Reduced Birth Weight and Exposure to Per- and Polyfluoroalkyl Substances: A Review of Possible Underlying Mechanisms Using the AOP-HelpFinder

Claudia Gundacker [1,*], Karine Audouze [2], Raimund Widhalm [1], Sebastian Granitzer [1], Martin Forsthuber [1], Florence Jornod [2], Maria Wielsøe [3], Manhai Long [3], Thórhallur Ingi Halldórsson [4,5], Maria Uhl [6] and Eva Cecilie Bonefeld-Jørgensen [3,7]

[1] Institute of Medical Genetics, Medical University of Vienna, 1090 Vienna, Austria
[2] Unit T3S, Université Paris Cité, Inserm U1124, 75006 Paris, France
[3] Department of Public Health, Aarhus University, 8000 Aarhus, Denmark
[4] Faculty of Food Science and Nutrition, University of Iceland, 102 Reykjavík, Iceland
[5] Department of Epidemiology Research, Statens Serum Institut, 2300 Copenhagen, Denmark
[6] Environment Agency Austria, 1090 Vienna, Austria
[7] Greenland Center for Health Research, Greenland University, Nuuk 3905, Greenland
* Correspondence: claudia.gundacker@meduniwien.ac.at; Tel.: +43-1-40160-56503

Abstract: Prenatal exposure to per- and polyfluorinated substances (PFAS) may impair fetal growth. Our knowledge of the underlying mechanisms is incomplete. We used the Adverse Outcome Pathway (AOP)-helpFinder tool to search *PubMed* for studies published until March 2021 that examined PFAS exposure in relation to birth weight, oxidative stress, hormones/hormone receptors, or growth signaling pathways. Of these 1880 articles, 106 experimental studies remained after abstract screening. One clear finding is that PFAS are associated with oxidative stress in in vivo animal studies and in vitro studies. It appears that PFAS-induced reactive-oxygen species (ROS) generation triggers increased peroxisome proliferator-activated receptor (PPAR)γ expression and activation of growth signaling pathways, leading to hyperdifferentiation of pre-adipocytes. Fewer proliferating pre-adipocytes result in lower adipose tissue weight and in this way may reduce birth weight. PFAS may also impair fetal growth through endocrine effects. Estrogenic effects have been noted in in vivo and in vitro studies. Overall, data suggest thyroid-damaging effects of PFAS affecting thyroid hormones, thyroid hormone gene expression, and histology that are associated in animal studies with decreased body and organ weight. The effects of PFAS on the complex relationships between oxidative stress, endocrine system function, adipogenesis, and fetal growth should be further explored.

Keywords: fetal growth; PFOS; PFOA; PFHxS; PFNA; PFDA

1. Introduction

Birth weight is a widely studied outcome in environmental health studies because it is an important predictor of neonatal health, easily and accurately measured, and sensitive to toxic effects [1,2].

By definition, the term "low birth weight" refers to a weight <2500 g [3]. Small for gestational age (SGA) describes newborns with a birth weight at least two standard deviations below the mean for gestational age in relation to a reference population [4]. This definition does not necessarily correspond to the more common one that classifies SGA as birth weight below the tenth percentile of gestational age [4,5]. The prevalence of SGA births is 10–12% in U.S., Chinese, and European populations [6–8], but can deviate significantly up to a prevalence of 42% in term SGA infants, for example, in South Asia [9]. According to Ludvigsson et al. [5], SGA occurs in more than 30 million infants each year and is associated with an increased risk of stillbirth, neonatal mortality, and death in infancy.

About 70% of the total variation in birth weight is explained by genetics, and the remaining variance is attributed to the environment, including pollution [4,9–11]. In a prospective cohort of dichorionic twin births, the contribution of fetal genetics to estimated fetal weight (EFW) peaked at 71% in the second trimester, whereas shared environment explained most of the phenotypic variation in fetal growth in the first trimester (54% contribution to EFW) [12].

The maternal factors associated with lower birth size include maternal birth weight, maternal height and age, previous stillbirth, preterm birth, and SGA birth, parity (as nulliparous women have higher risk for delivering SGA births), low socioeconomic status, smoking, drug consumption, and diseases such as infections and anemia. Together, maternal and paternal body size contribute to 3–12% of the variation in birth weight. Paternal factors, in general, are less well-studied [4].

The key regulators of fetal growth are fetal insulin and insulin-like growth factor (IGF)1 and IGF2 with their binding proteins and receptors, which modulate the action of IGF1 and 2 [4,13,14]. IGF1 regulates size prenatally and postnatally until adolescence, whereas maternally imprinted (paternally expressed) IGF2 is primarily a growth factor for fetal growth [14]. The placenta mediates maternal and fetal oxygen and nutrient exchange and has endocrine functions, thereby having a significant impact on birth size [15–17]. The levels of placental growth hormone variant (GHV) and placental growth factor are significantly associated with placental and fetal growth [4,18,19]. Numerous pathological conditions, including vascular impairment of the placenta, can result in SGA births [20,21].

Pollutants that may affect fetal growth include endocrine disrupting per- and polyfluoroalkyl substances (PFAS) [22]. PFAS are industrial chemicals with very stable C-F bonds in the perfluoroalkyl moiety, which allows an exceptionally wide range of applications but also results in high persistence [23,24]. Perfluoroalkane sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) are particularly persistent and bioaccumulate in wildlife and humans [24]. Human exposure occurs mainly through food and drinking water and, to a lesser extent through inhalation and dermal uptake [23,25]. Prenatal exposure is of high concern because it can affect fetal growth [24,26,27] and has therefore been the subject of numerous epidemiological studies. Mostly, fetal growth has been analyzed as a continuous outcome (birth weight, birth length, head circumference, ponderal index) and less frequently as SGA or birth weight Z-score, another weight-at-age index. Existing studies have been systematically reviewed and, to some extent, reanalyzed in several papers [6,28–33].

