Developmental toxicant exposures and sex-specific effects on epigenetic programming and cardiovascular health across generations

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Abstract

Despite substantial strides in diagnosis and treatment, cardiovascular diseases (CVDs) continue to represent the leading cause of death in the USA and around the world, resulting in significant morbidity and loss of productive years of life. It is increasingly evident that environmental exposures during early development can influence CVD risk across the life course. CVDs exhibit marked sexual dimorphism, but how sex interacts with environmental exposures to affect cardiovascular health is a critical and understudied area of environmental health. Emerging evidence suggests that developmental exposures may have multi- and transgenerational effects on cardiovascular health, with potential sex differences; however, further research in this important area is urgently needed. Lead (Pb), phthalate plasticizers, and perfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants with numerous adverse human health effects. Notably, recent evidence suggests that developmental exposure to each of these toxicants has sex-specific effects on cardiovascular outcomes, but the underlying mechanisms, and their effects on future generations, require further investigation. This review article will highlight the role for the developmental environment in influencing cardiovascular health across generations, with a particular emphasis on sex differences and epigenetic mechanisms. In particular, we will focus on the current evidence for adverse multi and transgenerational effects of developmental exposures to Pb, phthalates, and PFAS and highlight areas where further research is needed.

Key words: sex differences; toxicoepigenetics; DOHaD; cardiovascular disease; epigenetics

Introduction

Developmental environment and cardiovascular disease

The developmental origins of health and disease (DOHaD) hypothesis posits that environmental insults during critical windows of development can lead to diseases later in life [1, 2]. While the majority of DOHaD studies have focused on pregnancy and lactation, development continues throughout childhood and puberty. Thus, vulnerability to environmental exposures under the DOHaD paradigm spans well beyond pregnancy. Likewise, a role for paternal environmental exposures in the programming of offspring health is increasingly evident but remains less studied [3, 4]. Early environmental insults have been linked to an increased risk of several non-communicable diseases later in life, including diabetes, cancer, neurodegenerative diseases, and the focus of this review—cardiovascular diseases (CVDs) [2, 5-7]. In this review article, we focus on CVDs, which refer to a broad array of health conditions, including atherosclerosis, myocardial infarction, stroke, cardiac arrhythmia, hypertension, heart failure, heart valve disease, and congenital heart defects [2, 8]. In many cases, as are noted throughout the manuscript, direct links between various chemicals and CVDs may not yet be established. However, we have noted that some are linked to conditions that are not classically considered CVD but are nevertheless intimately linked to cardiovascular health, such as obesity, disruptions in hormone signaling, and hyperglycemia [2, 8, 9]. For this reason, we have chosen to include these in the manuscript where relevant. It is important to note that, because CVDs are multifactorial, it is often difficult to ascertain whether effects of chemical exposures on cardiovascular health are direct or are secondary to effects on body weight, endocrine function, inflammation, and other factors. In vitro studies using traditional and new approach methodologies will likely shed light on this important distinction but are beyond the scope of this review.

The developing cardiovascular system is exquisitely sensitive to exogenous influences. Several decades ago, Dr David Barker observed that adverse conditions in utero increased the risk of coronary heart disease later in life, highlighting the importance of this critical stage of life in influencing long-term health trajectory. One of the most famous examples of Dr Barker’s work illustrating how early developmental environment affects cardiovascular health is the Dutch hunger winter of 1944–45, during which there was a restriction of food to the western part of the Netherlands as a result of World War II [10]. Women who were pregnant during this period were thus exposed to severe caloric restriction. Because medical records and food rations were

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well-documented, the effects of the famine on children born to women during this time have been extensively investigated. The Dutch Famine birth cohort documented that nutrient restriction in utero in the first trimester was associated with increased risk of obesity in women and dyslipidemia and cardiovascular disease in both sexes. Restriction in the third trimester, on the other hand, was associated with decreased risk of obesity in men, independent of birth weight [10-12]. Other studies in animal models have shown consistent effects of in utero nutrient restriction on epigenetic programming of genes controlling metabolic homeostasis and CVD risk [13]. Subsequent research has demonstrated that maternal obesity, hyperglycemia, and poor diet (high fat, high sucrose, or low protein) influence CVD risk in offspring [14-17]. Likewise, adverse childhood experiences have also been linked to poor cardiovascular health later in life [18-20]. In addition to nutritional and psychosocial factors, early exposure to environmental toxicants can also influence cardiovascular development and disease risk across the life course. A number of toxicants have been investigated in this regard, including air pollution [21], metals [22-24], phthalates [25, 26], perfluoroalkyl substances (PFAS) [27], pesticides [28], and hormone mimics such as diethylstilbestrol (DES) [29]. A summary of the factors discussed here can be found in Table 1.

**Sex differences in environment-induced cardiovascular disease**

Although the early developmental environment plays a role in CVD risk, the effects of many environmental influences differ based on sex in both animals and humans [30-35]. However, the sex-specific effects of the developmental environment on cardiac health have received little attention. It is well-established that the incidence, pathogenesis, and prognosis of CVDs differ substantially between sexes [36, 37]. For example, although acute myocardial infarction is one of the leading causes of death in both men and women, women experience this condition later in life and exhibit atypical symptoms, resulting in delayed diagnosis [36]. Likewise, while ischemic heart disease is frequently characterized by coronary artery occlusion in men, women are more likely to exhibit microvascular dysfunction [37]. Hypertension is more common among men in young adults, but this disparity shifts in advanced age, where a greater number of women experience hypertension [38, 39]. Other conditions such as heart failure, genetic cardiomyopathies, and drug-induced arrhythmias also exhibit sexual dimorphism [37, 40, 41]. It is therefore imperative that we understand the sex differential effects that the early environment has on CVD risk and pathogenesis. Many factors noted above, including maternal obesity, diet, and diabetes [16, 42-44], psychosocial stressors [45], and toxicant exposures [46-48], show sex-specific effects on cardiovascular health. In this review, we will discuss what is known regarding the sex-specific multi and trans-generational effects of toxicant exposures on cardiac health. Each section contains a summary table of the studies discussed, which includes information on sex differences, if any, observed in each study.

**Epigenetics and multi and transgenerational inheritance**

Epigenetics refers to the study of mitotically heritable changes in gene regulation that do not involve an alteration to the DNA sequence itself. Several mechanisms of epigenetic regulation have been identified, including modifications to the 5-position of cytosine bases in DNA (5-methylcytosine, 5-hydroxymethylcytosine, 5-carboxylcytosine, and 5-formylcytosine), modifications to histone proteins (methylation, acetylation, phosphorylation, and ubiquitination), and actions of non-coding RNA. The most extensively studied epigenetic modification is methylation of the 5-position of cytosine bases [5-methylcytosine (5mC)], which plays a role in X-inactivation, genomic imprinting, and repression of transposable elements [49-52]. The regulation of gene expression by DNA methylation is associated with genomic location. For example, DNA methylation at promoters is generally associated with repression of genes, while intragenic DNA methylation is associated with gene activation [53, 54]. The 5-hydroxymethylcytosine (5hmC) modification plays a critical role post-fertilization and in primordial germ cells (PGCs) during the dynamic reprogramming of DNA methylation [55-57] but has also been shown to be a stable epigenetic mark present in a variety of mammalian tissues [58-60], and we have shown that it is stably reprogrammed by perinatal exposures in mice and humans [61, 62].

Two distinct waves of epigenetic reprogramming occur during early development, making this period vulnerable to environmental perturbations [63, 64]. The first wave of reprogramming occurs post-fertilization, in which parental epigenetic marks are erased and the somatic epigenetic marks of the developing embryo are established [65, 66]. The second wave of reprogramming occurs in the fetal primordial germ cells, in which sex and parent of origin-specific epigenetic patterning are established [67]. Environmental disruptions in the normal epigenetic patterning in germ cells that are not corrected during the development of the future generation may result in multi and transgenerational effects [68]. In a pregnant (F0) individual, toxicant-induced epigenetic changes may occur in their somatic and germ cells, as well as in the somatic and primordial germ cells of the developing fetus (Fig. 1) [68]. Toxicant exposure-induced effects occurring in the offspring (F1) and grand offspring (F2) generations are termed multigenerational effects, as F0 and F1 generations receive direct exposure during the pregnancy, and the primordial germ cells of the F2 generation are also directly exposed (Fig. 1) [68]. In contrast, trans-generational epigenetic inheritance refers to the transmission of environmental effects to future generations that did not receive the exposure directly through embryonic development or germ cells, i.e. F3 generations and beyond (Fig. 1) [68]. In males and non-gestating females, the F0 individual and F1 offspring are directly exposed, and transgenerational effects are observed in the F2 generation and beyond (Fig. 1). Transgenerational effects of environmental exposures have been reported in nematodes, plants, fruit flies, and mammals [68-71]. However, the prevalence of this phenomenon and its relevance to health, in particular human health, is the subject of significant debate and ongoing study [72, 73].

**Effects of environment on cardiovascular health across generations**

Several animal and human studies provide evidence that ancestral environmental exposures may induce cardiovascular health effects that span generations (Fig. 2). A summary of the manuscripts reviewed in this section can be found in Table 2. In animal studies, F1, F2, and F3 male progeny of rats exposed to global nutrient and calorie restriction during their entire pregnancy exhibited cardiovascular dysfunction, including high blood pressure, altered nitric oxide production, and impaired vasodilation in response to acetylcholine [74]. Maternal obesity may also produce transgenerational effects on cardiac health. Obese female mice produced both male and female offspring from F1
### Table 1: Developmental environmental factors linked to CVD

