PFOA decreased global methylation	Guerrero-Preston et al. (2010)
USA	44 mother-infant pairs	Maternal Serum (~16 weeks of pregnancy)	PFOA	Cord Blood[a]	Genome-wide DNA methylation	Illumina Infinium Methylation	PFOA decreased methylation of seven CpG sites located in three genes: *RAS P21 Protein Activator 3 (RASA3), Opioid Receptor Delta 1 (OPRD1), and Uridine-cytidine kinase 1 (UCK1)*	Kingsley et al. (2017)
Faroese Islands (Denmark)	72 mother-infant pairs	Cord Blood[a]	PFOS	Cord Blood[a]	Genome-wide DNA methylation	Illumina Infinium Methylation	PFOS resulted in differential methylation of 10,598 CpG sites in males	Leung et al. (2018)
Japan	190 mother-infant pairs	Maternal Serum (24–41 weeks of pregnancy)	PFOS, PFOA	Cord Blood[a]	Genome-wide DNA methylation	Illumina Infinium Methylation	PFOS and PFOA resulted in four replicated DMPs and eight DMRs	Miura et al. (2018)
Japan	177 mother-infant pairs	Maternal Serum (24–41 weeks of pregnancy)	PFOS, PFOA	Cord Blood[a]	Global DNA methylation (LINE-1)	Bisulfite pyrosequencing	No significant association	Kobayashi et al. (2017)
					Gene specific DNA methylation (*IGF2*, *H19*)	Bisulfite pyrosequencing	PFOA decreased *IGF2* methylation	
Taiwan	363 mother-infant pairs	Cord Blood[a]	PFOS, PFOA, PFNA, PFUdA	Cord Blood[a]	Global DNA methylation (LINE-1, Alu)	Bisulfite pyrosequencing	PFOS decreased Alu methylation	Liu et al. (2018)

[a] Cord blood samples were collected at delivery or immediately after birth.

Table 2
Previous *in vitro* studies examining the effects of PFAS exposure on epigenetic alterations.

Cell type	Compound	Experimental Duration	Experimental Concentration (μM)	Epigenetic mechanism	Methods	Observations	Reference
Human origin							
SK-N-SH (human neuroblastoma) cells	PFOS	48 h	50, 100, and 150 μM	Global DNA methylation (mRNA and protein expression of DNMTs)	RT-qPCR and western blotting	Decreased both gene and protein expression of DNMT1; increased both gene and protein expression of DNMT3b; decreased protein level of DNMT3a	Guo et al. (2017)
				Gene specific DNA methylation (BDNF promotor I and IV)	Bisulfite sequencing PCR	Altered methylation status of BDNF promoter I and IV; hypermethylation in the twentieth CpG site within promoter IV	
				microRNA expression (miR-16, miR-22, and miR-30a-5p)	RT-qPCR	Increased miR-16, miR-22, and miR-30a-5p expression	
SH-SY5Y (human neuroblastoma) cells	PFOS	48 h	10, 50, and 100 μM	microRNA expression	RT-qPCR	Decreased miR-16 expression and increased miR-22 expression	Li et al. (2015)
SK-N-AS (human neuroblastoma) cells	PFOS and PFOA	72 h	10 μM	Global DNA methylation	HPLC	No significant change	Bastos Sales et al. (2013)
HTR-8/Sv neo (first trimester human trophoblast) cells	PFOS	24 h and 48 h	1, 10, and 50 μM	Global DNA methylation	ELISA-like method (commercial kit)	Decreased global methylation at 48 h	Sonkar et al. (2019)
				Global DNA methylation (mRNA and protein expression of DNMTs)	RT-qPCR and western blotting	Decreased mRNA levels of *DNMT1*, *DNMT3a*, and *DNMT3b* at 24 h and decreased protein levels of DNMT1, DNMT3a, and DNMT3b at 48 h	
				microRNA expression (miR29-b)	RT-qPCR	Increased miR29-b expression	
				Global acetylation (protein lysine acetylation)	Western blotting	Increased global acetylation of protein at 48 h	
				Histone acetylation (mRNA and protein expression of SIRTs)	RT-qPCR and western blotting	Decreased mRNA levels of *SIRT1* and *SIRT3* at 24 h and decreased protein levels of SIRT1 and SIRT3 at 48 h	
L02 (human liver) cells	PFOA	72 h	12.1, 24.2, 60.4, 121, or 242 μM (=5, 10, 25, 50, or 100 mg/L)	Global DNA methylation	LC/ESI-MS	No significant change	Tian et al. (2012)
				Global DNA methylation (mRNA expression of *DNMT1, DNMT3a, DNMT3b*)	RT-qPCR	Increased mRNA expression of *DNMT3a*	
				Gene specific DNA methylation (GSTP, *p16*, *Erα*, and *PRB*)	Methylation-specific PCR	Higher methylation of *GSTP* at higher treatment groups; no effects on the promoter gene methylation of *p16, Erα, and PRB*	
				Gene specific DNA methylation (*GSTP* and *p16* promoter)	Bisulfite sequencing PCR	Increased methylation of *GSTP* promoter in a dose-dependent manner at the SP1 binding site	
hMSCs (human mesenchymal stem cells) differentiating into adipocytes	PFOS	10 d	10 μM	Genome-wide DNA methylation	Illumina Infinium HumanMethylation450 BeadChip	PFOS exposure resulted in DMRs in 2 adipogeneic genes (AXIN1 and DKK1) and 45 potential DMPs including a position in DKK1 and 15 positions in intergenic regions; caused hypomethylation of the majority of the positions. No clear association of DMRs in adipogenesis was found in pathway analysis	van den Dungen et al. (2017b)
N2A (murine neuroblastoma) cells	PFOS and PFOA	48 h	10 μM	Global DNA methylation	HPLC	No significant change	Bastos Sales et al. (2013)
	PFOA	48 h		Global DNA methylation		Decreased global methylation	Ma et al. (2018)

(*continued on next page*)

Table 2 (*continued*)