As summarized in Table 1, most analyses confirmed that prenatal exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonic acid (PFOS) is inversely associated with birth weight. In addition, Fan et al. [33] estimated the number of PFOA-associated low-birth-weight cases to be 461,635 (95% confidence interval: 57,418 to 854,645) in the last two decades, mainly in Asian regions. Yet, the observed decrements in birth weight were, on average, small and within the normal range of distribution, so they may have little or no direct effect on infant morbidity or mortality [1]. Accordingly, most authors [28–32] expressed that the significance of modest reductions in birth weight remains unclear. Govarts et al. [6] pooled data from seven European birth cohorts and found significantly higher SGA risk for cases with high PFOA exposure levels (OR: 1.64). Regarding PFOS, an increased SGA risk was present in cases where mothers had smoked during pregnancy (OR: 1.63); similar effects were reported by Rokoff et al. [34]. Higher odds of SGA birth were also found regarding PFOA (OR: 1.20) and PFNA (OR: 1.32) exposure in African American women [35].

Table 1. Reviews analyzing epidemiological studies for associations between prenatal PFAS exposure and birth weight indices.

Source	Type of Analysis	No of Studies (Publication Date)	Analyte(s)	Surveyed Birth Outcome	Main Results	Conclusions
Bach et al., 2015 [28]	Systematic review	14 (2004–2013)	PFOA, PFOS	Birth weight Low birth weight [a] Small for gestational age [b] Birth weight z-scores [c]	PFOA exposure associated with decreased measures of continuous birth weight in all studies at different magnitudes, with many results being statistically insignificant PFOS: no clear trend for effects on birth weight	"The impact on public health is unclear"
Negri et al., 2017 [29]	Systematic review	16 (up to 2015)	PFOA, PFOS	Birth weight	PFOA: $-12.8\,\mathrm{g/ng/mL}$ (-27.1 g per increase of $1\,\log_e$ ng/mL) PFOS: $-0.92\,\mathrm{g/ng/mL}$ (-46.1 g per increase of $1\,\log_e$ ng/mL)	" ... no quantitative toxicological evidence to support the epidemiological association, thus reducing the biological plausibility of a causal relationship"
Govarts et al., 2018 [6]	Pooled analysis	7 birth cohorts 5446 mother–child pairs	PFOA, PFOS	Small for gestational age [b]	PFOA: Higher levels associated with greater risk of SGA (OR: 1.64) PFOS: Higher levels associated with greater risk of SGA (OR: 1.63) in newborns of mothers who smoked during pregnancy (but decreased risk in newborns of non-smoking mothers (OR: 0.66))	"Prenatal environmental exposure to perfluorinated compounds with endocrine disrupting properties may contribute to the prevalence of SGA. We found indication of effect modification by child's sex and smoking during pregnancy. The direction of the associations differed by chemical and these effect modifiers, suggesting diverse mechanisms of action and biological pathways"

Table 1. *Cont.*

Source	Type of Analysis	No of Studies (Publication Date)	Analyte(s)	Surveyed Birth Outcome	Main Results	Conclusions
Dzierlenga et al., 2020 [30]	Random-effects meta-regression	29 (up to 2019)	PFOS	Birth weight	−3.22 g/ng/mL (all) −1.35 g/ng/mL (early group [d]) −7.17 g/ng/mL (later group [d])	"...when blood was drawn at the very beginning of pregnancy, there was essentially no relation of birth weight to PFOS", "stronger inverse association in Asian studies", "The evidence was weakly or not supportive of a causal association"
Cao et al., 2021 preprint [31]	Meta-analysis (fixed-effect and random-effect models)	6 (2009–2017)	PFOA, PFOS	Low birth weight [a]	PFOA: OR = 0.90 PFOS: OR = 1.32 (America: OR = 1.44)	"...study provided a systematic review and meta-analysis evidence for the relationship between maternal PFASs exposure and LBW of offspring through a small number of studies. Researchers should conduct further studies between different regions"
Lee et al., 2021 [32]	Systematic review	90 (2007–2021)	PFOA, PFOS, 11 other PFAS	Birth weight Birth length Ponderal Index [e] Gestational age	Most studies suggest that prenatal PFAS exposure (especially long-chain PFAS) may affect fetal growth	"The current epidemiologic evidence has mostly suggested that prenatal PFAS exposures may impair fetal growth... The mechanisms through which PFAS affect early-life physical development in humans remain unclear"

[a] Low birth weight: birth weight below 2500 g. [b] Small for gestational age (SGA): birth weight below the 10th growth percentile for gestational age. [c] Birth weight z-scores: birth weight standardized for sex and gestational age; [d] "early group": maternal blood drawn during early pregnancy; "later group": maternal blood drawn during late pregnancy. [e] Ponderal Index: birth weight in relation to birth length.

Most studies examine the effect of a single compound on fetal growth. However, pregnant women are exposed to a multitude of chemicals/stressors, including PFAS mixtures. Rokoff et al. [34] found concomitant prenatal exposure to maternal smoking, residential black carbon, and PFOS to be additively associated with lower birth weight z-scores. Exposure to a mixture of endocrine disrupting chemicals, i.e., PFAS, triclosan, phthalates, non-phthalate plasticizers, bisphenols, polycyclic aromatic hydrocarbons, pesticides, and polychlorinated biphenyls (PCBs), has been shown to be associated with lower birth weight z-scores and slower infant growth spurt rate, particularly in girls [22]. Both exposure to a

mixture of endocrine disrupting chemicals including PFAS or to actual serum mixture of PFAS has been shown to be associated with lower birth weight [22,36].

Prenatal PFAS exposures are also associated with other adverse pregnancy outcome, such as, for instance, PFOA and PFOS with late-onset preeclampsia [37]. This adds to the complexity of the situation, as the pathophysiology of preeclampsia, which particularly affects first-time pregnancies and is often associated with fetal growth restriction, is insufficiently understood [38].