| Environmental factor | Window of exposure | Model         | Phenotype                                                                                                                                                                                                 | Sex specificity | Reference |
|----------------------|--------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-----------|
| Famine               | First trimester in utero | Human        | Obesity, dyslipidemia (high LDL : HDL cholesterol ratio), cardiovascular disease (angina pectoris, Q waves on the ECG, or history of coronary revascularization)                                                |                | [10,12]              |
| Famine               | Third trimester in utero | Human        | Reduced risk of obesity                                                                                                                                                                                  |                | [11]               |
| Obesogenic diet (high fat and high sugar) and obesity during pregnancy | Gestation and lactation | Mouse | Reduction in cardiac function at 32 weeks of age—hypertrophy and diastolic dysfunction                                                                                                                    | Only effects on male offspring were reported | [14]               |
| Hyperglycemia        | Gestation          | Mouse        | In embryos, impaired formation of left-right axis in heart and left-right asymmetry in developing organs                                                                                               |                | [15]               |
| High-fat and high-sucrose diet | Gestation and lactation  | Mouse      | Higher left ventricular weight in males and females, smaller left ventricular relative wall thickness in females, higher left ventricular relative wall thickness in males, cardiac mitochondrial abnormalities in both sexes | Yes             | [16]               |
| Protein restriction  | Gestation and lactation | Rat         | Decreased mitochondrial oxidative phosphorylation, decreased antioxidant capacity, and increased reactive oxygen species                                                                             |                | [17]               |
| Adverse childhood experiences (abuse, neglect, violence, and household dysfunction) | First 18 years of life | Human | Impaired endothelial function and reduced circulating levels of SIRT1                                                                                                                                     | Only females were evaluated | [18]               |
| Adverse childhood experiences (abuse, neglect, and household dysfunction) | First 18 years of life | Human | Increased risk of ischemic heart disease (mediated by psychosocial factors)                                                                                                                             | Sex differences not reported | [19]               |
| Adverse childhood experiences (verbal and physical abuse, substance abuse, and poorly managed household) | First 18 years of life | Human | Increased multisystem health risk (high blood pressure, low heart rate variability, dyslipidemia, waist circumference, and other factors)                                                            | Sex differences not reported | [20]               |
| PM$_{2.5}$ exposure (73.61μg/m$^3$ for 6h/day, 7 days/week) | Gestation          | Mouse        | Left ventricular remodeling and dysfunction, inflammation, fibrosis, altered calcium homeostasis at 3 months of age                                                                                     | Effects only observed in male mice | [21]               |
| Pb exposure (0.2% in maternal drinking water) | Post-natal days 1–21 (birth until weaning) | Rat         | Increased total cholesterol, LDL and triglycerides at 4 and 18 months of age, necrosis, immune cell infiltration at 4 and 18 months of age                                                                | Only male offspring were evaluated | [22]               |
| Pb exposure          | Measured in pnatal maternal toenail samples | Human        | Increased risk of elevated systolic blood pressure in young children (average 5.5 years of age)                                                                                                           | Effects were stronger among boys | [23]               |
| Cadmium exposure (0, 1, and 50 ppm) | Gestation          | Mouse        | Increased relative heart weight at birth in both sexes, increased risk of hypertension in females at 6 months of age                                                                                      | Yes             | [24]               |
| DEHP (250 mg/kg, 500 mg/kg, and 1g/kg) | Embryonic day E6.5 to E14.5 | Mouse | Increased cardiac malformations (septal defects, ventricular myocardium noncompaction, and cardiac hypoplasia) at E15.5—potentially secondary to maternal toxicity and weight loss                               | Not reported    | [25]               |
| Phthalates and alkylphenolic compounds | Maternal occupational exposure assessed via job exposure matrix | Human        | Increased incidence of congenital heart defects, particularly in children who also carried ABC1 polymorphism                                                                                  | Not reported    | [26]               |
| PFAS (PFOA and PFHxS) | Gestation (maternal serum and cord blood) | Human        | Increased cardiometabolic risk score (insulin, waist circumference, and blood pressure) in adolescence                                                                                                 | No sex differences were detected | [27]               |
| DDT                  | Gestation (maternal serum samples)      | Human        | DDT exposure associated with hypertension at 39–47 years of age                                                                                                                                          | Only females were evaluated | [28]               |
| Diethylstilbestrol   | Gestation          | Human        | Increased incidence of coronary artery disease and myocardial infarction                                                                                                                              | Only females were evaluated | [29]               |
Figure 1: Multigenerational vs. transgenerational effects. Left: In a pregnant (F0) individual exposed to a toxicant, the F1 and F2 generations will also receive direct toxicant exposure, resulting in multigenerational effects. Transgenerational effects are those that occur in generations not directly exposed to the toxicant, i.e., F3 and beyond. Right: In males and non-gestating females, the F0 individual and F1 offspring are directly exposed (via germ cells), and transgenerational effects are observed in the F2 generation and beyond. The sex-specific effects of toxicants on transgenerational epigenetic inheritance are poorly understood.

Figure 2: Diet and exposure to toxicants such as Pb, PFAS, and phthalates can affect the epigenome of the parental germ cells, as well as the somatic and germline cells of a developing fetus, resulting in multigenerational and transgenerational effects on cardiovascular disease risk. The sex-specific generational effects of Pb, PFAS, and phthalates have not been adequately investigated.

To F3 with cardiac defects, including mitochondrial dysfunction and increased left ventricular mass [16]. Epigenetic or other mechanisms were not investigated in either of these studies, however, so the underlying molecular basis for their observations remains unclear. In zebrafish, paternal exposure to bisphenol A resulted in cardiac defects (pericardial edema, malformations in both cardiac chambers, ectopic heartbeats, and arrhythmias) in F1 progeny as well as altered expression of cardiac developmental genes [75]. Cardiac edema was also observed in F2 progeny exposed to the highest dose of BPA [75]. The authors observed...
no significant changes in global DNA methylation in sperm and testicular tissue from control vs. treated animals; however, other epigenetic or site-specific DNA methylation changes were not investigated [75].

Human studies provide further support for the hypothesis that the cardiovascular effects of the environment can span multiple generations. Research conducted on three cohorts of people born in 1890, 1905, and 1920 in Overkalix, a Municipality in Sweden, revealed that a dearth of food during a father’s slow growth period (the period between ages 9 and 12 years) resulted in a reduced risk of mortality from cardiovascular disease in the children [76]. However, if the paternal grandfather was exposed to an abundance of food during this critical period, then his grandchildren had a higher risk of mortality from diabetes [76]. Further investigation of this cohort of people showed that if a paternal grandmother lived through a period of sharp changes in food availability in early life up to puberty, then the daughters of her sons exhibited an increased risk for cardiovascular mortality [77]. In a separate study of a population exposed to the Chinese Famine (1959–61), prenatal exposure to famine was associated with an increased risk of hyperglycemia in adulthood for the exposed, as well as their offspring [78]. The effects on offspring hyperglycemia risk were independent of sex and occurred in offspring of both exposed mothers and fathers [78]. The molecular mechanisms underlying these effects are unclear; however, studies have demonstrated that epigenetic factors such as DNA methylation may underlie the transmission of stress across generations [79, 80].

Multi- and transgenerational effects of toxicant exposures have been reported for a wide variety of chemicals, including BPA [81, 82], phthalates [82], parabens, pesticides [83] and dioxin [84], fungicides [85], and jet fuel [86]. The transgenerational effects of chemical exposures on long-term cardiovascular health are poorly understood, and given the significant burden of morbidity and mortality posed by CVDs, further investigation into this important topic is urgently needed. Although a relatively small number of studies have investigated the transgenerational effects of environment specifically on cardiac health, significant animal and human evidence also links ancestral environment to conditions closely associated with CVD, including obesity, diabetes, and reproductive dysfunction. For example, ancestral exposure to BPA and phthalates [82], pesticides/herbicides [83, 87], tributyltin [6, 88], and high-fat diet [89] have been linked to an increased risk of obesity or adiposity, and some of these exposures exhibited sex differences. Obesity induced by methoxychlor was present in both F3 males and females, with a greater incidence in males [83]. Offspring consumption of a high-fat diet led to obesity in male but not female F4 mice after ancestral exposure to tributyltin [6]. A maternal high-fat diet led to increased body weight in F3 female mice, which was transmitted through the paternal line [89]. On the other hand, glyphosate and plastics-induced obesity was present in both F3 males and females at roughly equal frequency [82, 87]. These studies identified chemical-induced epigenetic changes that were present in the generations that did not receive direct exposure, providing evidence that transgenerational inheritance may be mediated by changes to the germline epigenome. Indeed, methoxychlor and BPA/phthalate-induced phenotypes were accompanied by changes in DNA methylation in the sperm of F3 mice [83, 84]. Glyphosate exposure led to altered DNA methylation in F1–F3 sperm [87]. Effects of ancestral exposure to tributyltin were associated with changes in DNA methylation and chromatin accessibility in F3 and F4 sperm and adipose tissue [6]. Increased body weight as a result of maternal high-fat diet was accompanied by changes in expression of paternally expressed imprinted genes, suggesting that stable epigenetic modifications at these genes may have been responsible for the observed effects on gene expression and body weight [89].

In the following sections, we will describe the sex-specific effects of developmental exposure to Pb, PFAS, and phthalates on cardiovascular health and epigenetic programming in human and animal studies, as well as the current evidence available regarding the transgenerational effects of these chemicals on cardiovascular health.

**Developmental Pb exposure and cardiovascular health**

The metal Pb is a ubiquitous environmental contaminant with a large number of deleterious human health effects. Sources of human Pb exposure include soil, water, food, contaminated household dust, consumer goods, folk remedies, smoking, and industrial sources [90, 91]. Human exposure to Pb occurs primarily through ingestion or inhalation, where it causes adverse neurological, hematological, renal, and cardiovascular effects [91]. A summary of the cardiovascular and epigenetic effects of developmental Pb exposure can be found in Tables 3 and 4, respectively.

**Cardiovascular effects of Pb exposure in animals**

Pb exposure in animals outside of the developmental period is widely reported to cause cardiotoxicity, heart failure, and hypertension [92-95]. Developmental exposures have been less extensively studied, but several have demonstrated adverse effects on blood pressure, cardiac function, and metabolic parameters. First, developmental Pb exposure in rats leads to hypertension in both sexes [96] or in male offspring (females were not included in the study) [97]. Exposure to Pb is also associated with altered angiogenesis in mouse embryos, suggesting that some Pb-induced developmental defects may be mediated by impaired blood flow [98]. Pb exposure during lactation impaired cardiac mitochondrial function and depleted antioxidant capacity in male rats [22], consistent with data in humans demonstrating direct effects of Pb on cardiac function [99]. Several additional studies have demonstrated that Pb exposure causes sex-specific changes in metabolic parameters that are closely linked to cardiovascular health, including weight, food intake, insulin signaling, blood glucose levels, and circulating IGF-1 [100-102]. Several additional Pb exposure studies have been conducted in young, recently weaned animals, a developmental period in which significant growth and hormonal changes are still occurring. Newly weaned male rabbits treated with Pb for 8 weeks showed serum and histological evidence of cardiac injury [103]. Young male rats exposed to Pb exhibited pathological changes in the structure of the aorta and myocardium [104]. Chronic Pb exposure in young male mice led to increased cardiac inotropy and blood pressure, concomitant with changes in circulating enzymes critical for blood pressure regulation [105].