Cell type	Compound	Experimental Duration	Experimental Concentration (μM)	Epigenetic mechanism	Methods	Observations	Reference
3T3-L1 (murine preadipocyte fibroblast) cells			0.024, 0.24, 2.4, 24, and 240 μM (=0.01, 0.1, 1, 10, and 100 mg/L)		ELISA-like method (commercial kit)		
				Global DNA methylation (mRNA expression of DNMT gene)	RT-qPCR	Increased mRNA expression of Dnmt1, Dnmt3a and Dnmt3b	
				Gene specific DNA methylation (PPARγ promoter)	Bisulfite sequencing PCR	Decreased methylation of PPARγ promoter	
mESCs (mouse embryonic stem cells)	PFOS	24 h	0.2, 2, 20, and 200 μM	microRNA expression	RT-qPCR	Increased miR-145 and miR-490−3p expression in a dose dependent manner	Xu et al. (2013)
mEBs (mouse embryoid bodies)	PFOS	6 d	0.2, 2, 20, and 200 μM	microRNA expression	RT-qPCR	Decreased miR-134, miR-145, and miR-490−3p expression	Xu et al. (2015)
ZFL (zebrafish liver) cells	PFBS and PFOS	48 h	23 μM (PFBS) or 93 μM (PFOS)	Global DNA methylation	LUMA assay	Only PFOS increased global methylation	Blanc et al. (2019)
				Global DNA methylation (*dnmt1*, *dnmt3aa*, *dnmt3ab*, and *dnmt3ba*), histone deacetylation (*hdac1*, and *hdac3*), and histone demethylation (*kdm5ba*)	RT-qPCR	PFBS decreased mRNA expression of *dnmt1*, *dnmt3aa*, *dnmt3ab*, *dnmt3ba*, *hdac1*, and *hdac3*; PFOS decreased *kdm5ba* expression	

2018; Sonkar et al., 2019; Tian et al., 2012; van den Dungen et al., 2017b; Xu et al., 2013; Xu et al., 2015). The majority of the *in vitro* studies targeted PFOS or PFOA. Blanc et al. (2019) tested the effects of perfluorobutane sulfonate (PFBS) on global DNA methylation of ZFL (zebrafish liver) cells and although no significant change in DNA methylation level was observed, PFBS altered the transcription of genes related to DNA methylation and histone modification, which suggests early molecular events induced by exposure may have influenced subsequent epigenetic changes.

Human (SK-N-SH, SH-SY5Y, and SK-N-AS) and murine neuroblast (N2A) cells were used in previous studies to examine the role of epigenetics in PFAS induced neurotoxicity (Bastos Sales et al., 2013; Guo et al., 2017; Li et al., 2015). For example, in human SK-N-SH cells, a 48 h PFOS exposure period significantly decreased the mRNA expression and protein levels of brain-derived neurotrophic factor (BNDF), altered the methylation of BDNF promotor I and IV, and increased the expression of BDNF-related-miRNAs such as miR-16, miR-22, and miR-30a-5p (Guo et al., 2017). These observations suggest the epigenetic involvement of the BDNF gene in the PFOS-induced neurotoxic pathway (Guo et al., 2017). BDNF plays an important role in survival and differentiation within the central nervous system and regulates learning and memory in adults (Cunha et al., 2010). Similar to these findings by Guo et al. (2017), an increased expression of miR-22 was also observed in SK-N-SH cells exposed to a similar concentration of PFOS, though decreased miR-16 expression was reported (Li et al., 2015).

Because DNA methylation during adipocyte differentiation is dynamic (Sakamoto et al., 2008), several studies have been conducted to test whether environmental chemicals affect adipocyte differentiation and lipid formation in order to explore to what extent DNA methylation may serve as an underlying mechanism. However, effects of PFAS on adipocytes vary greatly depending on the study; thus, potential associations with epigenetic markers seems unclear so far. Among the tested EDCs, Bastos Sales et al. (2013) found that neither PFOS nor PFOA induced significant effects on global DNA methylation in two neuroblastoma cell lines (i.e. SK-N-AS and N2A) or adipocyte differentiation of 3T3-L1 preadipocytes. PFOS ($\geq$10 μM) reduced lipid accumulation in two differentiating human adipocytes, specifically human mesenchymal stem cells (hMSCs) and Simpson-Golabi-Behmel syndrome (SGBS) cells (van den Dungen et al., 2017b). In the van den Dungen et al. (2017b) study, out of a total of seven selected POPs, PFOS, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and tributyl chlorotin (TBT) interfered with adipocyte differentiation and DNA methylation patterns in adipogenic and other genes (van den Dungen et al., 2017b), implicating that these compounds can induce differential DNA methylation in human differentiating adipocytes, even though gene expression profiles of adipogenic genes varied. Regarding the relationship between PFOA and adipocyte differentiation, Ma et al. (2018) reported that PFOA exposure caused promoter demethylation and up-regulated gene expression of peroxisome proliferator activated receptor gamma (PPARγ) in 3T3-L1 cells where preadipocyte proliferation and differentiation were induced, which may partially explain obesity development by PFOA exposure.

Several researchers have studied the influence of PFAS on epigenetic mechanisms in liver cells (Blanc et al., 2019; Tian et al., 2012). In human liver L02 cells exposed to PFOA, a dose-dependent increase in methylation of the promoter of detoxification enzyme glutathione-S-transferase Pi (GSTP) was observed at the transcription factor specificity protein 1 (SP1) binding site, indicating the possible involvement of aberrant DNA methylation in PFOA-mediated toxicity (Tian et al., 2012). Although most of the *in vitro* studies in Table 2 used mammalian cells, Blanc et al. (2019) measured epigenetic effects of several environmental chemicals in

both zebrafish embryos and ZFL cells. Among their study chemicals, which included two PFASs (PFOS and PFBS), bisphenols, methoxychlor, permethrin, vinclozolin and coumarin 47, only PFOS exposure significantly increased global DNA methylation in ZFL cells (Blanc et al., 2019). PFOS also significantly affected the expression of histone demethylase *kdm5ba* in both ZFL cells and zebrafish embryos (Blanc et al., 2019), suggesting *kdm5ba* as a sensitive biomarker in chemical induced epigenetic changes. Although these two studies showed the clear association between PFAS exposure and epigenetic modifications in liver, its relationship with specific health outcomes or diseases were not investigated.

To examine the effects of PFOS exposure on embryonic development, previous studies have used mouse embryoid bodies (mEBs) and embryonic stems cells (mESCs) (Xu et al., 2013, 2015). In both of these studies, PFOS exposure altered the expression levels of pluripotency factors such as Oct4, Sox2, and Nanog, and changed expression of miR-134, -145, and -490−3p (Xu et al., 2013, 2015). Additionally, Xu et al. (2015) reported an increase in mRNA and protein expression of polycomb group family members Cbx4, Cbx7, and Ezh2 following to PFOS exposure, which may cause a decrease in differentiation factors. Although the directions of the changes in miRNA and pluripotency factor expression differed between the two mouse embryonic cells (Xu et al., 2013, 2015), it seems clear that miRNA expression can modulate these disrupting effects of PFOS on expression of pluripotency factors combined with the regulating effects of these miRNAs on pluripotency markers, which could result in developmental toxicity (Tay et al., 2009; Xu et al., 2009; 2013).

The relationship of PFOS-induced oxidative stress via epigenetic regulation with pregnancy complications such as preeclampsia was studied using first trimester human trophoblast HTR-8/Sv neo cells by Sonkar et al. (2019). PFOS, which induced reactive oxygen species (ROS) generation, clearly decreased the gene and protein expression of DNA methyltransferases (DNMTs; DNMT1, 3A, and 3B) and histone deacetylase sirtuins (SIRTs; SIRT1 and 3) in placental cells (Sonkar et al., 2019). Through a knockdown study, these researchers suggested that miR-29 b expression regulates global DNA methylation and protein acetylation can result in ROS generation in the PFOS exposed placenta, which may lead to adverse pregnancy complications (Sonkar et al., 2019).