The many uncertainties in the observations on prenatal PFAS exposure and fetal growth raise the fundamental question of causality of the association. Several PFAS-related mechanisms have been proposed, including effects on the IGF axis [39], reduced blood vessel formation [40], and disturbed placental development and physiology that change placental weight [41–43] and placental endocrine function [44].

Overall, our knowledge of PFAS-induced mechanisms causing or contributing to reduced fetal growth is incomplete. One goal of the Human Biomonitoring Initiative for Europe (HBM4EU [45]) was to integrate data on mechanistic toxicology, human biomonitoring, and adverse outcome pathways (AOPs) to support human health risk assessment. As part of the HBM4EU project, our aim was to systematically search for PFAS-induced toxicity mechanisms. Using the AOP-helpFinder tool [46] we aimed to identify studies that investigated PFAS-related effects on hormones and hormone receptors, nutrients, oxidative stress, and growth signaling pathways. Based on the identified relevant experimental studies, we manually analyzed for modes of action (MoA), and if the mechanisms could be linked in a plausible way, these were used to propose new AOPs.

2. Materials and Methods

A combined literature and manual curation approach was conducted to rapidly identify and collect existing published and dispersed information on low birth weight and PFAS exposure to gain a better understanding of their MoA and to inform the development of future AOPs.

2.1. Development of the Search Term Lists

The first step was to create two lists in order to be able to run the AOP-helpFinder. First, a list of MeSH terms (a controlled vocabulary of the U.S. National Library of Medicines) and other free-text search terms related to the studied outcomes was compiled (Table 2). These search terms were complemented by a list of matching molecular initiating events (MIEs) and key events (KEs), which were selected by experts (Table 2). Then, a third list related to the PFAS compounds and their synonyms was generated, which includes five PFAS compounds (Table 3).

Table 2. Search terms on adverse outcomes and health effects including MIEs and KEs used in AOP helpFinder search.

MeSH Terms	Other Search Terms
Infant, Small for Gestational Age	Amino acids
Infant, Small for Gestational Age/growth and development	Nutrients
Infant, Small for Gestational Age/blood	Glucose
Infant, Small for Gestational Age/metabolism	Fatty acids
Infant, Small for Gestational Age/physiology	Fetal growth restriction
Premature Birth	Intrauterine growth retardation
Pre-Eclampsia	Intrauterine growth restriction
Receptor, Fibroblast Growth Factor, Type 1	Placenta malperfusion

Table 2. *Cont.*

MeSH Terms	Other Search Terms
Placenta Diseases	Vascular endothelial growth factor
Placenta Growth Factor	Flt-1
Placenta Growth Factor, PLGF-1 Isoform	Thiol adduct
Receptors, Vascular Endothelial Growth Factor	Thio/seleno-protein
Receptors, Androgen	Oxidative stress
Receptors, Estrogen	DNA polymerase gamma
	Small for Gestational Age
	Small for Gestational Age/growth and development
	Small for Gestational Age/blood
	Small for Gestational Age/metabolism
	Small for Gestational Age/physiology
	Fetal growth restriction
	Intrauterine growth retardation
	Intrauterine growth restriction
	IUGR
	Inhibition Cytochrome P450 enzyme activity
	Inhibition CYP17A1 activity
	Decreased Aromatase mRNA
	Decreased Cyp19a1 mRNA
Fetal growth	
AO	Increase, Growth inhibition
AO	Growth, reduction
AO	Decrease, Growth
MIE	Inhibition, VegfR2
KE	Decreased, angiogenesis
KE	Defect of Embryogenesis
KE	Decrease, Growth
KE	Reduction, Progesterone synthesis
Oxidative stress	
MIE	Activation, NRF2
KE	ROS formation
KE	Increase, Oxidative Stress
KE	Activation, PMK-1 P38 MAPK
KE	Down Regulation, GSS and GSTs gene
KE	Glutathione synthesis
KE	Glutathione homeostasis
MIE	Thiol group of chemicals interact with sulfhydryl groups of proteins to form thiol adducts
MIE	Inhibition of mitochondrial DNA polymerase gamma
KE	Dysfunction, Mitochondria

Table 2. *Cont.*

MeSH Terms	Other Search Terms
MIE	Binding, Thiol/seleno-proteins involved in protection against oxidative stress
Signaling pathways	
KE	Activation, AKT2
KE	Activation, HIF-1
KE	Activation, JAK/STAT pathway
KE	Activation, TGF-beta pathway
KE	Activation, JNK
MIE	Wnt ligand stimulation
KE	Inhibition, Wnt pathway
KE	Frizzled activation
KE	Alteration, Wnt pathway
Endocrine related pathways	
MIE	Activation, Androgen receptor
MIE	Decreased, Androgen receptor activity
MIE	Activation, Estrogen receptor
KE	Increased, Estrogen receptor activity
KE	Increased, ER activity
KE	Decrease, testosterone synthesis
KE	Decrease, testosterone level
KE	Decrease, dihydrotestosterone level
KE	Decrease, DHT level
KE	Decrease, androgen receptors (AR) activation
KE	Decrease, AR activation
KE	Reduction, 17-OH-pregnenolone conversion in DHEA
KE	Reduction, 17-OH-progesterone conversion in androstenedione
KE	Thyroid hormone disruption
Others	
MIE	Inhibition, Cytochrome P450 enzyme (CYP17A1) activity
MIE	Binding of substrate, endocytic receptor
MIE	Inhibition, Aromatase
KE	Decreased, Aromatase (Cyp19a1) mRNA
KE	Perturbation of cholesterol
KE	GSK3beta inactivation
KE	β-catenin activation

Abbreviations: AO, adverse outcome; MIE, molecular initiating event; KE, key event.