**Cardiovascular effects of Pb exposure in humans**

Although investigations into the effects of Pb on health have focused primarily on neurological outcomes, numerous studies also link Pb exposure to adverse cardiovascular outcomes, including high blood pressure, myocardial infarction, coronary artery disease, cardiac arrhythmias, heart failure, atherosclerosis, and stroke [99, 106-108]. Developmental exposures to Pb have also been linked to cardiovascular diseases in humans. First, there
Table 2: Generational effects of environment on cardiovascular health

| Environmental factor                      | Window of exposure | Model  | Epigenetic/molecular effect | Phenotype                                                                 | Sex specificity | Reference |
|------------------------------------------|--------------------|--------|-----------------------------|---------------------------------------------------------------------------|-----------------|-----------|
| Nutrient and calorie restriction         | Gestation          | Rat    | Not investigated            | High blood pressure and impaired vasodilation (F1, F2, and F3) at 16 weeks of age | Only male offspring were evaluated | [74]      |
| High-fat and high-sucrose diet           | Gestation and lactation | Mouse  | Not investigated            | Increased left ventricular mass in male (F1, F2, and F3) and female (F1 and F2) offspring, cardiac mitochondrial abnormalities in both sexes (F1, F2, and F3) at 8 weeks of age | Yes             | [16]      |
| Bisphenol A (1.00 and 2000 μg/l)         | Spermatogenesis    | Zebrafish | No changes in global DNA methylation in testicular cells or spermatozoa | Cardiac edema and malformations (defects in both heart chambers) in high dose exposed 7 dpf larvae (F1 and F2) | Not reported    | [75]      |
| Food availability                        | Slow growth period (before pre-pubertal peak in growth velocity) in males | Human  | Not investigated            | Increased risk of death from diabetes mellitus in grandchildren of men exposed to a abundant food during the slow growth period, decreased risk of death from CVD among grandchildren of men exposed to famine during the slow growth period | Not reported    | [76]      |
| Food availability                        | Period prior to puberty in females | Human  | Not investigated            | Increased risk of cardiovascular mortality in daughters of the sons from women exposed to sharp changes in food supply before puberty | Yes             | [77]      |
| Famine                                   | Gestation          | Human  | Not investigated            | Increased risk of hyperglycemia (F1 and F2) in adulthood                  | Not reported    | [78]      |
| BPA, DEHP and DBP at two doses: BPA 50 mg/kg BW/day, DEHP 750 mg/kg BW/day and DBP 66 mg/kg/BW/day and a second group with half of those doses via intraperitoneal injection | Gestation days 8–14 | Rat    | Differential DNA methylation in 197 regions in sperm of F3 generation | Obesity and pubertal abnormalities (primarily delayed puberty onset) in F3 males and females | Effects observed in both males and females | [82]      |
| Methoxychlor (200 mg/kg BW/day)          | Gestation days 8–14 | Rat    | Differential DNA methylation in sperm of F3 generation | Pubertal abnormalities in F1 males and females; obesity in F3 males and females, with a greater incidence in males; obesity in F4 generation males (transmitted through female) | Yes             | [83]      |
| A pesticide mixture (permethrin 150 mg/kg and DEET 40 mg/kg), a plastic mixture (bisphenol A 50 mg/kg, DBP 66 mg/kg, and DEHP 750 mg/kg), dioxin (TCDD 100 ng/kg) and a hydrocarbon mixture (jet fuel, JP8 500 mg/kg) or sesame oil control by intraperitoneal injection | Gestation days 8 to 14 | Rat    | Differentially methylated regions in the sperm of males from all exposure lineages | Early onset puberty in F3 females with exposure to plastics, dioxin and jet fuel; reduced number of ovarian follicles in F3 females with all exposures; increased sperm apoptosis in F3 males exposed to jet fuel | Yes             | [84]      |

(continued)
### Table 2: (Continued)

| Environmental factor | Window of exposure | Model | Epigenetic/molecular effect | Phenotype | Sex specificity | Reference |
|----------------------|--------------------|-------|-----------------------------|-----------|-----------------|-----------|
| Vinclozolin (100 or 200 mg/kg) or DMSO in sesame oil control | Gestation days 7–13 | Mouse | Differential DNA methylation in F3 sperm | Increased testis, prostate and kidney disease in F3 males; increase sperm apoptosis in F1, F2, and F3 males; increased ovarian cysts in F1, F2, and F3 females | Yes | [85] |
| Jet fuel (25% the oral LD50 dose) or DMSO in sesame oil by intraperitoneal injection | Gestation days 8 to 14 | Rat | Differential DNA methylation at 33 regions in F3 sperm | Renal abnormalities in F1 males and females; prostate and altered puberty timing in F1 males; loss of ovarian follicles and polycystic ovarian disease in F1 and F3 females; obesity in F3 males and females | Yes | [86] |
| Glyphosate (25 mg/kg body weight daily) | Gestation days 8–14 | Rat | Differential DNA methylation in sperm of F1 (264 regions), F2 (174 regions), and F3 (378 regions) generations | Delayed puberty in F1 and F2 males and F2 females; obesity in F2 and F3 males and females at 1 year of age | Yes | [87] |
| Tributyltin (50 nM in drinking water) | Gestation and lactation | Mouse | Changes in global DNA methylation in white adipose tissue from F4 males; changes in chromatin accessibility in F3 and F4 sperm | Increased obesity in response to a high-fat diet in F4 males | Yes | [8] |
| Tributyltin (5.42, 54.2, or 542 nM in drinking water) | 7 days prior to mating, through gestation | Mouse | Not investigated | Increased white adipose tissue and depots and adipocyte size and number in F1, F2, and F3 males and F1 and F2 females | Effects observed in both males and females | [88] |
| Maternal high-fat diet | 6 weeks prior to mating, through gestation and lactation | Mouse | Not investigated | Increased body weight in F3 females and improved glucose tolerance in F3 males | Yes | [89] |
### Table 3: Developmental Pb exposure and cardiovascular health

| Exposure details | Window of exposure | Model | Epigenetic/molecular effect | Phenotype | Sex specificity | Reference |
|------------------|--------------------|-------|-----------------------------|-----------|-----------------|-----------|
| 0.2% Pb acetate in drinking water | Gestation until adulthood (12, 20, and 28 weeks) | Rat | Not investigated | Elevated blood pressure, respiratory frequency, hyperactive baro- and chemoreflexes in adulthood in both sexes | Effects observed in both males and females | [96] |
| 500 ppm of Pb acetate in drinking water | Gestation and lactation | Rat | Not investigated | Elevated systolic blood pressure, increased reactivity to noradrenaline in adolescent and adult male offspring | Only male offspring were evaluated | [97] |
| 0.05% Pb acetate in drinking water | 1 week prior to mating until day 11.5 gestation | Mouse | Not investigated | Abnormal angiogenesis in yolk sac of embryos | Not investigated | [98] |
| 2.1 (low), 16 (medium), or 32 (high) ppm Pb acetate in drinking water | 2 weeks prior to mating through gestation and lactation | Mouse | Not investigated | Increased food intake and energy expenditure in both sexes in adulthood, increased body weight, body fat, and insulin response in males | Yes | [100] |
| 12 mg of Pb acetate per ml or sodium acetate control in drinking water | 30 days prior to breeding until 21 days of age | Rat | Not investigated | Delayed timing of puberty | Only female offspring were evaluated | [101] |
| 300 mg/l Pb acetate in drinking water | Gestation and lactation | Rat | Not investigated | Elevated blood glucose at 12 and 21 days post-natal | Not reported | [102] |
| 100 mg Pb/kg diet | 8 weeks treatment starting at 35 days of age | Rabbit | Not investigated | Reduced relative heart weight, increased creatine phosphokinase | Only males were evaluated | [103] |
| 1% Pb acetate in drinking water | 12 and 40 day treatments starting at 4-5 weeks of age | Rat | Increased levels of acetylated histone H3 in abdominal and thoracic aortas and cardiac tissue | Increased proliferating cell nuclear antigen in cardiac tissue, enlarged cardiac cells after 40 days, elevated blood pressure during first 17 days, altered internal elastic lamina of aorta | Not reported | [104] |
| 60 ppm of Pb acetate in drinking water | Weaning until 10 months of age | Rat | Not investigated | Increased diastolic and systolic blood pressure, increased cardiac inotropy | Only males were evaluated | [105] |
| Blood Pb levels in preschool | Early childhood | Human | Not investigated | Smaller left ventricle (intraventricular septum, left ventricular posterior wall, and left ventricular mass index), impaired left ventricular systolic function (fractional shortening and ejection fraction), increased inflammatory cell counts in both sexes | Effects observed in both males and females | [109] |
| Maternal hair Pb levels | Gestation | Human | Not investigated | Increased risk of congenital heart defects (septal, conotruncal, right-sided obstructive, left-sided obstructive, anomalous venous return, and others) | Not reported | [110] |
| Parental hair, toenail, and tooth Pb levels | Gestation | Human | Not investigated | Increased Pb levels in parents of children with congenital heart defects | Not reported | [111] |

(continued)
### Table 3: (Continued)

| Exposure details                                                                 | Window of exposure | Model         | Epigenetic/molecular effect                  | Phenotype                                                                 | Sex specificity                     | Reference |
|--------------------------------------------------------------------------------|--------------------|---------------|----------------------------------------------|---------------------------------------------------------------------------|-------------------------------------|-----------|
| Blood Pb levels in pregnant women at 17–40 weeks gestation                      | Gestation          | Human         | Not investigated                             | Increased blood Pb levels in mothers of children with congenital heart defects (conotruncal defects, right ventricle outflow tract obstructions, and septal defects) | Not reported                        | [112]     |
| Pb levels in first meconium samples of newborns                                | Gestation          | Human         | Not investigated                             | Increased Pb levels in meconium of infants with conotruncal heart defects  | Not reported                        | [113]     |
| Pb levels in blood of mothers with infants under 6 months of age                | Gestation          | Human         | Not investigated                             | Increased blood Pb levels in mothers of infants with congenital heart defects | Effects observed in both males and females | [114]     |
| Pb levels in prenatal cord blood and early childhood                            | Gestation and early childhood | Human         | Not investigated                             | Elevated cord blood Pb associated with increased systolic blood pressure; elevated early childhood Pb associated with increased peripheral vascular resistance and decreased stroke volume in response to acute stress | Not reported                        | [115]     |
| Blood Pb levels in childhood (9–11 years of age)                                | Early childhood    | Human         | Not investigated                             | Impaired response to cardiovascular stress (reduced stroke volume and increased peripheral vascular resistance) | Not reported                        | [116]     |
| Pb levels in maternal toenail samples collected at ∼28 weeks gestation and/or 6 weeks postpartum | Early and late prenatal periods | Human         | Not investigated                             | Early prenatal Pb exposure associated with significantly higher systolic blood pressure in children (mean age 5.5 years), with stronger effects among boys compared to girls | Yes                                 | [47]      |
| Blood Pb levels in pregnant women in second trimester                           | Gestation          | Human         | Not investigated                             | Higher maternal blood Pb associated with higher risk of elevated systolic blood pressure in children 4–6 years of age who had short gestation | Not reported                        | [117]     |
| Pb in maternal urine at ∼12 weeks gestation                                     | Gestation          | Human         | Not investigated                             | Increased risk of elevated blood pressure in early childhood (4–11 years of age), particularly in combination with molybdenum | Not reported                        | [118]     |
| Maternal blood Pb concentrations at gestational weeks 14 and 30                  | Gestation          | Human         | Not investigated                             | Late gestation Pb associated with reduced kidney volume in children ∼4.5 years of age, with a stronger association in girls | Yes                                 | [120]     |
| Maternal blood Pb levels                                                        | Gestation          | Human         | Not investigated                             | Lower Z-scores for total cholesterol, LDL and HDL in boys with maternal blood Pb levels ≥ 5 μg/dl | Yes—No effects noted in girls       | [121]     |
| Exposure details | Window of exposure | Model | Epigenetic/molecular effect | Phenotype | Sex specificity | Reference |
|------------------|--------------------|-------|----------------------------|-----------|----------------|-----------|
| Pb concentrations in maternal urine at gestational week 8 and erythrocytes at gestational week 14 | Early gestation | Human | Significant inverse association between urinary Pb and cord blood DNA methylation at nine CpGs, including several in Glycoprotein 6 gene | Not investigated | Effects observed in both sexes | [129] |
| Pb levels in prenatal maternal red blood cells (average 27.9 weeks gestation) and cord blood | Mid-late gestation | Human | Significant negative association between maternal RBC Pb and cord blood DNA methylation at four CpGs in both sexes; several differentially methylated CpGs specific to males and females, including NOTCH1 and GNAS in females | Not investigated | Yes—More significantly differentially methylated CpGs in female vs. male infants | [132] |
| Maternal blood Pb at ~12 weeks gestation | Early gestation | Human | Hypermethylation of the MEG3 imprinted regulatory domain in umbilical cord blood | Lower birth weight and rapid gains in adiposity at 2–3 years of age | Not reported | [133] |
| Serial blood Pb samples from birth to 78 months of age | Early childhood | Human | Mean blood Pb levels in childhood associated with a significant decrease in DNA methylation at IGF2/H19 and PEG3 loci and increased methylation at PLAG1/HYMA1 in peripheral blood in adulthood | Yes—Effects on PEG3 stronger in males; effects on IGF2/H19 stronger in females | Yes | [136] |
| 0, 100, or 500 ppb of Pb in petri dish water from <1 to 2 until 72h post-fertilization (end of embryogenesis) | Embryogenesis | Zebrafish | Significant reduction in global DNA methylation in whole embryos at both doses; reduced transcript expression of dmnt3 and dmnt4 (human DNMT3B ortholog) | Not investigated | Not reported | [138] |
| 0, 2, 10, and 17 μg/l of Pb(NO3)2 in culture plate | 3–4 h to 7 days post-fertilization | Zebrafish | Down-regulation of Dnmt1 and Dnmt3b transcript expression in embryos | Increased malformations and decreased heart rate with Pb in combination with elevated temperature | Not reported | [139] |
| Pb acetate in drinking water | 2 weeks prior to mating, through gestation and lactation | Mouse | Sex-specific differential methylation of hundreds of CpGs and 1000 base pair regions in male and female offspring hearts in adulthood | Not investigated | Yes | [146] |
is evidence that Pb alters normal cardiac development. Children exposed to Pb in e-waste had smaller left ventricles, decreased left ventricular function, and increased markers of inflammation, effects that were independent of sex [109]. Maternal Pb exposure has also been associated with congenital heart defects in several studies, although sex differences were not investigated [110-113], or effects did not differ by sex [114]. Cardiovascular function and reactivity are tightly regulated by the autonomic nervous system, and several lines of evidence link Pb exposure to altered autonomic regulation. Blood Pb levels were associated with significant autonomic and cardiovascular dysregulation in response to acute psychological stress in children, with some sex differences [115, 116]. Like adults, studies have also demonstrated that Pb exposure may lead to increased blood pressure in children. In utero exposure to Pb is associated with increased baseline blood pressure in children [47, 117, 118], with sex-dependent [47] and independent [117] associations observed. Importantly, some reported effects of Pb were significant at Pb levels well below 10μg/dl, levels that are still prevalent in the USA, particularly among urban minority populations [47, 117, 119]. Cardiovascular and renal functions are intimately linked, and Pb exposure was linked to altered kidney volume in children, particularly in females [120]. Finally, Pb may also impact cardiac health through alterations in blood lipids, an effect observed only in male children [121]. Although developmental Pb exposure has been linked to neurological defects later in life [5], the effects of early Pb exposure on adult cardiovascular health have not been investigated. Importantly, individuals in the USA who were highly exposed to Pb as children in the 1960s, 1970s, and early 1980s [122] are now in middle age, a stage of life when cardiovascular diseases emerge. It is thus imperative that the scientific community better understand the effects of early Pb exposure on long-term cardiovascular health.