Because epigenetic control and gene expression patterns can be tissue or species specific, it is not surprising that results from different *in vitro* studies contradicted each other regarding effects of PFAS on global methylation. Several studies with human cells including neuroblastoma cells (Guo et al., 2017), trophoblast cells (Sonkar et al., 2019), and mesenchymal stem cells differentiating into adipocytes (van den Dungen et al., 2017b) reported decreased DNA methylation as a result of PFOS exposure, whereas ZFL cells showed significantly increased global DNA methylation (Blanc et al., 2019). Though PFOA did not significantly alter human liver L02 cells (Tian et al., 2012) or human and murine neuroblastoma cells (Bastos Sales et al., 2013), a significant decrease in global DNA methylation along with increased expression of DNMT genes was observed in 3T3-L1 murine preadipocytes (Ma et al., 2018). Several mammalian cell studies measured mRNA and/or protein expression of DNMT genes such as DNMT1, DNMT3A, and DNMT3B (Blanc et al., 2019; Guo et al., 2017; Ma et al., 2018; Sonkar et al., 2019; Tian et al., 2012). DNMTs methylate cytosine at the C_5 position with a methyl donor S-adenosyl-l-methionine (SAM), and there are five known isoforms of DNMTs in mammals including DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L (Hermann et al., 2004). Among them, DNMT1 functions as a major maintenance methyltransferase while DNMT3A and DNMT3B are involved in de novo methylation, playing pivotal roles in normal development and disease (Okano et al., 1999). As shown in Table 2, previous *in vitro* studies reported decreased DNMT1 and DNMT3A gene expression elicited by PFOS exposure (Guo et al., 2017; Sonkar et al., 2019) and increased expression caused by PFOA (Ma et al., 2018; Tian et al., 2012). In contrast, no significant changes in the expression of DNMTs was found in ZFL cells exposed to PFOS (Blanc et al., 2019). Unlike most of the epidemiological studies, which focused on DNA methylation (Table 1), several *in vitro* studies examined effects of PFOS exposure on miRNA expression profiles (Guo et al., 2017; Li et al., 2015; Sonkar et al., 2019; Xu et al., 2013, 2015).

2.3. Mammalian studies

As summarized in Table 3, the majority of mammalian studies have examined effects of PFOS on DNA methylation patterns or miRNA profiles (Dong et al., 2016; Tian et al., 2019; Wan et al., 2010; Wang et al., 2015). A few studies reported PFOA-induced changes on DNA methylation (Wen et al., 2020) or histone modification (Li et al., 2019). Several animal studies have conducted either early life stage or *in utero* exposure to PFOS or PFOA, and focused on adverse outcomes in offspring resulting from multigenerational epigenetic alterations (Li et al., 2019; Tian et al., 2019; Wan et al., 2010; Wang et al., 2015). Many previous papers examined PFAS effects in liver, primarily focusing on understanding the epigenetic mechanism of liver cancer (Dong et al., 2016; Li et al., 2019; Wan et al., 2010; Wang et al., 2015) or of hepatic steatosis (Wen et al., 2020). One study by Tian et al. (2019) focused on the testes, and researched the epigenetic mechanisms causing reproductive toxicity induced by PFOS exposure.

In livers of male adult rats treated with PFOS for 28 days, microarray analysis showed that several genes related to the PPARα pathway and thyroid hormone regulation were affected, including the CAR/PXR pathway, phase II/III enzymes, and deiodinase type 1 and 3 (Dong et al., 2016). Though expression of 38 hepatic miRNAs was significantly altered, the fold changes were relatively subtle. These findings included up-regulation of miR-200a-3p and miR-200 b-3p and down-regulation of miR-21−5p and miR-122−5p (Dong et al., 2016). The pathway analysis of miRNAs and gene expression found the involvement of the miRNAs in PFOS-induced hepatotoxicity, and the role of miRNA regulation of epithelial-to-mesenchymal transition in liver cancer (Dong et al., 2016).

Following *in utero* exposure to PFOS in the Sprague-Dawley rat model, Wan et al. (2010) measured promoter methylation of two tumor suppressor genes, GSTP and p16, which are sensitive biomarkers in liver carcinogenesis. In this previous study, the researchers found increased GSTP promoter methylation at several critical sites (+79, +81, and +84) and no significant alteration in p16 promoter methylation in livers of postnatal weaned offspring at day 21. The observed increased GSTP promoter methylation combined with decreased global methylation (observed through both the ELISA and the LINE-1 methylation assays) supported the potential effect of PFOS exposure on hepatocarcinogenesis (Wan et al., 2010).

Similarly, Wang et al. (2015) investigated changes in miRNA expression induced by PFOS in prenatal and neonatal Albino Wistar rat liver in order to understand the underlying mechanism of cancer initiation and tumorigenesis (Wang et al., 2015). After exposing pregnant female rats to 3.2 mg/kg of PFOS from gestational day 1 (GD 1) to postnatal day 7 (PND 7), miRNAs were isolated from rat livers on both PND 1 and PND 7; several oncogenic and tumor-suppressing miRNAs, including miR-125a and miR-494, aberrantly expressed in the offspring rat livers, and some inconsistent directions of alteration were observed between those two time points (Wang et al., 2015). Based on the results in the miRNA expression profile, the authors proposed a molecular pathway of PFOS-induced carcinogenesis involving direct and indirect

Table 3
Previous mammalian studies examining the effects of PFAS exposure on epigenetic alterations.