Table 3. Search terms on PFAS.

PFAS, General Terms	
PFAS, Perfluoroalkyl substances	
Perfluoroalkyl substances	
Perfluoroalkyl acids	
Perfluoroalkyl carboxylates (PFCAs)	
Perfluroalkylated substances	
PFC, Perfluorinated compound	
Perfluorinated sulfonates (PFSAs)	
PFAS compounds *	
PFCAs	
C8	PFOA, perfluorooctanoic acid
C9	PFNA, perfluorononanoic acid
C10	PFDA, perfluorodecanoic acid or perfluoro-n-decanoic acid
PFSAs	
C6	PFHxS, perfluorohexanesulfonic acid or perfluoro-1-hexanesulfonate
C8	PFOS, perfluorooctane sulfonic acid or perfluorooctane sulfonate

* PFAS compounds listed according to functional group (i.e., carboxylated or sulfonated) and carbon chain length (C6–C10).

2.2. Running the AOP-helpFinder Tool

The full available literature (>33 millions of publications) in the *PubMed* database (accessed March 2021) was used for the screening. First, all publications related to at least one of the PFAS compounds were identified and kept for the text mining. Then, the AOP-helpFinder was applied, which is a tool based on artificial intelligence [47,48] that was previously successfully applied to develop new AOP [49–51]. This tool allows to automatically identify co-occurrence between terms from both lists, i.e., between an outcome search term and a PFAS, in published abstracts was run. Then, we kept only abstracts co-mentioning at least one outcome search term and one PFAS. Default parameters were used (i.e., screening the full abstracts and calculation of the two scoring systems to identify as much as possible the relevant associations) [52].

2.3. Manual Curation

We excluded abstracts for further investigation that were in duplicate or not significant for the proposed study focus. Identified abstracts co-mentioning outcome search terms and PFAS were manually investigated, and when necessary, the full publications were read to confirm the linkage and help building the AOPs.

2.4. Flowchart

Overall, 1880 abstracts were retrieved and reviewed for eligibility and significance. After exclusion of ineligible abstracts and abstract screening, 106 experimental studies remained (Figure 1). The experimental studies were searched for molecular initiating events (MIEs) and key events (KEs) (see Tables 4–7, Tables 8–11 for specific references).
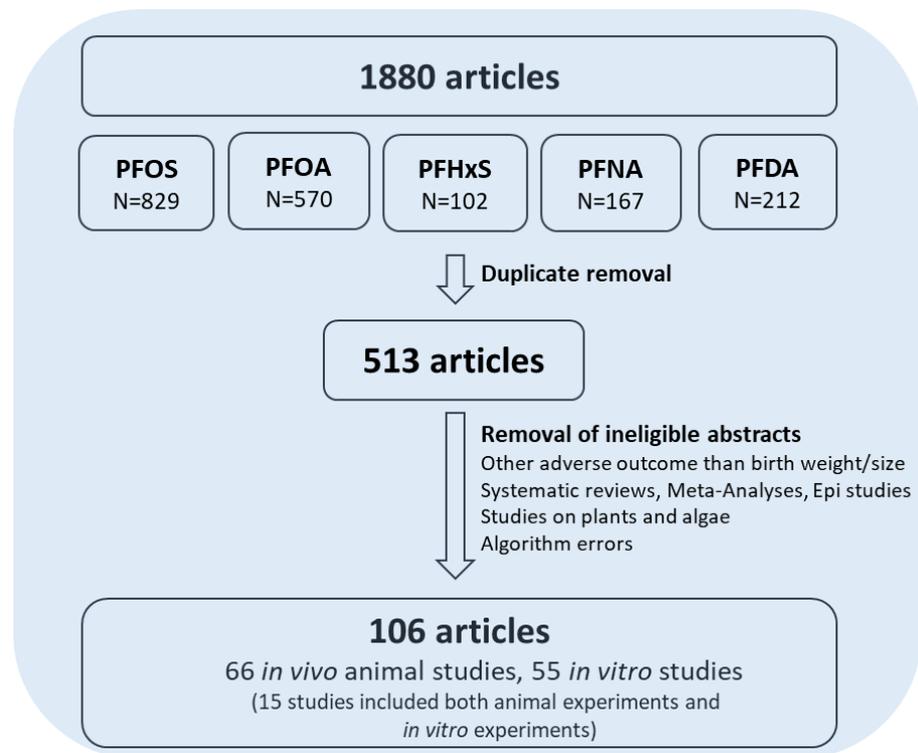
Figure 1. The sequence of the AOP-helpFinder search.

Table 4. In vivo results related to PFAS exposures and oxidative stress.

Authors	Species	Solvent	Body Weight	Fetal Weight	Offspring Weight	Rep/Dev	Amino acids	Glucose	Lipids	Liver	Oxidative Stress	Lethality	Specific Genes	Other AO
PFOS														
Kim et al., 2020 [53]	*C. elegans*					↓	↑↓		↑↓		↑	↑		
Kim et al., 2021 [54]	Drosophila	Aceton	↓			↓			↑↓			↑		
Lee et al., 2015 [55]	Female CD-1 mice	DMSO							↑↓		↑			
Li et al., 2020 [56]	Adult CD-1 mice	DMSO												DNA Methylation
Li et al., 2020 [57]	*C. elegans*	Water							↑↓					
Ortiz-Villanueva et al., 2018 [58]	Zebrafish	DMSO				↑↓								Metabolome
Park et al., 2020 [59]	*Macrophthalmus japonicus crab*													Involvement of MAPK/p38