**Effects of developmental Pb exposure on the epigenome**

Pb exposure throughout the life course has been linked to alterations in DNA methylation, histone modifications, and non-coding RNAs in various tissues [123]. Significant evidence also links developmental Pb exposure to epigenetic alterations in offspring. Human studies investigating the effects of developmental Pb exposure on the epigenome have focused primarily on the measurement of Pb in maternal blood, maternal tibia or patella, cord blood, or neonatal blood spots as proxies for Pb exposure during pregnancy. These studies have repeatedly demonstrated that Pb exposure is associated with changes in DNA methylation and hydroxymethylation in offspring blood [62, 124-126], and sex differences have been reported [125-127]. Moreover, recent work suggests that maternal Pb-induced changes in DNA methylation may be transmitted to grandchildren [128]. Few studies to date, however, have investigated the effects of Pb on the epigenome in the context of the cardiovascular system and cardiovascular disease. Pb-associated changes in DNA methylation have been identified at genes relevant to cardiovascular disease, although the long-term implications of these findings for cardiovascular health are unclear. For example, developmental Pb exposure leads to a sex-independent reduction in methylation of the glycoprotein VI gene, which plays an important role in platelet activation and blood clotting [129]. Likewise, maternal blood Pb exposure was associated with altered cord blood DNA methylation at numerous CpGs, particularly in female infants, including several associated with cardiovascular development and diseases such as NOTCH1 and GNAS [130-132].

Developmental Pb exposure-induced changes in DNA methylation at imprinted genes, which play a critical role in growth and cardiometabolic health, have also been reported. In addition to changes in DNA methylation at the imprinted GNAS locus noted above [132], maternal blood Pb levels were associated with altered DNA methylation of the MEG3 imprinted gene in cord blood, concomitant with reduced birth weight and rapid increases in adiposity, both of which are associated with long-term risk of cardiovascular disease [133]. Given the small sample size, sex differences were not identified. Roles for MEG3 have been identified in the regulation of cardiac and vascular remodeling in the context of cardiac fibrosis and angiogenesis [134, 135]. Prenatal Pb exposure is also associated with changes in DNA methylation in the imprinted gene IGF2 [136], which have also been implicated in cardiovascular disease [137].

Perinatal Pb exposure has also been linked to epigenetic changes in animal models, although effects on cardiovascular tissues have not been adequately investigated. Pb exposure affects Dnmt expression and activity in zebrafish embryos [138, 139]. In particular, Pb exposure and thermal stress cooperate to affect heart rate in zebrafish embryos, concomitant with altered expression of Dnmt1 and Dnmt3b [139]. Although these studies did not address cardiovascular disease directly, modulation of the functions of Dnmt1, Dnmt3a, and Dnmt3b has been linked to atherosclerosis and heart failure in animal models [140, 141], and single nucleotide polymorphisms in Dnmt1 are associated with coronary artery disease risk in humans [142]. In mice, Pb exposure during pregnancy and lactation led to sex and tissue-specific alterations in DNA methylation at murine IAP transposons, although heart tissue was not examined [143]. Given the established role for Pb as a neurotoxicant, multiple studies have demonstrated that perinatal Pb exposure causes epigenetic changes in rodent brain [144, 145]. Few studies, however, have investigated the effects of Pb on the cardiac epigenome. We recently discovered that perinatal Pb exposure results in sex-specific changes in DNA methylation in the hearts of offspring mice that are present in adulthood, long after cessation of the exposure [146]. The implications of these changes for cardiac health are under investigation.

**Developmental PFAS exposure and cardiovascular health**

PFAS are a large family of manmade chemicals with a common structure, consisting of a chain of carbon atoms bound to fluorine atoms, with a functional group at the end of the molecule [147]. Because of the strength of the carbon fluoride bond, PFAS are environmentally persistent, with long half lives in the human body and in the environment [147]. PFAS are widely used in the design of consumer products that resist grease, water, stains, and oil, as well as firefighting foams and some cosmetics [147]. Humans are exposed to PFAS primarily through ingestion of food and water, inhalation of indoor air, and contact with other contaminated sources [147]. PFAS chain lengths vary, with longer chain chemicals exhibiting longer half lives in the human body [147]. The health effects of longer chain legacy PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are the most extensively studied, while the effects of shorter chain PFAS on human health are still poorly understood. A summary of the cardiovascular and epigenetic effects of developmental PFAS exposures can be found in Tables 5 and 6, respectively.
| Exposure details | Model | Ectopic/molecular effect | Phenotype | Sex specificity | Reference |
|------------------|-------|--------------------------|-----------|----------------|-----------|
| PFOA at dose of 2 mg/kg egg weight injected into eggs | Chicken | Not investigated | Embryonic days 0–10 | Not investigated | [148] |
| PFOA at doses of 0, 0.5, 1, and 2 mg/kg of egg weight injected into eggs | Chicken | Not investigated | Embryonic days 0–19 | Not investigated | [149] |
| PFOA (2 mg/kg) or HFPO-DA (1, 2, 4, and 8 mg/kg) injected into eggs | Chicken | Not investigated | Embryonic days 0–21 | Not investigated | [150] |
| PFOS in doses 10 μM to 2 mM | Xenopus | Not investigated | Embryonic days 0–10 | Not investigated | [151] |
| PFOA 1, 10, and 20 mg/kg via intraperitoneal injection | Mouse | Not investigated | Gestational days 5–9 | Not investigated | [153] |
| 1, 25, 50, and 100 μg/l PFOA in culture medium | Zebrafish | Not investigated | 2–30 h post-fertilization | Not investigated | [154] |
| Six PFAS (Me-PFOSA-AcOH, PFDA, PFNA, PFOS, PFOA, and PFHxS) in maternal serum during third trimester | Human | Not investigated | Late gestation | Not investigated | [155] |
| Levels of 10 PFAS (PFOS, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFDoA, PFBS, PFHpA, and PFOSA) in maternal blood during early pregnancy | Human | Not investigated | Early gestation | Not investigated | [156] |

(continued)
Table 5: (Continued)

| Exposure details                                                                 | Window of exposure | Model  | Epigenetic/molecular effect | Phenotype                                                                                                                                                                                                 | Sex specificity | Reference |
|---------------------------------------------------------------------------------|--------------------|--------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-----------|
| First trimester maternal plasma levels of PFOA, PFOS, and PFHxS                 | Early gestation    | Human  | Not investigated            | Increased risk of preeclampsia with PFHxS in women carrying female fetuses; increased risk of hypertension with PFOS and PFHxS in women carrying male fetuses                                                             |                 | [160]     |
| Levels of 27 PFAS in maternal blood and cord blood plasma before and during delivery | Late gestation  | Human  | Not investigated            | Increased odds of septal defects with exposure to 6 m-PFOS or PFDA in maternal blood; increased odds of conotruncal defects with exposure to PFOS or PFDoA in maternal blood                                             |                 | [165]     |
| Estimated serum PFOA concentration during pregnancy                             | Gestation          | Human  | Not investigated            | Weak association between maternal serum PFOA concentrations and congenital heart defects                                                                                                                   |                 | [166]     |
| Estimated serum PFOA concentration during pregnancy                             | Gestation          | Human  | Not investigated            | No significant association between maternal serum PFOA concentrations and congenital heart defects                                                                                                         |                 | [167]     |
| PFOS exposure, assessed by Little Hocking Water Association service category    | Gestation          | Human  | Not investigated            | No significant association between maternal PFOS exposure and congenital defects of any kind                                                                                                                 |                 | [168]     |
| Serum levels of 12 PFAS in children between 12 and 20 years of age              | Adolescence        | Human  | Not investigated            | Strong positive association between PFOS and diastolic blood pressure in males in linear model, but no significant association in non-linear model                                                              | Yes—Effects in males but not females | [48]      |
| Serum levels of PFOA, PFOS, PFHxS, and PFNA in obese children 8–12 years of age | Pre-adolescence    | Human  | Not investigated            | Risk of elevated systolic blood pressure and LDL/total cholesterol with PFNA, elevated LDL cholesterol with PFOA and PFOS; elevated total cholesterol with PFOA                                                                 | No              | [169]     |
| Serum concentrations of 18 PFAS in adolescents aged 15–19 years                  | Adolescence        | Human  | Not investigated            | Positive association between PFOS, PFNA, PFDA, PFUnDA, and apolipoprotein B, total- and LDL cholesterol; positive association between total PFAS, PFOS, PFNA, PFDA, PFUnDA, and risk of dyslipidemia; positive association between total PFAS, PFHxS, PFOS, PFOA, and hypertension; positive association between PFHpS and PFHxS and risk of obesity | Not reported    | [170]     |
| Levels of PFHxS, PFOS, PFOA, and PFNA in first trimester maternal plasma        | Early gestation    | Human  | Not investigated            | Positive association between prenatal PFHxS and triglycerides; positive association between PFNA and cardiometabolic risk score at 4 years of age                                                                 | No              | [171]     |
| Serum concentrations of 12 PFAS in adolescents aged 14–19 years or children aged 8–11 years | Childhood and adolescence | Human | Not investigated            | Increased total and LDL cholesterol with all PFAS in adolescents; increased total, LDL and HDL, cholesterol with PFOS and PFNA in children; increased HDL cholesterol with PFOA and PFHxS in children; lower BMI z-score with higher concentrations of PFAS | Yes—Stronger associations observed in girls | [172]     |
| Levels of 5 PFAS in cord blood in World Trade Center birth cohort               | Gestation          | Human  | Not investigated            | Higher total cord blood lipids with PFOS, PFOA, and PFHxS levels; higher total cholesterol and lower triglycerides with PFDS; higher triglycerides with PFOA and PFHxS exposure                                                                 | Not reported    | [173]     |