Species	Sex and Age	Experimental Duration	Compound	Experimental Concentration	Target Tissue	Epigenetic Mechanism	Methods	Observations	Reference
Sprague-Dawley rats	Male adults	28 d	PFOS	50 mg/kg diet (oral)	Liver	miRNA expression	miRNA microarray analysis (results confirmed by RT-qPCR)	Resulted in altered expression of 38 miRNAs; three members of the miR-200 family were the most increased while miR-122−5p and miR-21−5p were the most decreased	Dong et al. (2016)
CD-1 mice	Female adults (30 d old)	10 d	PFOA	1, 5, 10 and 20 mg/kg/d dissolved in water (oral)	Liver	Global DNA methylation	ELISA-like method (5-mC level; commercial kit)	Decreased global methylation at 5, 10, and 20 mg/kg/d in a dose dependent manner	Wen et al. (2020)
						Global DNA methylation (*Dnmt1, Dnmt3a, Dnmt3b; Tet1, Tet2, Tet3*)	RT-qPCR	Decreased *Dnmt3a* expression at 10 and 20 mg/kg/d; increased *Tet1* expression at 5, 10, and 20 mg/kg/d; PFOA had a dose dependent effect on both *Dnmt3a* and *Tet1*	
Sprague-Dawley rats	Pregnant female	GD 2-21	PFOS	0.1, 0.6, and 2.0 mg/kg/d (gavage)	Livers from weaned offspring rats (PND 0 and PND 21)	Global DNA methylation	ELISA-like method (commercial kit)	Decreased global methylation at 2 mg/kg/d	Wan et al. (2010)
						Global DNA methylation (LINE-1 regulatory region)	Combined bisulfite restriction analysis (COBRA)	Decreased LINE-1 regulatory region methylation at 2 mg/kg/d	
						Global DNA methylation (Dnmt1, Dnmt3a, Dnmt3b)	RT-qPCR and western blotting	Increased Dnmt3a expression	
						Gene specific DNA methylation (GSTP and p16 promoter)	Combined bisulfite restriction analysis (COBRA)	Increased GSTP promoter methylation; no significant changes in p16 promoter methylation	
Albino Wistar rats	Pregnant female	GD 1-PND 7	PFOS	3.2 mg/kg rodent chow or feed (oral)	Livers from weaned offspring rats (PND 1 and PND 7)	miRNA expression	miRNA microarray analysis (results confirmed by RT-qPCR)	PFOS resulted in altered expression of 46 miRNAs on PND 1 and 9 miRNAs on PND 7; four miRNAs (miR-125a-3p, miR-23a, miR-25, and miR-494) were aberrantly expressed in rat liver on both PND 1 and PND 7.	Wang et al. (2015)
Sprague-Dawley rats	Neonatal F0 (PND 5)	PND 6-60	PFOS	F0 exposure: 5 mg/kg/d PFOS (oral), 5 mg/kg/d PFOS (oral) + 16 mg/kg/d SAM (ip), 5 mg/kg/d PFOS (oral) + 0.15 mg/kg/d DAC (ip)	Testes from weaned male offspring rats on PND 60 (F1; mature without drug treatment)	Global DNA methylation (DNMT1, DNMT3a, DNMT3b)	cDNA microarray	PFOS increased *Dnmt3a* mRNA expression in all exposure groups but did not change *Dnmt1* and *Dnmt3b* expression. mRNA level of *Dnmt3a* was increased solely in the PFOS only group.	Tian et al. (2019)
Kunming mice	Pregnant female	GD 0-17	PFOA	1, 2.5, 5, and 10 mg/kg (gavage)	Livers from weaned female offspring mice (PND 21)	Histone modification	HAT and HDAC activity/inhibition assay	PFOA significantly reduced HAT activity and increased HDAC activity	Li et al. (2019)
						Histone modification	Western blot analysis	PFOA significantly decreased protein expression of anti-acetyl-histone H3 (H3Ac) and acetyl-histone H4 (H4Ac) in a dose dependent manner	

regulation of cancer development through target genes. Additionally, the finding that more miRNAs were altered on PND 1 (46 miRNAs) than on PND 7 (9 miRNAs) suggested that prenatal rat liver can be more sensitive to PFOS exposure than that of neonatal rats (Wang et al., 2015).

In order to evaluate the role of methylation in PFOS-induced reproductive toxicity, three groups of Sprague Dawley rats (F0 generation) were administered 5 mg/kg/d PFOS, 5 mg/kg/d PFOS + 16 mg/kg/d SAM (a methyl donor), or 5 mg/kg/d PFOS + 0.15 mg/kg/d decitabine (DAC, a hypomethylating agent) from PND 6 to PND 60 (Tian et al., 2019). Development and fertility of offspring (F1) was then monitored after rodents were normally fed for two months without PFOS exposure. The F1 female rats were not pregnant in the F0 PFOS group and the F0 PFOS + DAC group whereas the F1 females in the F0 PFOS + SAM group were pregnant. While cDNA microarray analysis revealed that the *DNMT3a* gene in F1 testes was statistically enriched in all F0 PFOS exposure groups, the mRNA level of *DNMT3a* was increased in the F0 PFOS only group, but not in the F0 PFOS + SAM or F0 PFOS + DAC groups, indicating that PFOS might affect the regulation of *Dnmt3a* gene expression. This evidence, along with the findings in spermatogonia stem cells in F1 testis of the F0 PFOS + SAM group regarding gene expression and proliferation, suggested that methyl donor SAM may provide a self-protection mechanism to infertility induced by parental PFOS exposure through DNA methylation events (Tian et al., 2019).

Similar to the epidemiological and *in vitro* studies reviewed above, most of the previous mammalian work has focused on PFOS and PFOA. In a study by Wen et al. (2020), PFOA exposure in adult female mice resulted in global hypomethylation, decreased *Dnmt3a* expression, and increased *Ten-eleven translocation methylcytosine dioxygenase 1* (*Tet1*) expression in the livers (Wen et al., 2020). Like DNMTs, TETs also play an important role in the maintenance of DNA methylation status by causing demethylation in CpG islands. Although the authors found hepatocellular hypertrophy and increased lipid deposits, which may indicate fatty liver disease, a direct association with an epigenetic mechanism was not clearly revealed.

Liver toxicity in female offspring of Kunming mice following maternal exposure to PFOA was studied by Li et al. (2019). Decreased body weight and survival rate, morphological changes in the liver, and increased serum levels of oxidative stress and liver cell damage markers exhibited the effects of PFOA exposure during pregnancy on the growth, development, liver function of the pups. In addition, the maternal PFOA exposure significantly reduced histone acetyltransferase (HAT) activity, increased histone deacetylase (HDAC) activity in the livers of female offspring mice (Li et al., 2019). This study found that PFOA induced oxidative stress can lead to elevated HDAC activity, which inhibits HAT. Additionally, Li et al. (2019) suggested increased HDAC activity may aggravate downstream fatty acid metabolism disorders through activation of the PPARα pathway based on changes in oxidative stress markers, histone modification markers, and expression of PPARα mRNA and downstream related genes in the offspring liver.

Due to the large variation in tested species and target tissues, there are a very limited number of comparable results between *in vitro* (Table 2) and *in vivo* studies (Table 3). For example, an *in vitro* study in which L02 human liver cells were exposed to PFOA did not show significant alteration in global DNA methylation, but exhibited increased mRNA expression of *DNMT3a* (Tian et al., 2012). However, the livers of adult CD-1 mice showed a dose-dependent decrease in 5-mC level, indicating hypomethylation, along with decreased expression of the *Dnmt3a* gene after exposure to PFOA for 10 days (Wen et al., 2020). Many more studies with varied experimental designs are needed to confirm and reveal precisely how various PFAS induce epigenetic change. Although previous mammalian studies have reported developmental, reproductive, or multigenerational effects of PFOS and PFOA, a very limited set of adverse outcomes potentially associated with epigenetic changes were investigated, such as hepatotoxicity or liver cancer (Table 3). Future studies are needed to examine other PFASs and more diverse endpoints, including endocrine disruption and modulation, neurotoxicity, and metabolic disease, which have been related to PFAS exposure.