Table 4. *Cont.*

Authors	Species	Solvent	Body Weight	Fetal Weight	Offspring Weight	Rep/Dev	Amino acids	Glucose	Lipids	Liver	Oxidative Stress	Lethality	Specific Genes	Other AO
Qiu et al., 2016 [60]	Male ICR mice	DMSO	–									↑		
Seyoum et al., 2020 [61]	Daphnia	Water	↓			↓			↑↓			↑		
Wan et al., 2020 [62]	CD1 mice	DMSO		↓		↓							SNAT4	
Wang et al., 2020 [63]	*Dugesia japonica*	DMSO									↑		SOD, CAT, GPx1	
Xia et al., 2018 [64]	*Anodonta woodiana*	DMSO										↑		
Yue et al., 2020 [65]	*C. elegans*	Water	↓			↓			↑↓			↑		Metabolism
Zhang et al., 2020 [66]	Manila clam	DMSO									↑↓			Metabolism/Genes
PFOA														
Du et al., 2018 [67]	Male Balb/c mice	DMSO	↓					↑	↑			↑		
Guruge et al., 2006 [68]	Male Sprague–Dawley rats		↑↓				↑↓		↑↓				Transcriptome	Gene expression
Kim et al., 2020 [53]	C. elegans					↓	↑↓		↑↓		↑	↑		Metabolism; Lipidomic
Li et al., 2019 [69]	Kunming mice			↓						↑↓	↑	↑		Gene expression
Li et al., 2021 [70]	M. edulis	Water									↑		CAT/SOD/GPx	
Liu et al., 2015 [71]	Male mice	Water				↓					↑			
Liu et al., 1996 [72]	Male rats	unclear	↓							↑				
Salimi et al., 2019 [73]	Mouse		↓								↑			
Seyoum et al., 2020 [61]	Daphnia		–			↓			↑↓			↑		
Wang et al., 2010 [74]	Drosophila		↓		↓	↓								Reduced longevity of males
Xia et al., 2018 [64]	*Anodonta woodiana*	DMSO										↑		
Yan et al., 2015 [75]	Male Balb/c mice	Water						↑↓	↑↓	↑↓			Akt, GSK	
Yang 2010 [76]	*Oryzias latipes*	Water									–		PPR alpha	

Rep/Dev, effects on reproduction and/or development; green cell (↑), increase; orange cell (↓), decrease; blue cell, changes in different directions; grey cell (–), no effect. For more details, see Supplementary Table S1.

Table 5. In vitro results related to PFAS-induced oxidative stress and reduced cellular health.

Publication Information			Study Setup							
Authors	Cell System	Species	Reproduction	Amino Acids	Glucose	Lipids/Fats	Oxidative Stress	Cytotoxic/Reduced Cell Number	Spec. Genes	Other AO
PFOS										
Chiu et al., 2018 [77]	Not described	Tox-Screening; ToxCast; Tox21					↑			Many different AO
Gorrochategui et al., 2014 [78]	JEG-3	Human						↑		
Gorrochategui et al., 2016 [79]	A6 Kidney Epithelial Cells	Xenopus laevis						↑		
Li et al., 2020 [56]	HTR-8/SVneo	Human						↑		
Reistad et al., 2013 [80]	Cerebellar granule cells	Rat					↑	↑		
Sun et al., 2018 [81]	SH-SY5Y Cell	Human					↑	↑	NRF2, HO-1	
Sun et al., 2019 [82]	SH-SY5Y Cell	Human					↑	↑	JNK-1	
Tang et al., 2017 [83]	ES cell line D3	Mouse				↑	↑	↑	Mfn1, Mfn2, mTOR, RICTOR	Ca2+ flux is impaired
Wang et al., 2015 [84]	HAPI microglial cells	Rat					↑	↑	ERK, JNK, p38	
Wei et al., 2009 [85]	Primary hepatocytes	Gobiocypris rarus (fish)				↑	↑	↑		Gene expression
Xu et al., 2016 [86]	3T3-L1 pre-adipocytes	Mouse			↑	↑↓		↑	NRF2, Lpl, NQo1, PPAR, FABP4	
Zarei et al., 2018 [87]	Lymphocytes	Human					↑	↑		
PFOA										
Chiu et al., 2018 [77]	Not described	Tox-Screening; ToxCast; Tox21					↑			Many different AOs
Gorrochategui et al., 2014 [78]	JEG-3	Human						↑		
Gorrochategui et al., 2016 [79]	A6 Kidney Epithelial Cells	Xenopus laevis						↑		
Lu et al., 2016 [88]	Sperm cells	Mouse	↓			↓	↑	↑	FABP3/4/6/ KAR/ELOVL5	AKT
Mashayekhi et al., 2015 [89]	Rat mitochondria (liver/brain)	Rat					↑	−		No changes in GSH levels
Reistad et al., 2013 [80]	Cerebellar granule cells	Rat					↑			

Table 5. *Cont.*

Authors	Cell System	Species	Reproduction	Amino Acids	Glucose	Lipids/Fats	Oxidative Stress	Cytotoxic/Reduced Cell Number	Spec. Genes	Other AO
Suh et al., 2017 [90]	RIN-m5F cells	Rat					↑	↑		
Tang et al., 2018 [91]	Primary lymphocytes	C. auratus					↑	↑		
Tian et al., 2021 [92]	RAW264.7	Mouse		↑↓		↑↓	↑	↑		
Wei et al., 2009 [85]	Primary hepatocytes	*Gobiocypris rarus* (fish)					↑	↑	–	
PFNA										
Gorrochategui et al., 2014 [78]	JEG-3	Human						↑		
Wei et al., 2009 [85]	Primary hepatocytes	*Gobiocypris rarus* (fish)					↑	↑	–	
PFHxS										
Gorrochategui et al., 2014 [78]	JEG-3	Human						–		
Lee et al., 2014 [93]	Neuronal cells	Rat					↑	↑		
Lee et al., 2014 [94]	PC12	Rat					↑	↑		
PFDA										
Dong et al., 2017 [95]	AGS gastric epithelial cells	Human						↓		
Kleszczyński et al., 2011 [96]	HCT116	Human								Ca ions inside mito-chondria
Wei et al., 2009 [85]	Primary hepatocytes	*Gobiocypris rarus* (fish)					↑	–		
Xu et al., 2019 [97]	Hepatic cells	Mouse					↑			DNA Damage
Mixture										
Wei et al., 2009 [85]	Primary hepatocytes	*Gobiocypris rarus* (fish)					↑	–		

Green cell (↑), increase; orange cell (↓), decrease; blue cell, changes in different directions; grey cell (–), no effect. For more details, see Supplementary Table S2.