(continued)
| Exposure details                                                                 | Window of exposure               | Model | Epigenetic/molecular effect                  | Phenotype                                                                                                                                                                                                 | Sex specificity | Reference |
|--------------------------------------------------------------------------------|----------------------------------|-------|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-----------|
| Blood PFOS, PFHxS, and PFOA levels in children 8–14 years of age               | Childhood-adolescence            | Human | Not investigated                            | Increased blood glucose (2 h oral glucose tolerance test) with PFOA and PFHxS, or increased glucose area under the curve for PFHxS; significant alterations in lipids and amino acids with PFAS exposure                                 | No              | [174]    |
| PFAS levels in maternal plasma (median 9.7 weeks gestation) and in children (median 7.7 years of age) | Early gestation and childhood    | Human | Not investigated                            | Higher levels of PFOS, PFOA, PFDeA associated with higher total cholesterol and/or LDL cholesterol among girls; higher HDL cholesterol with PFAS among boys and girls                                                       | Yes             | [175]    |
| Levels of PFAS (PFOS, PFOA, PFNA, PFDA, and PFHxS) in 9-year-old children from the European Youth Heart Study | Childhood                        | Human | Not investigated                            | Inverse correlation between PFOA, PFDA, PFHxS, and leptin; positive and negative associations between adiponectin and PFOA or PFHxS, respectively, in boys                                                 | Yes             | [176]    |
| Maternal serum PFOA levels at gestational week 30                               | Late gestation                   | Human | Not investigated                            | Increased risk of overweight or obesity, larger waist circumference, serum insulin, and leptin with PFOA exposure in girls at 20 years of age                                                                         | Yes             | [177]    |
| Maternal PFOA and PFOS levels in serum at gestational age 24±10 weeks           | Mid-gestation                    | Human | Not investigated                            | Increased risk of waist to height ratio > 0.5 with PFOA or PFOS at 5–9 years of age, with the PFOS association slightly greater in Greenlandic girls than boys                                                 | Yes             | [178]    |
| Levels of PFOS and PFOA in newborn dried blood spots                            | Late gestation                   | Human | Not investigated                            | Lower BMI with PFOA and PFOS, with stronger associations observed for girls                                                                                                                                   | Yes             | [179]    |
| Levels of PFOS and PFOA in maternal plasma during 1st and 2nd trimester, and cord blood | Gestation                        | Human | Not investigated                            | Inverse association between maternal PFOA, PFOS, and children’s weight and body mass index in first year of life in boys, with no association observed in girls                                                   | Yes             | [180]    |
| Levels of PFOA and four other long-chain PFAS in maternal serum during 3rd trimester | Late gestation                   | Human | Not investigated                            | Inverse association between PFNA, PFDeA, PFUnDA, PFDoDA, and birth weight in girls; increased odds of small for gestational age with PFDeA, PFUnDA in girls; lower average height z-score with PFDeA, PFUnDA, PFDoDA; reduced childhood height in boys with PFNA, PFDoDA exposure | Yes             | [181]    |
| Maternal plasma levels of PFOS and PFOA during 1st and 2nd trimester           | Early gestation                  | Human | Not investigated                            | No significant associations between maternal PFOS, PFOA and body mass index, risk of overweight, and waist circumference at 7 years of age                                                                                 | Not reported    | [182]    |
| Levels of PFOS, PFOA, and PFHxS in maternal serum, median gestational age 15 weeks | Early-mid gestation              | Human | Not investigated                            | Inverse relationship between PFAS and birth weight; positive relationship between PFOA and body weight at 20 months of age                                                                                     | Only females were evaluated | [183]    |
Cardiovascular effects of developmental PFAS exposure in animals

Numerous animal studies, conducted in rodents, zebrafish, Xenopus, and chicken embryos, have demonstrated that developmental PFAS exposures are associated with adverse cardiovascular outcomes. PFOA exposure in chicken embryo cardiomyocytes (exposures occurring while in the embryo or ex-vivo) led to reduced viability, altered cell morphology, and de-regulated intracellular calcium levels [148]. Additional work demonstrated that developmental exposure to PFOA, or the PFOA replacement, hexafluoropropylene oxide dimer acid (HFPO-DA, GenX) in chicken embryos led to altered cardiac function and morphology [149, 150]. Developmental PFAS exposures have been shown to cause cardiac developmental defects in Xenopus embryos [151] or in weanling rats [152] or mice [153], although the sex-specific effects of these chemicals were not investigated. At a molecular level, single cell analysis of zebrafish embryos revealed that developmental PFOA exposure disrupts the expression of genes associated with cardiac contractility and differentiation in the cardiac cell population [154]. Additional work has demonstrated that gestational exposure to PFAS alter maternal and offspring lipids, with potential implications for long-term cardiovascular health. Gestational GenX exposure led to altered maternal and offspring glucose and lipid metabolism in rats, although sex-specific effects were unclear [155]. Low-dose developmental PFOA exposure in female mice led to an increased rate of weight gain and an increase in serum leptin and insulin in mid-life [156]. Collectively, these data suggest that PFAS exposures in early life may have adverse cardiovascular consequences. However, given substantial differences in PFAS dosing and toxicokinetics between humans and animals, the drawing of direct parallels between species must be done with caution.

Cardiovascular effects of developmental PFAS exposure in humans

Early life PFAS exposures are linked to adverse cardiovascular effects in humans, although some studies have produced conflicting and difficult to interpret findings. Several endpoints have been investigated, including maternal metabolic and cardiovascular effects, as well as offspring congenital heart defects, blood pressure, blood glucose, lipid profiles, levels of hormones regulating appetite and obesity, BMI, and growth. In pregnant women, PFAS exposures are linked to adverse metabolic and cardiovascular effects. Maternal levels of PFAS are positively associated with total cholesterol and triglycerides [157], as well as higher blood glucose and increased risk of gestational diabetes mellitus [158]. PFAS exposures during pregnancy are also associated with an increased risk of hypertension or preeclampsia [159, 160], with differences observed based on the sex of the fetus [160]. Although the implications for the long-term health of the offspring are unclear, adverse effects of maternal diabetes, hyperglycemia, preeclampsia, and high cholesterol on offspring cardiovascular health have been reported in human observational studies and experimental models [14, 15, 161-164].

Table 6: developmental PFAS exposure and the epigenome in the context of cardiovascular health

| Exposure details | Window of exposure | Model | Epigenetic/molecular effect | Phenotype | Sex specificity | Reference |
|-----------------|-------------------|-------|-----------------------------|-----------|----------------|-----------|
| PFOS and PFOA concentrations in maternal serum | Gestation | Human | In cord blood, 4 differentially methylated CpGs met FDR cutoff; several CpGs showed changes that overlapped between 2 cohorts; 1 differentially methylated region identified met FWER cutoff; several CpGs, regions map to CVD-relevant genes, including ZFP57, NTN-1 | Not investigated | Not reported | [187] |
| Cord blood levels of PFOS, PFOA, PFHxS, PFBA, and PFNA | Gestation | Human | In cord blood, 10598 differentially methylated CpGs with PFOS in males, linked to pathways including cardiac proliferation | Not investigated | Yes—15% of male-specific CpGs linked to X chromosome | [192] |
| PFOS and PFOA levels in umbilical cord serum | Gestation | Human | Inverse correlation between serum PFOA and global DNA methylation in umbilical cord serum | Not investigated | Not reported | [193] |
| Concentrations of PFOA, PFOS, PFNA, and PFUA in cord blood | Gestation | Human | Inverse association between PFOS and Alu methylation in cord blood | Not investigated | Not reported | [194] |
| Levels of PFOA and PFOS in newborn dried blood spots | Gestation | Human | In dried blood spots, >90th percentile PFOA concentration correlated with DNA methylation CpG near SCRT2, SRXN1; >90th percentile PFOS concentration was related to a CpG in GVIN1 in boys and a CpG in ZNF26 in girls). Log-scaled, continuous PFOS concentration associated with methylation of CpG in PTBP1, a gene associated with cardiovascular disease and development | Not investigated | Yes | [195] |
An increasing number of studies have begun to assess the effects of PFAS on children and adolescents. With regard to PFAS and congenital heart defects in humans, data are limited, with conflicting results. One study found an association between maternal levels of several PFAS and congenital heart defects in both sexes [165], while a second study also found a weak association between PFOA and congenital heart defects [166]. However, other studies have found no association [167, 168]. Effects of PFAS on blood pressure have also been reported, with several studies showing modest increases in blood pressure with PFAS exposure. For example, PFAS have been associated with increased blood pressure in several different populations of children and adolescents, with some studies identifying sex differences [48] and others not [169, 170]. Other studies, however, have observed no effects on blood pressure [171, 172].

PFAS exposures at multiple time points during early life have also been linked to altered blood glucose, lipids, and metabolic homeostasis in children and adolescents. Maternal blood levels of several PFAS were linked to increased lipids in cord blood [173]. Gestational exposure to PFOA and perfluorohexanesulfonic acid (PFHxS) were associated with unfavorable cardiometabolic risk scores in adolescence, driven by altered insulin levels and insulin resistance, blood pressure, waist circumference, and other factors [27]. Several studies have demonstrated that blood PFAS levels in children and adolescents are associated with increased blood glucose and/or alterations in lipids and amino acids [170, 174, 175], with some investigations identifying sex differences [175]. Other researchers found no effects of PFAS on blood lipids or glucose [171]. PFHxS levels in children were associated with altered levels of adipokines, including adiponectin and leptin, with effects on adiponectin found only in boys [176]. Effects of developmental exposure to various PFAS on childhood BMI and growth have been investigated, with PFAS being associated with increased BMI or growth [177, 178], decreased BMI or growth [179-181], no change [182], or effects that differed based on age [183]. Sex differences in the effects of these chemicals on BMI have been reported [177, 180].