2.4. Aquatic species

In recent years, aquatic organisms, particularly fish models, have been used more frequently in human health focused toxicology studies, including epigenetic efforts, because they are cost- and time-effective vertebrate alternatives to using mammalian models, while often accounting for animal welfare concerns (Brooks et al., 2020). For example, DNA methylation and histone modification have lately been studied in adult and embryonic zebrafish exposed to various environmental compounds such as BPA, metals, dioxins, polychlorinated biphenyls, benzo(a)pyrene, and tris (1, 3-dichloro-2-propyl) phosphate (Aluru et al., 2015, 2018; Bouwmeester et al., 2016; Corrales et al., 2014; Laing et al., 2016; Olsvik et al., 2014; Santangeli et al., 2016; Volz et al., 2016). Kamstra et al. (2015) previously reviewed the usefulness of zebrafish in epigenetic studies and reported that tissue distribution of 5-mC or 5-hydroxymethylcytosine (5-hmC) as well as basic DNA (de)methylation mechanisms are highly conserved between zebrafish and mammalian models.

A few studies have examined epigenetic changes induced by PFBS, PFOS, and PFOA (Table 4) in zebrafish (Blanc et al., 2019; Bouwmeester et al., 2016; Zhang et al., 2011), in addition to PFAS studies with other aquatic species (e.g., planaria and sea urchins) (Ding et al., 2015; Zhang et al., 2019). The majority of aquatic studies examined DNA methylation, while one study reported the miRNA profile after PFOS exposure (Zhang et al., 2011).

Bouwmeester et al. (2016) individually exposed zebrafish embryos to sublethal concentrations of eleven compounds (including PFOA) from 0 to 72 h post fertilization (hpf) to assess the applicability of the zebrafish embryo as a screening model for DNA methylation studies. While global DNA methylation was not significantly affected after exposure to the environmental compounds, the estrogenic compounds together with PPARγ agonist PFOA showed that DNA methylation effects were more sensitive than morphological response threshold, and suggested the promoter methylation of *vasa* (new name: *ddx4*), which is actively expressed during development, could be a potential informative marker in future studies. This report was one of the first efforts to examine the applicability of zebrafish embryos for DNA methylation studies in environmental toxicology. As the authors noted, long-term effects of embryonic exposure are not sufficiently studied, which warrants additional efforts in the future.

In another zebrafish embryo study, exposure to the EC_{10} level of PFOS (17.5 mg/L) or PFBS (10.5 mg/L) did not show clear alterations in the gene expression of epigenetic regulators involved in DNA methylation or histone modification (Blanc et al., 2019), whereas significant down-regulation of many of those genes were observed in the PFBS exposed ZFL cells as discussed previously (Table 2). This discrepancy might not be surprising because it is represents a comparison between whole body (embryo) and specific tissue (liver) responses, which warrants careful interpretation of the data.

After exposure of zebrafish embryos to 1 mg/L of PFOS from 6 hpf until 24 or 120 hpf, miRNA microarray analysis showed that expression patterns of a total of 39 and 81 miRNAs (respectively) were significantly altered among 219 known zebrafish miRNAs

Table 4
Previous studies examining the effects of PFAS exposure on epigenetic alterations in aquatic organisms.

Species	Experimenta1 Duration	Compound	Experomental Concentrations	Epigenetic Mechanism	Methods	Observation	Resference
Zebrafish embryo (1 hpf)	Until 96 hpf	PFOS, PFBS	PFOS: 17.5 mg/L (=35 μM) PFBS: 10.5 mg/L (=35 μM)	Global DNA methylation (*dnmt1, dnmt3aa, dnmt3ab, dnmt3ba*), histone deacetylation (*hdac1, hdac3*), and histone demethylation (*kdm5ba*)	RT-qPCR	PFOS caused a small but significant decrease in *dnmt3ab* expression at 96 hpf; PFBS had no significant effect	Blanc et al. (2019)
Zebrafish embryo (0 hpf)	Until 72 hpf	PFOA	4.14–133 mg/L (=10–320 μM)	Gene specific DNA methylation (*vasa*, *vtg1*, and *cyp19a2* promoters)	Bisulfite pyrosequencing	PFOA decreased methylation at CpG3 in *vasa* and increased methylation at CpG1 in *vtg1*	Bouwmeester et al. (2016)
Zebrafish embryo (6 hpf)	Until 24 or 120 hpf	PFOS	1 mg/L	Global DNA methylation miRNA expression	HPLC miRNA microarray analysis (results confirmed by stem-loop RT-qPCR)	No significant effect PFOS significantly increased expression of 20 miRNAs and decreased expression of 19 miRNAs at 24 hpf; increased expression of 41 miRNAs and decreased expression of 40 miRNAs at 120 hpf	Zhang et al. (2011)
Planarian (*Dugesia japonica*)	10 d	PFOA	15 mg/L PFOA; 10 mg/L BA; 15 mg/L PFOA + 10 mg/L BA; 15 mg/L PFOA + 20 mg/L BA	Global DNA methylation Global DNA methylation (*dnmt1*)	ELISA-like method (commercial kit) RT-qPCR	PFOA increased 5-mC contents and decreased 5-hmC contents on days 1, 3, 7, and 10 PFOA decreased *dnmt1* mRNA expression on days 1 and 3, and increased on days 7 and 10	Zhang et al. (2019)
Adult sea urchin (*Glyptocidaris crenularis*)	21 d exposure followed by 7-d depuration period	PFOS	0.01, 0.1, and 1 mg/L	DNA methylation rate	MSAP method	PFOS increased the DNA methylation polymorphism, methylation rate, and demethylation rate in gonad with increasing exposure time, but these measures all decreased after the depuration period. Methylation events were dominant throughout.	Ding et al. (2015)

(Zhang et al., 2011). The significantly altered miRNAs were involved in development, apoptosis, the cell signal pathway, cell cycle progression, cell proliferation, oncogenesis, hormone secretion, and adipose metabolism (Zhang et al., 2011), suggesting the involvement of miRNA expression in PFOS-induced developmental toxicity in zebrafish.

In contrast to increasing studies with fish models, epigenetic changes induced by PFAS are limited in aquatic invertebrates. In a study by Zhang et al. (2019) using a planarian, the protective effects of blueberry anthocyanin (BA) against oxidative stress induced by PFOA were examined. While exposure to 15 mg/L PFOA significantly increased 5-mC, decreased 5-hmC contents, and caused a decrease followed by an increase in *dnmt1* expression, these alterations were slightly reduced by the co-treatment with BA.