Table 6. In vivo results related to PFAS exposures and cellular signaling pathways.

Authors	Species	Target Tissue	P-AKT (Thr308)	p-AKT (S473)	P38 mRNA	PPARα mRNA	PPARγ mRNA
PFOS							
Park et al., 2020 [59]	Crab (*Macrophthalmus japonicus*)	Gill, hepatopancreas			↑		
Qiu et al., 2016 [60]	Mouse (male ICR mice 8 weeks of age)	Testes		↑			
Xu et al., 2016 [86]	Mouse (C57BL/6 mice 10 weeks of age)	Epididymal white adipose tissue					↑
Zhang and Sun et al., 2020 [66]	Clam (*R. philippinarum*)	Hepatopancreas					↑
PFOA							
Du et al., 2018 [67]	Mouse (male Balb/c mice 6–7 weeks of age)	Adipose tissue		↓			
Lu et al., 2016 [88]	Mouse (male Balb/c mice 6–8 weeks of age)	Epidymis	↑				
Yan et al., 2015 [75]	Mouse (male Balb/c mice 6–8 weeks of age)	Liver	↑	↑			
Yan et al., 2015 [75]	Mouse (male Balb/c mice 6–8 weeks of age)	Muscle		↑			
Yan et al. 2015 [75]	Mouse (male Balb/c mice 6–8 weeks of age)	White adipose tissue		↓			
Yang 2010 [98]	Fish (male medaka fish)	Liver				↑	

Green cell (↑), increase; orange cell (↓), decrease. For more details, see Supplementary Table S3.

Table 7. In vitro results related to PFAS exposures and cellular signaling pathways.

Authors	Species	Cell Type	P-ERK	P-JNK	P-p38	PPARγ mRNA
PFOS						
Qiu et al., 2016 [60]	Mouse (male ICR mice 8 weeks of age)	Primary Sertoli cells			↑	
Sun et al., 2019 [82]	Human	SH-SY5Y (neuroblastoma)		↑		
Wang et al., 2015 [84]	Rat	HAPI (microglia-like cell line)	↑	↑	–	
Xu et al., 2016 [86]	Mouse	Adipocytes derived from 3T3-L1 preadipocyte cell line				↑
PFHxS						
Lee et al., 2014 [94]	Rat	PC12 (adrenal gland)	↑	↑	↑	
Lee et al., 2014 [93]	Rat (7-day old Sprague–Dawley rat pups)	Primary cerebella granular cells	↑	↑	↑	

Green cell (↑), increase; grey cell (–), no effect. For more details, see Supplementary Table S3.

3. Results

Exposure to PFAS and associated outcomes was investigated in a variety of experimental in vivo and in vitro studies (Tables 4–7, Tables 8–11). The reviewed studies encompass a large variety of different species used as in vivo models (fish, mollusks, crustacea, insects, nematodes, amphibians, and rodents) as well as in vitro models (yeast, fish, amphibian, rodent, monkey, and human cells). Most studies focused on PFOA or PFOS exposure, while fewer studies also included perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), or perfluorodecanoic acid (PFDA). Mixture exposure were also studied in a small number of in vivo and in vitro studies (Table 5, Tables 8 and 9). The PFAS treatments used in these studies can only approximate the human exposure situation. Often, superphysiological concentrations were tested over short exposure times ranging from hours/days in vitro to several weeks in vivo.

3.1. PFAS-Associated Cytotoxicity and Oxidative Stress

The in vivo studies demonstrate that PFOA and PFOS exposure can cause various outcomes, including reduced body weight and fetal and offspring weight, adverse effects on reproduction and development, changes in metabolism (i.e., altered levels of amino acids, lipids, and glucose), and changes in liver function and the (epi-)genome. The direction of these effects varied greatly (very often, effects in both directions were observed), so firm conclusions on these PFAS-induced adverse outcomes cannot be drawn (Table S1).

However, both PFOA and PFOS showed co-occurrence of oxidative stress and animal death or increased cellular damage in 3 in vivo and 13 in vitro studies (Tables 4 and 5). Although the effects were observed to occur in a dose-dependent manner, the effect concentrations differed between the model of investigation. In general, the concentrations at which oxidative stress appeared were often lower than those at which cytotoxicity was induced, suggesting that oxidative stress may precede animal lethality or cell damage (Tables S1 and S2). Information on cell models, (animal) species, effect concentrations, and exposure route and time is given in Tables S1 and S2. In addition to PFOA and PFOS, the in vitro studies also showed consistent increase of oxidative stress upon PFNA, PFHxS, or PFDA exposure going along with increased cell damage (including reduced cell number, increased apoptosis, increased cytotoxicity, and decreased viability) after exposure to PFHxS. In the case of PFNA and PFDA, only a few studies have been conducted to investigate cell damage, and these do not provide a conclusive picture regarding the direction of the effect (Table 5). Possible reasons for this inconsistency are the different model systems (whole organism versus cell culture) and differences in treatment concentrations and exposure time.