Conflicting research findings into the effects of PFAS on cardiovascular health are likely due to differences in the toxicokinetics and toxicodynamics of the various PFAS, the timing of outcome measurement (pre vs. post-puberty), the timing of exposure, the experimental methods employed, sample sizes, as well as lifestyle habits and other confounding factors among the populations studied. Therefore, further investigations into the effects of developmental PFAS on human cardiovascular health are necessary.

**Effects of developmental PFAS exposure on the epigenome**

A relatively modest number of studies have examined the effects of PFAS exposures on the epigenome. Moreover, the effects of PFAS on the cardiac epigenome, and the implications these changes have for cardiovascular disease, have not been investigated. Studies into the epigenetic effects of PFAS have been conducted in cells in vitro, in animal models, and in human populations, with the overwhelming majority of the research focused on DNA methylation. Likewise, most studies thus far have focused on legacy PFAS such as PFOS and PFOS, and very little published work to date has examined newer generation PFAS.

Several human studies have demonstrated associations between blood levels of PFAS and DNA methylation at various loci, including LINE-1 elements, which are linked to cardiovascular diseases [184]. Notably, recent work has also linked PFAS exposures to altered expression of miRNAs, where exposure in women was associated with changes in miRNAs that have functions in cardiovascular and Alzheimer’s diseases [185]. Among the predicted target genes for the miRNAs was DNMT3A, suggesting potential crosstalk between miRNA and DNA methylation [185]. A second study in this group identified PFAS-associated changes in DNA methylation in several biological pathways, including cardiac hypertrophy [186]. Investigations into the effects of developmental exposure to PFAS on the human epigenome have utilized maternal or offspring blood, cord blood, and blood spots. PFAS levels in maternal serum were associated with changes in DNA methylation in cord blood at several loci implicated in cardiovascular disease and development [187-191]. Additional work has produced similar findings in cord blood global or site-specific DNA methylation [192-194], with some studies identifying sex differences [192] and others finding no evidence of sex specificity [193, 194]. Concentrations of PFOS and PFOA in infant blood spots were associated with sex-specific changes in DNA methylation at a small number of loci, including the gene FKBPI, related to cardiovascular disease and development [195].

Several additional studies in vitro and in animal models have demonstrated PFAS-induced epigenetic changes; however, few investigations into the effects of PFAS on the cardiac epigenome have been conducted thus far. Some in vitro evidence suggests that PFAS exposures moderate the epigenome in undifferentiated cells. PFOS exposure leads to increased oxidative stress, altered expression of DNA methyltransferases and sirtuins, and decreased DNA methylation in human trophoblast cells [196]. Likewise, PFOS exposure altered DNA methylation in differentiating adipocytes [197]. In mouse embryo bodies, PFOS increased expression of the Polycomb complex members and markers of pluripotency and decreased expression of factors associated with differentiation [198]. Exposure to a mixture of PFAS along with the polychlorinated biphenyl PCB126 led to increased expression of dnmt1 in zebrafish embryos [199]. The effects of these chemicals on cardiac differentiation in vitro or in vivo, however, have not been investigated.

**Developmental phthalate exposure and cardiovascular health**

Phthalates are a large class of chemicals that are divided into two groups based on their molecular weight and chemical properties. High molecular weight phthalates are found in medical tubing, food packaging, vinyl toys, and building products, while low molecular weight phthalates are found in personal care products such as perfumes, lotions, nail polish, and shampoos [200]. Human exposure to phthalates occurs through inhalation, skin absorption, ingestion, and intravenous injection [200]. Phthalate exposures are linked to a wide array of adverse metabolic, neurodevelopmental, reproductive, and cardiovascular health effects [201]. A summary of the cardiovascular and epigenetic effects of developmental phthalate exposures can be found in Tables 7 and 8, respectively.

**Cardiovascular effects of developmental phthalate exposure in animals**

Animal and in vitro studies provide substantial evidence that phthalate exposures are deleterious to cardiovascular health. In mice, maternal exposure to diethylhexyl phthalate (DEHP) caused cardiac developmental defects [25, 202]. Work in zebrafish yielded similar findings, where exposure to DEHP [203], butyl benzyl phthalate (BBP) [204], or dibutyl phthalate (DBP) [205] during
### Table 7: Developmental phthalate exposure and cardiovascular health

| Exposure details                                                                 | Window of exposure                  | Model                     | Epigenetic/molecular effect | Phenotype                                                                 | Sex specificity   | Reference |
|---------------------------------------------------------------------------------|-------------------------------------|---------------------------|-----------------------------|---------------------------------------------------------------------------|-------------------|-----------|
| DEHP (500 mg/kg) during pregnancy                                               | Embryonic days 6.5–14.5             | Mouse                     | Not investigated            | Reduced fetal weight, increased septal defects, and ventricular myocardium noncompaction | Not reported       | [202]    |
| DEHP (0.02 pg) injected into the yolk sac of the embryo                          | 1–4 days post-fertilization         | Zebrafish                 | Not investigated            | Increased mortality at 2 and 3 days post-fertilization; reduced heart rate at 3 and 4 days post-fertilization; pericardial edema and heart looping disorder 3 days post-fertilization | Not reported       | [203]    |
| Benzyl butyl phthalate (0.03, 1.9, and 3.8 μM) in petri dish water              | 4–72 h post-fertilization           | Zebrafish                 | Not investigated            | Dose-dependent cardiac malformations (pericardial edema, elongated and string-shaped heart, looping abnormalities), reduced heart rate | Not reported       | [204]    |
| Dibutyl phthalate (0.36, 1.8, and 3.6 μM) in petri dish water                  | 4–72 h post-fertilization           | Zebrafish                 | Not investigated            | Dose-dependent increase in pericardial edema, structural abnormalities; reduced heart rate at highest dose | Not reported       | [205]    |
| DEHP administered to dams (0, 1, 10, and 100 mg/kg/day via oral gavage)         | Lactation—postpartum days 1–21      | Rat                       | Not investigated            | Dose-dependent increased fasting blood glucose; decreased insulin receptor expression and insulin receptor substrate phosphorylation in female hearts at postnatal day 60 | Only females were evaluated | [206]    |
| DEHP administered to dams (1, 10, and 100 mg/kg/day) or olive oil control by oral gavage | Lactation—postpartum days 1–21      | Rat                       | Not investigated            | Increased blood glucose, decreased glucose uptake and oxidation, and decreased insulin receptor expression in male hearts at postnatal day 22 | Only males were evaluated | [207]    |
| DEHP (1, 10, 100, and 300 mg/kg) administered intragastric under anesthesia     | 5–6 weeks of age, for a period of 35 days | Mouse                    | Not investigated            | Inhibition of fatty acid beta oxidation and TCA cycle; increased glycolysis; inhibition of synthesis and transport of fatty acids in hearts of male mice | Only males were evaluated | [208]    |
| DEHP (50 and 100 μg/mL) or DMSO vehicle in culture medium                        | 72 h                                | Neonatal rat cardiomyocytes from 1-day-old rats | Not investigated | Increase in cardiomyocyte fatty acid transport and beta oxidation; increased expression of genes associated with fatty acid transport and beta oxidation | Not reported       | [209]    |
| DEHP (300 mg/kg/day) by oral gavage                                             | Gestational day 14 until birth       | Rat                       | Not investigated            | Decreased activity in post-natal day 60 offspring, decreased activity and blood pressure in post-natal day 200 offspring | Only males were evaluated | [210]    |
| Phthalate exposure estimated via job exposure matrix                             | Reported occupational exposure during periconceptional period | Human                    | Not investigated            | Maternal phthalate exposure associated with perimembranous ventricular septal defect, patent ductus arteriosus, secundum atrial septal defect, and pulmonary valve stenosis; paternal exposures associated with perimembranous ventricular septal defect and pulmonary valve stenosis | Not reported       | [215]    |
| Exposure details                                           | Window of exposure                                      | Model   | Epigenetic/molecular effect | Phenotype                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Sex specificity | Reference |
|-----------------------------------------------------------|---------------------------------------------------------|---------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-----------|
| Phthalate exposure estimated via job exposure matrix      | Reported occupational exposure during periconceptional period | Human   | Not investigated            | Paternal phthalate exposure associated with increased incidence of congenital heart defects; no significant associations observed for maternal exposures                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Not reported    | [216]     |
| Maternal urinary phthalate metabolites in 1st, 2nd, and 3rd trimester | Gestation                                               | Human   | Not investigated            | Increased phthalic acid in 1st trimester associated with increased pericardial fat index at 10 years of age                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Effects observed in both sexes | [217]     |
| Maternal urinary phthalates in 1st, 2nd, and 3rd trimester | Human                                                   | Human   | Not investigated            | 3rd trimester high molecular weight phthalates associated with lower systolic and diastolic blood pressure in girls (mean age 9.7 years); no associations observed for boys                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Yes             | [46]      |
| Urinary phthalate metabolites in children 6–8 years of age | Childhood                                               | Human   | Not investigated            | Significant positive association between several phthalate metabolites and blood pressure z-score, pulse pressure, mean arterial pressure; monomethyl phthalate associated with increased risk of high blood pressure                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Not reported    | [218]     |
| Urinary metabolite concentrations of three phthalates (low molecular weight, high molecular weight, and DEHP) | Childhood, adolescence—ages 6–19                        | Human   | Not investigated            | Positive association between metabolites of DEHP and systolic blood pressure                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Not reported    | [219]     |
| Maternal urinary phthalate concentrations in 1st, 2nd, and 3rd trimester | Gestation                                               | Human   | Blood leukocyte DNA methylation at H19 or HSD11B2 do not mediate association between phthalate exposure and adiposity | 1st and 2nd trimester phthalate metabolites positively (MBP and MIBP) or negatively (MBzP) associated with adiposity in adolescent girls; 2nd trimester MBzP associated with increased BMI and waist circumference in boys Early gestational phthalate metabolites (monobenzyl, monocarboxyoctyl, and monocarboxynonyl phthalate) negatively associated with 8-isoprostane at 9 years of age; early gestational mono(2-ethylhexyl) and mono(2-ethyl-5-carboxypentyl) phthalate positively associated with 8-isoprostane at 14 years of age | Yes             | [220]     |
| Maternal urinary phthalate metabolites at 13 and 26 weeks gestation | Early-mid gestation                                     | Human   | Not investigated            | Early gestational phthalate metabolites (monobenzyl, monocarboxyoctyl, and monocarboxynonyl phthalate) negatively associated with 8-isoprostane at 9 years of age; early gestational mono(2-ethylhexyl) and mono(2-ethyl-5-carboxypentyl) phthalate positively associated with 8-isoprostane at 14 years of age | Not reported    | [221]     |
## Table 8: Developmental phthalate exposure and the epigenome in the context of cardiovascular health