Another study with an invertebrate model exposed adult sea urchins to PFOS for 21 days (Ding et al., 2015). In this study, exposure significantly lowered motor and feeding ability and changed antioxidant enzyme activities (i.e., superoxide dismutase and catalase) in the coelomic fluid of urchins. Additionally, PFOS exposure increased gonadal DNA methylation polymorphism, the methylation rate, and the demethylation rate with increasing exposure time based on the methylation sensitive amplified polymorphism (MSAP) method (Ding et al., 2015). Further, methylation events were dominant throughout the experiment while demethylation rates were lower than methylation rates, which could indicate that sea urchins survive PFOS exposure by using hypermethylation as a self-protection mechanism (Ding et al., 2015).

3. Conclusion and recommendations

An increasing number of epidemiological, *in vitro*, and *in vivo* experimental studies have reported associations among PFAS exposure, epigenetic modification, and health outcomes (Tables 1–4). However, these epigenetic changes are not often directly linked to altered gene expression or altered phenotypes. Whereas epidemiological studies have reported the relationship between PFAS exposure and numerous health outcomes, only a limited number of molecular studies have been conducted, focusing on a specific type of adverse outcome such as cancer, metabolic diseases, neurotoxicity or reproductive toxicity. Few studies have been performed to elucidate the involvement of epigenetic regulation in PFAS induced thyroid disruption or kidney dysfunction. Moreover, although a variety of PFASs have consistently been found in humans and the environment, previous studies have largely focused on epigenetic alterations by two major PFASs, PFOS and PFOA. Even here, these effects on DNA methylation in humans and animal models vary. Future studies examining other PFAS and endpoints associated with various adverse outcomes are warranted. Based on our current review, it is likely that oxidative stress induced by early life exposure to PFOS or PFOA may serve as a key link between the multiple epigenetic mechanisms and some adverse health outcomes later in life (Li et al., 2019; Sonkar et al., 2019; Zhang et al., 2019). Our group recently suggested that perfluoroalkyl phosphinic acid, an emerging PFAS of concern, can affect neurobehavior possibly through altered DNA methylation caused by excessive oxidative stress based on gene expression and DNA methylation changes in zebrafish larvae (Kim et al., 2020). Future studies should focus on PFAS induced oxidative stress and investigate the effects on various epigenetic changes associated with long term health indicators, including histone modification and miRNA expression.

Epigenetic alterations can serve as potential molecular initiating events within adverse outcome pathways (AOPs) of environmental chemicals such as PFAS. In fact, within the AOP wiki (aopwiki.org/), an increasing number of AOPs including DNA methylation (KEs 228, 662, 1452, 1453, 1620, 1622, 1698, 1773, and 1778) and histone modifications (KEs 1502 and 1503) as key events have been suggested. For example, decrease global methylation induced by DNMT inhibition leads to population decline (AOP 336 and 337) while promoter demethylation of PPARγ results in adipogenesis (AOP 72). However, many of these AOPs are not directly focused on specific stressors such as PFAS. Future studies of epigenetic effects by PFAS exposure are needed within an AOP context to define hazards and risks to public health and the environment. Such efforts also promise to identify sustainable substitutions and to advance the design of less hazardous chemicals, an important principle of green chemistry (Anastas and Warner 1998). Considering the tissue specific regulation of epigenetic mechanisms, a combined *in silico, in vitro* and *in vivo* evaluation strategy using as toxicogenomics further promises to advance a molecular mechanistic understanding of epigenetic changes elicited by environmental PFAS exposure (Fig. 1).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aluru, N., Kuo, E., Helfrich, L.W., Karchner, S.I., Linney, E.A., Pais, J.E., et al., 2015. Developmental exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin alters DNA methyltransferase (dnmt) expression in zebrafish (*Danio rerio*). Toxicol. Appl. Pharmacol. 284, 142–151.

Aluru, N., Karchner, S.I., Krick, K.S., Zhu, W., Liu, J., 2018. Role of DNA methylation in altered gene expression patterns in adult zebrafish (*danio rerio*) exposed to 3, 3', 4, 4', 5-pentachlorobiphenyl (PCB 126). Environmental Epigenetics 4, dvy005.

Baccarelli, A., Bollati, V., 2009. Epigenetics and environmental chemicals. Curr. Opin. Pediatr. 21, 243.

Bastos Sales, L., Kamstra, J.H., Cenijn, P.H., van Rijt, L.S., Hamers, T., Legler, J., 2013. Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicol. Vitro 27, 1634–1643.

Bhan, A., Mandal, S.S., 2014. Long noncoding rnas: emerging stars in gene regulation, epigenetics and human disease. ChemMedChem 9, 1932–1956.

Blanc, M., Ruegg, J., Scherbak, N., Keiter, S.H., 2019. Environmental chemicals differentially affect epigenetic-related mechanisms in the zebrafish liver (zf-l) cell line and in zebrafish embryos. Aquat. Toxicol. 215, 105272.

Bollati, V., Baccarelli, A., 2010. Environmental epigenetics. Heredity 105, 105–112.

Bouwmeester, M.C., Ruiter, S., Lommelaars, T., Sippel, J., Hodemaekers, H.M., van den Brandhof, E.-J., et al., 2016. Zebrafish embryos as a screen for DNA methylation modifications after compound exposure. Toxicol. Appl. Pharmacol. 291, 84–96.

Braun, J.M., 2017. Early-life exposure to edcs: role in childhood obesity and neurodevelopment. Nat. Rev. Endocrinol. 13, 161–173.

Brooks, B.W., Sabo-Attwood, T., Choi, K., Kim, S., Kostal, J., LaLone, C.A., et al., 2020. Toxicology advances for 21st century chemical pollution. One Earth 2, 312–316.

Corrales, J., Fang, X., Thornton, C., Mei, W., Barbazuk, W.B., Duke, M., et al., 2014. Effects on specific promoter DNA methylation in zebrafish embryos and larvae following benzo[a]pyrene exposure. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 163, 37–46.

Chang, E.T., Adami, H.-O., Boffetta, P., Wedner, H.J., Mandel, J.S., 2016. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans. Crit. Rev. Toxicol. 46, 279–331.

Cunha, C., Brambilla, R., Thomas, K.L., 2010. A simple role for bdnf in learning and memory? Front. Mol. Neurosci. 3, 1.

Ding, G., Wang, L., Zhang, J., Wei, Y., Wei, L., Li, Y., et al., 2015. Toxicity and DNA methylation changes induced by perfluorooctane sulfonate (PFOS) in sea urchin glyptocidaris crenularis. Chemosphere 128, 225–230.