3.2. PFAS-Associated Activation of PPAR, AKT, and MAPK Signaling Pathways

The here-reviewed in vivo and in vitro studies show that PFAS exposure resulted in an increased gene expression of transcription factors peroxisome proliferator-activated receptor (PPAR)α and PPARγ. In addition, PFAS were found to enhance the phosphorylation of key cellular signaling molecules including AKT, also known as Protein kinase B, and the mitogen-activated-protein kinases (MAPKs) ERK, JNK, and p38, thereby activating them (Tables 6, 7 and S3). Information on (animal) species, cell models, effect concentration, and exposure time is given in Table S3. The aforementioned effects on gene expression and phosphorylation were independent of the PFAS compound used in the in vivo and in vitro studies (PFOA, PFOS, and PFHxS). However, there was an unexpected finding regarding tissue-specific response to PFOA exposure (Table 6). AKT activity reduced upon PFOA exposure in adipose tissue. This phenomenon was not found in liver and muscle tissue from the same mice, where AKT activity was actually increased after PFOA treatment.

3.3. PFAS-Associated Endocrine Effects

PFAS-Associated Estrogenic and Androgenic Effects

The in vivo studies generally found increased estrogen levels and decreased testosterone levels after PFAS exposure (Tables 8 and S4). The effect doses ranged from 1–25 mg/kg/day in rodent and 25–250 μg/L in fish (Table S4). The animal species, exposure route, range, and time are given in Table S4. Increased estrogen receptor (ER) levels (RNA and/or protein) were also found in the majority of the here-reviewed in vivo studies, whereas the androgen receptor (AR) levels (RNA and/or protein) were decreased. The changes in hormone levels might relate to changes in the steroidogenesis cytochrome (CYP) enzyme levels, which were found altered in several studies. Many in vivo studies also measured the vitellogenin (VTG) protein and reported increased levels conforming the estrogenic activities of PFAS. Other estrogenic and androgenic effects found in the in vivo studies include altered sperm genesis, altered gene expression of the hypothalamic–pituitary–gonadal–liver (HPGL) axis, and reduced anogenital distances (AGD) and testicular weights in male offspring (Tables 8 and S4). The results from the in vitro studies (Tables 9 and S5) confirm the in vivo results. The specific cell model and exposure range and time are given in Table S5. The estrogen production was generally increased, and the testosterone production decreased after PFAS exposure. The majority of studies found agonistic estrogenic activities of the PFAS in the reporter gene assays; however, only one study found estrogenic effects with the E-screen assay. Of the six studies investigating androgenic receptor activities of PFAS, only one study [99] reported antagonistic effects. The H295R steroidogenesis assay or aromatase activity assay were used to investigate in vitro effect on the steroidogenesis, and the results were conflicting. Some found increased expression of the steroidogenesis CYP enzymes and decreased aromatase activity [99–101], while many others found no effect. Other estrogenic-related outcomes in the in vitro studies include altered expression of estrogen-responsive biomarker genes and increased progesterone and estrone level (Tables 9 and S5).

Table 8. In vivo results related to PFAS exposures and estrogen and androgen pathways.

Publication information	Study Setup	Estrogenic and Androgenic Related Results								
Authors	Species	Estrogen levels	ER Transcription (mRNA)	ER Expression (Protein)	VTG	Testosterone Levels	AR Transcription (mRNA)	AR Expression (protein)	CYP	Other Directly Estrogenic/Androgenic Related Effects
PFOS										
Bao et al., 2019 [102]	Female zebrafish	↑↓	↑↓		↑↓					Altered gene expression along the HPGL axis
Bao et al., 2020 [103]	Male zebrafish		↑		↑		↓			↓FSH and LH receptor in gonads, ↓ expression of GnRH, GNRHr, FSH, and LH in brain, impaired sexual behavior
Benninghoff et al., 2011 [104]	Juvenile rainbow trout				–					

Table 8. *Cont.*

Publication information	Study Setup	Estrogenic and Androgenic Related Results								
Authors	Species	Estrogen levels	ER Transcription (mRNA)	ER Expression (Protein)	VTG	Testosterone Levels	AR Transcription (mRNA)	AR Expression(protein)	CYP	Other Directly Estrogenic/Androgenic Related Effects
Biegel et al., 1995 [105]	Rats (male CD)	↑				↑			↑CYP19	
Chen et al., 2016 [106]	Zebra fish (Post-fertilization)	↑	↑			↓			CYP19A (↑female) / (↓male)	↑amh (gonad), structural changes in gonads
Du et al., 2013 [107]	Zebrafish embryo		↑↓						↓CYP17, CYP19a, CYP19b	
Qu et al., 2016 [108]	Mouse (C57 male)	–	↑↓			↓				↓sperm concentration, vacuolations observed in spermatogonia, spermatocytes and Leydig cells, ↑incidence of apoptotic cells (testes)
Qiu et al., 2020 [109]	Famale Spague Dawley rat	↑		↑						
Qiu et al., 2021 [110]	Mouse (ICR male)	–				↓				No effect on LH or FSH, ↓ sperm count, damaged testicular interstitium morphology
Rodríguez-Jorquera et al., 2019 [111]	Fathead minnow (*Pimephales promelas*)		↑							
Rosen et al., 2017 [112]	Mouse (wt and ppara-null)									Gene-expression: ↓male-specific genes, ↑female-specific genes
Xin et al., 2020 [113]	Zebra fish	↑	↑		↑				↑CYP19a, ↓CYP19b	Altered spermgenesis
Xu et al., 2017 [114]	Mouse (-/- and +/+ ERβ)			↑						Only in ERβ +/+ mice: hydropic degeneration and vacuolation in hepatocytes, increase cholesterol and bile acid, altered liver genes.