| Exposure details | Window of exposure | Model | Epigenetic/molecular effect | Phenotype | Sex specificity | Reference |
|------------------|--------------------|-------|----------------------------|-----------|----------------|-----------|
| Maternal urinary phthalate concentrations at 13 and 26 weeks gestation | Early-mid gestation | Human | Monoethyl phthalate levels in early and mid-gestation associated with Alu repeat methylation in cord blood; association with LINE-1 methylation present but weaker; increased concentration of di-(2-ethylhexyl) phthalate metabolites mid gestation associated with reduced methylation of Alu repeats in blood at 9 years of age | Not investigated | Not reported | [223] |
| Maternal urinary phthalate concentrations during 3rd trimester | Late gestation | Human | Negative association between placental LINE-1 methylation and concentrations of urinary phthalate metabolites (MEHHP and sum of DEHP) | Significant positive association between concentrations of several metabolites MEHHP, MEOHP; sum of DEHP, and fetal growth restriction | Not reported | [224] |
| 1st trimester urinary concentrations of 11 phthalate metabolites | Early gestation | Human | Significant inverse association between placental H19 methylation and the sum of phthalate metabolites and low molecular weight phthalate metabolites; concentrations of sum and low molecular weight metabolites inversely associated with methylation of IGF2DMR0 in placenta | No significant effects of DNA methylation or imprinted gene expression on birth length or weight | Yes—Altered methylation of IGF2DMR0 with phthalate exposure more likely to occur in female placenta | [227] |
| Maternal urinary phthalate concentrations from patients with or without gestational diabetes mellitus in 2nd trimester | Mid gestation | Human | In maternal serum, increased miR-16-5p expression with MBzP levels; increased miR-29a-3p expression with adjusted MEHP; negative association between miR-29a-3p expression and unadjusted and adjusted MBP concentration; negative association between miR-29a-3p expression and unadjusted MiBP concentration | MEHP levels higher in non-diabetic patients compared to gestational diabetes | Not reported | [230] |
| DEHP (5, 50, 100μg/mL) or DMSO vehicle | 5 days, followed by differentiation into cardiomyocytes | Mouse P19 embryonic carcinoma cell line | In P19 cells, increased expression of Dnmt1, Dnmt3a at higher doses; increased methylation of CpGs within Ppara and Pparg1 genes | Increased rate of differentiation; increased beat rate | Not applicable | [231] |
| DEHP in chow (25 mg DEHP/kg chow in 7% corn oil) | 2 weeks prior to mating, through gestation and lactation | Mouse | In mouse heart, sex-specific changes in DNA methylation at hundreds of CpGs and regions at 5 months of age; several genes with differential DEHP-induced methylation were also differentially methylated in heart samples from patients with heart failure | Elevated blood glucose, insulin resistance, reduced insulin receptor, and reduced glucose uptake and oxidation in gastrocnemius muscle at 2 months of age in both sexes | Effects observed in both sexes | [232] |
| DEHP (0, 1, 10, and 100 mg/kg per day) or olive oil vehicle via oral gavage | Gestation days 9-21 | Rat | In embryos, hypomethylation of nppa and ctnf genes related to cardiac development and upregulation of gene expression; hypermethylation of tnhb gene and down-regulation of expression with highest DBP dose | Increased heart rate, pericardial edema, apoptosis | Not reported | [234] |
| DEHP or DBP (50, 250μg/l) or acetone vehicle | Starting 1.5–1.7 h post-fertilization and lasting 4 days | Zebrafish | In embryos, hypomethylation of nppa and ctnf genes related to cardiac development and upregulation of gene expression; hypermethylation of tnhb gene and down-regulation of expression with highest DBP dose | | | [235] |
development led to cardiac developmental defects. However, whether there are sex differences in phthalate-induced developmental defects is unclear. Additional work has linked lactic acid exposure to DEHP to systemic and cardiac-specific changes in insulin signaling in female offspring rats [206] and in male offspring [207], and young male mice exposed to DEHP developed cardiac mitochondrial dysfunction [208]. These in vivo changes in metabolism have also been observed in vitro, where DEHP exposure in neonatal rat cardiomyocytes led to shifts in cellular metabolism associated with cardiac disease states [209]. Developmental exposure to DEHP led to decreased blood pressure in adult male rats, suggesting that DEHP can cause long-term changes in cardiovascular physiology [210]. Finally, work in cell lines and animal tissue demonstrates that phthalates interfere with synthesis of prostaglandins, lipid signaling molecules with important roles in cardiovascular health [211].

**Cardiovascular effects of developmental phthalate exposure in humans**

In human epidemiologic studies, phthalate exposures in adults are linked to a variety of adverse cardiovascular outcomes, including decreased heart rate variability, coronary heart disease, and atherosclerosis [212-214]. Likewise, a number of human studies have demonstrated associations between early life phthalate exposure and cardiovascular diseases. First, several investigations have linked phthalates to congenital heart defects. Phthalate exposure during pregnancy cooperated with genetic factors to increase the risk of congenital heart defects in a population of Chinese children [26]. Maternal or paternal [215] or only paternal [216] occupational exposures to phthalates were associated with an increased risk of congenital heart defects. Sex-specific effects of phthalate exposures on congenital heart defects are unclear, as they were not investigated in these studies. Notably, assessments of exposure were based on questionnaires rather than measurement of phthalate levels in biological samples, so further investigation is warranted to confirm these findings. An additional investigation demonstrated that maternal urinary phthalate levels were associated with a significant increase in pericardial fat in children at 10 years of age, with no sex differences observed [217], suggesting that phthalates may alter fat deposition in organs.

Phthalate exposures have also been correlated with changes in blood pressure. Maternal urinary phthalate levels were associated with decreased blood pressure in female children [46]. However, additional studies that measured phthalate concentrations in the urine of children found that metabolites of several phthalates were associated with increased blood pressure in children and adolescents [218, 219]. Discrepancies in the findings are likely due to differences in the timing of exposure or outcome measurement (maternal vs. offspring), distinct toxicokinetics and toxicodynamics of the various phthalates, different doses and windows of exposure, as well as variations in genetics, diet, lifestyle and other factors of the populations investigated.

Additional work demonstrated associations between phthalate exposures and adiposity in children that were dependent upon sex, the specific phthalate, and the timing of exposure. In girls, their mothers’ urinary levels of phthalate metabolites during pregnancy were associated with increased adiposity in peri-adolescence [220]. Whether these changes in body composition persist and the long-term implications of these findings for cardiovascular health are unclear. Lastly, prenatal phthalate exposures have been linked to age-dependent alterations in levels of 8-isoprostane, a marker of oxidative stress associated with CVD, in the blood of children and adolescents [221].

Findings in humans and animals thus collectively provide compelling evidence that phthalate exposures during early life are linked to adverse cardiovascular effects. However, more work is necessary to determine the sex-specific effects of these chemicals, as well as the effects of developmental phthalate exposures on cardiovascular health across the life course, including middle and old age where cardiovascular diseases are most prevalent. Moreover, as most research thus far has focused only on DEHP, further investigation into other phthalates is warranted.

**Effects of developmental phthalate exposure on the epigenome**

Numerous human, animal, and in vitro studies demonstrate that phthalate exposures are associated with changes in the epigenome. Similar to Pb and PFAS, the majority of the studies thus far have focused on DNA methylation, and few studies have investigated the effects of phthalates on the epigenome specifically in the cardiovascular system. Phthalate-induced epigenetic changes have been reported for both early life and adult exposures. In adolescent and young adult Taiwanese, urinary metabolites of DEHP were associated with an increased in carotid intima-media thickness (CIMT), a marker of subclinical atherosclerosis, as well as increased global DNA methylation, and the authors proposed that DNA methylation may mediate the effect of DEHP on CIMT. Although both males and females were included in the study, sex differences were not investigated [222].

Several studies have linked maternal phthalate exposures to altered DNA methylation at repetitive elements. Maternal urine levels of monoethyl phthalate or DEHP were associated with decreased DNA methylation at Alu repetitive elements in cord blood or in the blood of children at 9 years of age, respectively [223], or with decreased LINE-1 methylation in the placenta and fetal growth restriction [224]. Sex differences were not reported in either study. Although the implications of these findings for cardiovascular health are unclear, methylation of Alu and LINE-1, as well as fetal growth restriction, has all been associated with CVD [225, 226]. Prenatal phthalate exposure has also been associated with sex-specific dysregulation of DNA methylation and expression of the imprinted genes H19 and IGF2 [227], both of which have important roles in cardiovascular disease and development [228, 229]. Additional work has identified a link between maternal exposure to several phthalates and alterations in serum miRNA levels linked to gestational diabetes [230], a condition linked to increased risk of cardiovascular disease [162].

Additional work in vitro and in animals corroborates the findings in humans. In vitro, exposure of P19 cells to DEHP prior to cardiac differentiation led to altered expression of DNMT1 and DNMT3A before and during differentiation [231]. As in humans, few studies have investigated the effects of early life phthalate exposures specifically on the cardiovascular epigenome. Recent work from our lab demonstrated that maternal exposure to DEHP during gestation and lactation led to sex-specific changes in DNA methylation in the hearts of offspring mice at 5 months of age, long after cessation of exposure [232]. Many of the genes differentially methylated after DEHP exposure also exhibited differential methylation in cardiac tissue from human heart failure patients, suggesting that DEHP exposure alters DNA methylation at disease-relevant genes [232]. Maternal exposure to a mixture of plastics including phthalates led to sex and dose-specific alterations in puberty onset, including early onset puberty in F3 females, as well as obesity in F3 males and females [82].
Although cardiovascular outcomes were not specifically investigated in this study, precocious puberty is a phenomenon associated with obesity and cardiovascular disease in humans [233]. In rats, exposure to DEHP during pregnancy led to increased global DNA methylation and expression of Dnmt1, Dnmt3a, and Dnmt3b transcript and protein in the gastronemius muscle of both male and female offspring, concomitant with altered systemic glucose homeostasis [234]. The cardiovascular implications of this work are unclear; however, systemic glucose homeostasis is closely coupled to cardiovascular health [9]. Epigenetic effects of phthalates have also been demonstrated in zebrafish, where DEHP and di-n-butyl phthalate (DBP) exposure led to altered DNA methylation of transcription factors critical for normal cardiac development, concomitant with cardiac developmental defects [235].

Multi- and transgenerational effects of Pb, PFAS and phthalates on cardiovascular health

To date, there have only been a small number of studies that have investigated multi- or transgenerational effects of exposure to Pb, PFAS, or phthalates. A summary of the studies outlined below can be found in Table 9.

Pb exposure

There are currently no studies that have investigated multi- or transgenerational effects of Pb exposure on cardiovascular tissues. However, a small number of studies have identified epigenetic effects on genes linked to cardiovascular development and disease in other tissues. Using neonatal bloodspots in humans, grandmothers’ exposure to Pb was associated with altered DNA methylation in the blood of grandchildren, with alterations occurring at several genes (NDRG4, APOA5, NINJ2, and TRPV2) with important functions in cardiovascular development and disease [128, 236-239]. In zebrafish, ancestral exposure to Pb resulted in neurobehavioral deficits as well as sex-specific, differential expression of genes involved in epigenetic regulation [240]. Notably, in females, cardiovascular disease-related genes were also significantly enriched among differentially expressed genes [240]. In mice, prenatal Pb exposure led to smaller F3 offspring in addition to altered corticosterone levels in F3 females [241]. Both males and females showed region-specific alterations in DNA methylation in the brain [241]. Cardiovascular health outcomes and DNA methylation were not investigated; however, the findings of altered size at birth and glucocorticoid levels, both associated with cardiovascular disease in humans [226, 242], suggest that cardiovascular effects should be investigated in future studies.