Dong, H., Curran, I., Williams, A., Bondy, G., Yauk, C.L., Wade, M.G., 2016. Hepatic mirna profiles and thyroid hormone homeostasis in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS). Environ. Toxicol. Pharmacol. 41, 201–210.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, 1339–1342.
Gore, A., Chappell, V., Fenton, S., Flaws, J., Nadal, A., Prins, G., et al., 2015. Executive summary to EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, 593–602.
Guerrero-Preston, R., Goldman, L.R., Brebi-Mieville, P., Ili-Gangas, C., Lebron, C., Witter, F.R., et al., 2010. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. Epigenetics 5, 539–546.
Guo, X.X., He, Q.Z., Li, W., Long, D.X., Pan, X.Y., Chen, C., et al., 2017. Brain-derived neurotrophic factor mediated perfluorooctane sulfonate induced-neurotoxicity via epigenetics regulation in sk-n-sh cells. Int. J. Mol. Sci. 18.
Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O., 2001. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environ. Sci. Technol. 35, 766–770.
Hermann, A., Gowher, H., Jeltsch, A., 2004. Biochemistry and biology of mammalian DNA methyltransferases. Cellular and Molecular Life Sciences CMLS 61, 2571–2587.
Ho, S.-M., Johnson, A., Tarapore, P., Janakiram, V., Zhang, X., Leung, Y.-K., 2012. Environmental epigenetics and its implication on disease risk and health outcomes. ILAR J. 53, 289–305.
Jian, J.-M., Chen, D., Han, F.-J., Guo, Y., Zeng, L., Lu, X., et al., 2018. A short review on human exposure to and tissue distribution of per-and polyfluoroalkyl substances (PFASs). Sci. Total Environ. 636, 1058–1069.
Kamstra, J.H., Alestrom, P., Kooter, J.M., Legler, J., 2015. Zebrafish as a model to study the role of DNA methylation in environmental toxicology. Environ. Sci. Pollut. Control Ser. 22, 16262–16276.
Kazazian Jr., H.H., Goodier, J.L., 2002. Line drive: retrotransposition and genome instability. Cell 110, 277–280.
Kim, M.J., Moon, S., Oh, B.-C., Jung, D., Ji, K., Choi, K., et al., 2018. Association between perfluoroalkyl substances exposure and thyroid function in adults: a meta-analysis. PloS One 13 e0197244-e0197244.
Kim, S., Cho, Y.H., Won, S., Ku, J.-L., Moon, H.-B., Park, J., et al., 2019. Maternal exposures to persistent organic pollutants are associated with DNA methylation of thyroid hormone-related genes in placenta differently by infant sex. Environ. Int. 130, 104956.
Kim, S., Stroski, K.M., Killeen, G., Smitherman, C., Simcik, M.F., Brooks, B.W., 2020. 8: 8 perfluoroalkyl phosphinic acid affects neurobehavioral development, thyroid disruption, and DNA methylation in developing zebrafish. Sci. Total Environ. 139600.
Kingsley, S.L., Kelsey, K.T., Butler, R., Chen, A., Eliot, M.N., Romano, M.E., et al., 2017. Maternal serum PFOA concentration and DNA methylation in cord blood: a pilot study. Environ. Res. 158, 174–178.
Kobayashi, S., Azumi, K., Goudarzi, H., Araki, A., Miyashita, C., Kobayashi, S., et al., 2017. Effects of prenatal perfluoroalkyl acid exposure on cord blood igf2/h19 methylation and ponderal index: the hokkaido study. J. Expo. Sci. Environ. Epidemiol. 27, 251–259.
Kwiatkowski, C.F., Andrews, D.Q., Birnbaum, L.S., Bruton, T.A., DeWitt, J.C., Knappe, D.R.U., et al., 2020. Scientific basis for managing PFAS as a chemical class. Environ. Sci. Technol. Lett. 7, 532–543.
Laing, L., Viana, J., Dempster, E., Trznadel, M., Trunkfield, L., Uren Webster, T., et al., 2016. Bisphenol a causes reproductive toxicity, decreases dnmt1 transcription, and reduces global DNA methylation in breeding zebrafish (*Danio rerio*). Epigenetics 11, 526–538.
Leter, G., Consales, C., Eleuteri, P., Uccelli, R., Specht, I.O., Toft, G., et al., 2014. Exposure to perfluoroalkyl substances and sperm DNA global methylation in arctic and european populations. Environ. Mol. Mutagen. 55, 591–600.
Leung, Y.K., Ouyang, B., Niu, L., Xie, C., Ying, J., Medvedovic, M., et al., 2018. Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics 13, 290–300.
Li, D., Zhang, L., Zhang, Y., Guan, S., Gong, X., Wang, X., 2019. Maternal exposure to perfluorooctanoic acid (PFOA) causes liver toxicity through ppar-alpha pathway and lowered histone acetylation in female offspring mice. Environ. Sci. Pollut. Control Ser. 26, 18866–18875.
Li, W., He, Q.Z., Wu, C.Q., Pan, X.Y., Wang, J., Tan, Y., et al., 2015. PFOS disturbs bdnf-erk-creb signalling in association with increased microrna-22 in sh-sy5y cells. BioMed Res. Int. 2015, 302653.
Liu, C.Y., Chen, P.C., Lien, P.C., Liao, Y.P., 2018. Prenatal perfluorooctyl sulfonate exposure and alu DNA hypomethylation in cord blood. Int. J. Environ. Res. Publ. Health 15, 1066.
Ma, Y., Yang, J., Wan, Y., Peng, Y., Ding, S., Li, Y., et al., 2018. Low-level perfluorooctanoic acid enhances 3T3-L1 preadipocyte differentiation via altering peroxisome proliferator activated receptor gamma expression and its promoter DNA methylation. J. Appl. Toxicol. 38, 398–407.
Mariussen, E., 2012. Neurotoxic effects of perfluoroalkylated compounds: mechanisms of action and environmental relevance. Arch. Toxicol. 86, 1349–1367.
Miura, R., Araki, A., Miyashita, C., Kobayashi, S., Kobayashi, S., Wang, S.L., et al., 2018. An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: the hokkaido study. Environ. Int. 115, 21–28.
Okano, M., Bell, D.W., Haber, D.A., Li, E., 1999. DNA methyltransferases dnmt3a and dnmt3b are essential for *de novo* methylation and mammalian development. Cell 99, 247–257.
Olsvik, P.A., Williams, T.D., Tung, H-s, Mirbahai, L., Sanden, M., Skjaerven, K.H., et al., 2014. Impacts of TCDD and MeHg on DNA methylation in zebrafish (*Danio rerio*) across two generations. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 165, 17–27.
Organisation for Economic Cooperation and Development (OECD), 2018. Toward a New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs): Summary Report on Updating the Oecd 2007 List of Per and Polyfluoroalkyl Substances (PFASs).
Park, S.S., Skaar, D.A., Jirtle, R.L., Hoyo, C., 2017. Epigenetics, obesity and early-life cadmium or lead exposure. Epigenomics 9, 57–75.
Pogribny, I.P., Beland, F.A., 2009. DNA hypomethylation in the origin and pathogenesis of human diseases. Cell. Mol. Life Sci. 66, 2249–2261.
Rappazzo, K.M., Coffman, E., Hines, E.P., 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. Int. J. Environ. Res. Publ. Health 14, 691.
Ruiz-Hernandez, A., Kuo, C.-C., Rentero-Garrido, P., Tang, W.-Y., Redon, J., Ordovas, J.M., et al., 2015. Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence. Clin. Epigenet. 7, 55.
Sakamoto, H., Kogo, Y., Ohgane, J., Hattori, N., Yagi, S., Tanaka, S., et al., 2008. Sequential changes in genome-wide DNA methylation status during adipocyte differentiation. Biochem. Biophys. Res. Commun. 366, 360–366.
Santangeli, S., Maradonna, F., Gioacchini, G., Cobellis, G., Piccinetti, C.C., Dalla Valle, L., et al., 2016. BPA-induced deregulation of epigenetic patterns: effects on female zebrafish reproduction. Sci. Rep. 6, 21982.
Schulz, K., Silva, M.R., Klaper, R., 2020. Distribution and effects of branched versuched and linear PFAS in the abiotic environms linear isomers of PFOA, PFOS, and PFHXS: a review of recent literature. Sci. Total Environ. 733, 139186.
Shatseva, T., Lee, D.Y., Deng, Z., Yang, B.B., 2011. Microrna mir-199a-3p regulates cell proliferation and survival by targeting caveolin-2. J. Cell Sci. 124, 2826–2836.
Singh, S., Li, S.S.-L., 2012. Epigenetic effects of environmental chemicals bisphenol a and phthalates. Int. J. Mol. Sci. 13, 10143–10153.
Sonkar, R., Kay, M.K., Choudhury, M., 2019. PFOS modulates interactive epigenetic regulation in first-trimester human trophoblast cell line htr-8/svneo. Chem. Res. Toxicol. 32, 2016–2027.
Stanifer, J.W., Stapleton, H.M., Souma, T., Wittmer, A., Zhao, X., Boulware, L.E., 2018. Perfluorinated chemicals as emerging environmental threats to kidney health: a scoping review. Clin. J. Am. Soc. Nephrol. 13, 1479–1492.
Temkin, A.M., Hocevar, B.A., Andrews, D.Q., Naidenko, O.V., Kamendulis, L.M., 2020. Application of the key characteristics of carcinogens to per and polyfluoroalkyl substances. Int. J. Environ. Res. Publ. Health 17, 1668.
Tian, J., Xu, H., Zhang, Y., Shi, X., Wang, W., Gao, H., et al., 2019. Sam targeting methylation by the methyl donor, a novel therapeutic strategy for antagonize pfos transgenerational fertilitty toxicity. Ecotoxicol. Environ. Saf. 184, 109579.
Tian, M., Peng, S., Martin, F.L., Zhang, J., Liu, L., Wang, Z., et al., 2012. Perfluorooctanoic acid induces gene promoter hypermethylation of glutathione-s-transferase pi in human liver l02 cells. Toxicology 296, 48–55.
Tsai, M.-M., Huang, H.-W., Wang, C.-S., Lee, K.-F., Tsai, C.-Y., Lu, P.-H., et al., 2016. Microrna-26b inhibits tumor metastasis by targeting the kpna2/c-jun pathway in human gastric cancer. Oncotarget 7, 39511.
United States Environmental Protection Agency (USEPA), 2018. Fact sheet: 2010/2015 pfoa stewardship program. (Accessed 21 October 2020).
van den Dungen, M.W., Murk, A.J., Kampman, E., Steegenga, W.T., Kok, D.E., 2017a. Association between DNA methylation profiles in leukocytes and serum levels of persistent organic pollutants in Dutch men. Environmental Epigenetics 3, dvx001.
van den Dungen, M.W., Murk, A.J., Kok, D.E., Steegenga, W.T., 2017b. Persistent organic pollutants alter DNA methylation during human adipocyte differentiation. Toxicol. Vitro 40, 79–87.
Vieira, V.M., Hoffman, K., Shin, H.-M., Weinberg, J.M., Webster, T.F., Fletcher, T., 2013. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environ. Health Perspect. 121, 318–323.
Volz, D.C., Leet, J.K., Chen, A., Stapleton, H.M., Katiyar, N., Kaundal, R., et al., 2016. Tris (1, 3-dichloro-2-propyl) phosphate induces genome-wide hypomethylation within early zebrafish embryos. Environ. Sci. Technol. 50, 10255–10263.
Wan, Y-j, Li, Y-y, Xia, W., Chen, J., Lv, Z-q, Zeng, H-c, et al., 2010. Alterations in tumor biomarker gstp gene methylation patterns induced by prenatal exposure to pfos. Toxicology 274, 57–64.
Wang, F., Liu, W., Jin, Y., Wang, F., Ma, J., 2015. Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in aberrant changes in mirna expression profile and levels in developing rat livers. Environ. Toxicol. 30, 712–723.
Wang, J., Zhang, Y., Zhang, W., Jin, Y., Dai, J., 2012. Association of perfluorooctanoic acid with hdl cholesterol and circulating mir-26b and mir-199-3p in workers of a fluorochemical plant and nearby residents. Environ. Sci. Technol. 46, 9274–9281.
Watkins, D.J., Wellenius, G.A., Butler, R.A., Bartell, S.M., Fletcher, T., Kelsey, K.T., 2014. Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation. Environ. Int. 63, 71–76.
Wen, Y., Chen, J., Li, J., Arif, W., Kalsotra, A., Irudayaraj, J., 2020. Effect of pfoa on DNA methylation and alternative splicing in mouse liver. Toxicol. Lett. 329, 38–46.
Xu, B., Chen, X., Mao, Z., Chen, M., Han, X., Du, G., et al., 2013. Perfluorooctane sulfonate disturbs nanog expression through mir-490-3p in mouse embryonic stem cells. PloS One 8.
Xu, B., Ji, X., Chen, X., Yao, M., Han, X., Chen, M., et al., 2015. Effect of perfluorooctane sulfonate on pluripotency and differentiation factors in mouse embryoid bodies. Toxicology 328, 160–167.
Yang, A.S., Estécio, M.R.H., Doshi, K., Kondo, Y., Tajara, E.H., Issa, J.-P.J., 2004. A simple method for estimating global DNA methylation using bisulfite pcr of repetitive

DNA elements. Nucleic Acids Res. 32 e38-e38.
Zhang, J., Wang, B., Zhao, B., Li, Y., Zhao, X., Yuan, Z., 2019. Blueberry anthocyanin alleviate perfluorooctanoic acid-induced toxicity in planarian (dugesia japonica) by regulating oxidative stress biomarkers, atp contents, DNA methylation and mrna expression. Environ. Pollut. 245, 957–964.
Zhang, L., Li, Y.Y., Zeng, H.C., Wei, J., Wan, Y.J., Chen, J., et al., 2011. Microrna expression changes during zebrafish development induced by perfluorooctane sulfonate. J. Appl. Toxicol. 31, 210–222.