PFAS exposures

Few investigations into the multi- or transgenerational effects of PFAS exposures have been conducted to date. Among the small number of studies available, the majority have been performed in insect and worm species (summarized in Table 9). Exposure to perfluorobutane sulfonate (PFBS), a replacement for PFOS, led to transgenerational effects on reproductive function in fish [243], as well as movement defects in the F1 generation of Caenorhabditis elegans [244]. Continuous exposure to low-dose PFBS in worms ancestrally exposed to PFBS resulted in significantly shortened lifespan in F4 and F5 generations [244]. FFHx5 and PFBS also caused transgenerational alterations in lipid metabolism pathways in C. elegans [70]. In zebrafish, ancestral F-53B exposure led to altered expression of several thyroid hormone regulated genes, a trend toward increased thyroxine levels, as well as impaired swim bladder inflation in animals not directly exposed to the chemical [245]. Epigenetic effects were not investigated in any of these studies.

The effects of PFAS on the germline epigenome are also poorly understood. In mice, exposure to PFOA led to significant alterations in several miRNAs in the testes [246]. In a more recent human study, an investigation into the effects of PFAS on sperm DNA methylation in men from three regions in Europe and the Arctic yielded inconclusive results [247]. No consistent changes in global DNA methylation were observed with PFAS exposures; however, small but statistically significant changes in DNA methylation were observed with specific PFAS in specific populations [247]. Moreover, the study only included partners of pregnant women, potentially selecting for men with healthier reproductive function [247]. Given the paucity of investigations into the germline and transgenerational effects of PFAS in mammals, more research in this area is urgently needed.

Phthalate exposures

Compared to Pb and PFAS, more studies have been conducted into the multi and transgenerational effects of phthalates, although they have focused primarily on hormonal functions, with effects observed in both males and females. In males, studies in rats and mice demonstrated that exposure to DEHP or DBP during pregnancy led to reduced sperm counts and mobility [248, 249], as well as impaired spermatogonial stem cell function [249]. Altered sperm function in F3 rats was accompanied by lower global levels of DNA methylation [248]. As noted previously in rats, maternal exposure to a mixture of plastics (BPA and phthalates) led to reproductive alterations and obesity in F3 animals of both sexes [82].

In females, separate studies with rats and mice showed that ancestral exposure to DEHP had adverse effects on reproductive function in F3 animals, although whether epigenetic mechanisms played a role in these effects were not investigated [250, 251]. Exposure of pregnant mice to a mixture of six phthalates led to premature reproductive aging in F3 females [252], a phenomenon associated with cardiovascular diseases in humans [253]. Ancestral exposure to DEHP led to female-specific changes in levels of corticosterone in response to stress, as well as altered expression of the imprinted gene, Gnas [254], which has important functions in cardiovascular health [131, 255]. Further work showed multigenerational effects of DEHP exposure on DNA methylation at imprinted loci in oocytes [256], suggesting that these chemicals may have effects on germ cell function across multiple generations.

Although transgenerational effects of phthalates on cardiovascular outcomes have not been investigated, the well-established links between hormonal dysregulation and cardiovascular health [242, 253, 257] suggest that phthalates may also affect cardiovascular health across generations. This important question should be addressed in future studies.

Conclusion

Although environmental factors play a critical role in the etiology of cardiovascular diseases, how their effects differ by sex is poorly understood. Likewise, future studies are needed to better understand how toxicant exposures impact cardiovascular health across generations (Fig 2). Indeed, although the studies outlined in this review and documented in Tables 1–9 demonstrate that chemical exposures during early development can have adverse cardiovascular health effects, the precise window(s) of vulnerability are still unclear. Systematic investigation into this question,
### Table 9: Multi- and transgenerational effects of Pb, PFAS, and phthalates

| Factor                                                                 | Window of exposure | Model      | Epigenetic/molecular effect                                                                                                                                                                                                                                                                                                                                 | Phenotype                                                                                                                                                                                                 | Sex specificity | Reference |
|------------------------------------------------------------------------|--------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|-----------|
| Pb, measured in maternal (reflecting grandmother’s exposure) and child (reflecting mother’s exposure) neonatal dried blood spots, as well as child blood | Gestation          | Human      | Altered DNA methylation in the blood of grandchildren at genes associated with cardiovascular development and disease (NDRG4, APOA5, NINJ2, and TRPV2)                                                                                                                                                                                                 | Not investigated                                                                                                                                                                                       | Not reported    | [128]    |
| F0 generation exposed as embryos to Pb(NO₃)₂ in water at 10 μM      | <2 h post-fertilization until 24 h | Zebrafish  | In embryos, differential expression of genes involved in epigenetic modifications in F2 generation; genes associated with CVD significantly differentially expressed in F2 females                                                                                                                                                                                                 | Not investigated                                                                                                                                                                                       | Not investigated | [240]    |
| 100 ppm Pb acetate dissolved in distilled deionized drinking water   | 2 months prior to mating | Mouse      | Altered DNA methylation of Th in the cortex in F3 females; altered DNA methylation of Bdnf in the hippocampus of F3 males                                                                                                                                                                                                 | Reduced body weight; decreased corticosterone in F3 females                                                                                                                                               | Yes            | [241]    |
| PFBS (0, 1.0, 2.9, and 9.5 μg/l) exposure in F0 generation            | Embryo stage until reaching sexual maturity (6 months of age) | Zebrafish  | Reduced global DNA methylation levels in F1 embryos                                                                                                                                                                                                                                                                                                            | Decreased weight in F1 generation eggs; increased weight of F2 eggs                                                                                                                                     | Yes—effects on F0 female reproduction | [243]    |
| PFBS (0.0005, 0.01, 0.1, 0.5, 1, and 2 mM—equivalent to 0.15, 3.0, 300, 150, 300, and 600 mg/l) in growth medium | L4 stage larvae were exposed for 48 h | C. elegans | Not investigated                                                                                                                                                                                                                                                                                                                                           | Movement defects in F1 generation; reduced lifespan in F4 and F5 generations continuously exposed to low-dose PFBS                                                                                         | Not reported    | [244]    |
| PFBS and PFHxS in growth medium (1 ng/l)                              | Egg stage, for 72 h | C. elegans | In larvae, altered expression of enzymes and genes important for lipid metabolism                                                                                                                                                                                                                                                                                           | Not investigated                                                                                                                                                                                       | Not reported    | [70]     |
| 6.2 chlorinated polyfluorinated ether sulfonate (F-53B, 0, 5, 50, or 500 μg/l) in growth medium | 180 days           | Zebrafish  | Altered expression of thyroid axis genes in F1 and F2 embryos/larvae                                                                                                                                                                                                                                                                                           | Increased T4 levels in F1 embryos; increased mortality; impaired swim bladder formation; increased T3 and T4 levels in F1 larvae; impaired swim bladder formation in F2 larvae                                                                 | Not reported    | [245]    |
| Levels of PFAS in serum from partners of pregnant women             | Paternal exposure  | Human      | Negative association between PFNA and global sperm DNA methylation in all populations combined; positive association between PFOA and LINE-1 methylation in Kharkiv; negative association between PFHxS and sperm global DNA methylation in Greenland; negative association between PFOS and sperm global DNA methylation in Warsaw; positive association between PFOS and satellite alpha repeat methylation in Kharkiv | Not investigated                                                                                                                                                                                       | Only males were evaluated | [247]    |
|                                                                        |                     |            |                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                          |                |          |

(continued)
| Factor | Window of exposure | Gestational days | Model | Epigenetic/molecular effect | Phenotype | Sex specificity | Reference |
|--------|-------------------|------------------|-------|-----------------------------|-----------|----------------|-----------|
| Di-n-butyl phthalate (0 and 500 mg/kg) or corn oil vehicle via oral gavage | Gestational days 8–14 | Rat     | Increased levels of betaine and betaine homocysteine S-methyltransferase, global DNA hypomethylation in F1 and F3 testis at 60 days of age | Decreased sperm counts in F1–F3 generations at 60 days of age | Only males were evaluated | [248] |
| DEHP (500 mg/kg) or corn oil vehicle by oral gavage | Gestational days 7–14 | Mouse   | Not investigated | Decreased sperm count and mobility in F1–F3 generations; reduced sperm count and decreased motility in F2 and F4 generations; decreased luteinizing hormone in F3 | Only males were evaluated | [249] |
| DEHP (20 and 200 μg/kg/day and 500 and 750 mg/kg/day) or corn oil control by oral gavage | Gestational day 10.5 until birth | Mouse   | Not investigated | Accelerated puberty, disruptions in estrous cycle and decreased anogenital distance in female offspring | Only females were evaluated | [250] |
| DEHP (1, 20, 50, or 300 mg of DEHP/kg/day) or oil control by oral gavage | Gestational day 14 until birth | Rat     | Not investigated | Decreased rate of pregnancy, lower body weight, increased litter size, and decreased pup weight in F3 | Only females were evaluated | [251] |
| Phthalate mixture: 35% DEP, 21% DEHP, 15% DBP, 8% DiBP, 15%DiNP, and 5% BzBP (20 μg/kg–500 mg/kg) or corn oil vehicle via oral gavage | Gestational day 10 until birth | Mouse   | Not investigated | Decreased testosterone and inhibin B, increased follicle stimulating hormone and luteinizing hormone in F1; lower testosterone and reduced percentage of antral follicles in F2; altered estrous cycle, increased ovarian weight, decreased luteinizing hormone, a lowered number of follicles in F3 | Only females were evaluated | [252] |
| DEHP (150 and 200 mg/kg) or corn oil control daily via oral gavage | From 0.5 to 18.5 days after mating | Mouse   | Higher pituitary Gnas expression with stress and ancestral DEHP exposure in both F3 sexes | Lower corticosterone concentrations after stress in F3 females; dose-dependent behavioral changes and reduced seminal vesicle weight in F3 males | Yes | [254] |
| DEHP (0 and 40 μg DEHP/kg) or 0.1% DMSO vehicle daily via oral gavage | Gestational days 7–14 | Mouse   | Reduced DNA methylation in Igf2r and Peg3 regulatory regions in F1 male and female primordial germ cells and in F2 oocytes | Not investigated | Yes | [256] |
testing the effects of chemical exposures during different stages of development and during other potentially vulnerable points across the life course (childhood, adolescence, and reproductive senescence) is necessary to gain a deeper understanding of the threat posed by environmental toxicants to cardiovascular health.

This need for additional insight into the adverse generational and sex-specific health effects of chemicals is exemplified by Pb, PFAS, and phthalates. Of note, among the studies identified in Tables 3–9 addressing the cardiovascular, epigenetic, and trans-generational effects of these toxicants, the vast majority do not provide an analysis of sex specificity. Although research practices have become more inclusive, in part through guidance from the National Institutes of Health [258], the importance of sex as a biological variable is still underappreciated in the scientific community at large [259]. Of the tens of thousands of chemicals registered for use in the USA, only a minority have undergone government safety evaluations, highlighting the significant burden on consumers and researchers to elucidate their potential health effects. Given the profound economic and health burden posed by cardiovascular diseases, more research into this important area of public health is urgently needed.

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Data availability

Not applicable.

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Author contributions

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Environmental Epigenetics, 2022, Vol. 00, No. 00

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