Table 8. *Cont.*

Authors	Species	Estrogen levels	ER Transcription (mRNA)	ER Expression (Protein)	VTG	Testosterone Levels	AR Transcription (mRNA)	AR Expression(protein)	CYP	Other Directly Estrogenic/Androgenic Related Effects
Zhang and Lu et al., 2020 [115]	Rats (pregnant Sprague-Dawley)					↓			↓CYP11A1, CYP17A1, Hsd17b3	↓Dhh and SOX9 (sertoli cells), affected proliferation (leydig stem cells)
Zhao et al., 2014 [116]	Rats (pregnant Sprague-Dawley)					↓			↓Cyp11a1 Cyp17a, Hsd3b1	↓ AGD and testicular weights (male pups), impaired fetal Leydig cells, ↓ fetal Leydig cells number
Zhong et al., 2016 [117]	Mouse (C57BL/6)	↑				↓				
PFOA										
Benninghoff et al., 2011 [104]	Juvenile rainbow trout				↑					
Lu et al., 2019 [118]	Rat (Sprague-Dawley with eliminated Leydig cells)					↓			↓CYP11A1, CYP17A1	No effect on serum FSH and LH, ↓expression of Lhcgr, Scarb1, Star, Hsd3b1 and Hsd11b1 in leydig cells, affected proliferation of stem Leydig cells
Qiu et al., 2020 [109]	Female Sprague Dawley rat	↑		↑						
Rosen et al., 2017 [112]	Mouse (wt and ppara-null)									↓expression of male-specific genes, ↑expression of female-specific genes
Wei et al., 2007 [119]	Freshwater rare minnow		↑		↑					↓ Degenerating vitellogenic-stage oocytes
Xin et al., 2019 [120]	Zebra fish	↑			↑					
Yao et al., 2014 [121]	Female CD-1 mouse									No effect of ER target genes

Table 8. *Cont.*

Authors	Species	Estrogen levels	ER Transcription (mRNA)	ER Expression (Protein)	VTG	Testosterone Levels	AR Transcription (mRNA)	AR Expression (protein)	CYP	Other Directly Estrogenic/Androgenic Related Effects
Zhao et al., 2010 [122]	Female C57Bl/6 mice	–		↑						↑serum progesterone, ↑mammary gland responses to estrogen and progesterone, ↑liver steroid hormone metabolic enzyme gene expressions, no effect on SHBG
PFNA										
Benninghoff et al., 2011 [104]	Juvenile rainbow trout				↑					
Feng et al., 2009 [123]	Rat (Sprague–Dawley male)	↑				↑↓				No effect on FSH and LH
Rosen et al., 2017 [112]	Mouse (wt and ppara-null)									↓ expression of male-specific genes, ↑ expression of female-specific genes
Singh et al., 2019 [124]	Mouse (prepubertal Parkers male)								↓CYP11A	
Singh et al., 2019 [125]	Mouse (prepubertal Parkers male)						↓	↓		↓ Impairment in testicular functions, Decreased overall germ cell transformation
PFHxS										
Rosen et al., 2017 [112]	Mouse (wt and ppara-null)									↓expression of male-specific genes, ↑expression of female-specific genes
PFDA										
Benninghoff et al., 2011 [104]	Juvenile rainbow trout				↑					

Table 8. *Cont.*

Publication information	Study Setup	Estrogenic and Androgenic Related Results								
Authors	Species	Estrogen levels	ER Transcription (mRNA)	ER Expression (Protein)	VTG	Testosterone Levels	AR Transcription (mRNA)	AR Expression (protein)	CYP	Other Directly Estrogenic/Androgenic Related Effects
Mixture										
Benninghoff et al., 2011 [104]	Juvenile rainbow trout				↑					
Rodríguez-Jorquera et al., 2019 [111]	Fathead minnow (Pimephales promelas)		↑							

Green cell (↑), increase; orange cell (↓), decrease; blue cell, alteration or change in different directions; grey cell (–), no effect. For more details, see Supplementary Table S4. ER, estrogen receptor; AR, androgen receptor; VTG, vitellogenin; CYP, cytochromes P450; HPGL, hypothalamus–pituitary–gonadal–liver; GnRH, gonadotropin-releasing hormone; GNRHr, gonadotropin-releasing hormone receptor; FSH, follicle-stimulating hormone; LH, luteinizing hormone; Dhh, desert hedgehog.

Most in vivo studies showed that PFOS, PFHxS, and PFOA exposure affect the level of thyroid hormone (TH) by decreasing T4 (three increase, eight decrease), while T3 level varied in different studies (four increase, five decrease) (Table 10). The animal species, exposure route, range, and time are given in Table S6. The effect doses ranged from 1–63 mg/kg/day in rodent and 0.2–0.5 g/L in fish in regard to the thyroid effects (Table S6). Interestingly, in the three zebrafish PFAS exposure studies, the T3 level increased, whereas in rodent rat studies, the T3 levels decreased, but for the single mice study, the T3 increased. Two studies show, respectively, a PFOS-related decrease of TSH and thyroglobulin (TG), whereas another study observed an increase in TSH-receptor (TSHR) and thyroperoxidase (TPO). In addition, for thyroid cell histology, PFOS elicited a decrease in nuclear area in zebrafish embryos, and a PFOS substitute (F-538) caused thyroid follicular hyperplasia in adult female rats. Thus, the in vivo studies also observed that PFAS exposure could result in decreased embryo mass or pup birth weight and abnormal morphology in thyroid cell. The reviewed 18 studies reported that PFASs influences the expression of thyroid-hormones-related genes in zebra fish embryos, adult rats, amphibians/Xenopus laevis, pregnant mice, and chicken embryos (Tables 10 and S6).

For the in vitro studies, overall results are shown in Table 11, and the specific cell type, exposure range, and time are given in Table S7. Ten of the in vitro studies showed that PFAS (PFOS, PFOA, PFNA, PFHxS, and PFDA) bind to the human thyroid hormone transport protein transthyretin (TTR) although the binding potency was lower than TH (Table 11). Thus, the in vitro studies indicate that PFAS can interfere with TH transport in vivo by competitively displacing TH from TTR. PFAS also bind to TH receptors (TRα and TRβ) and activate their transcriptional activity and/or displace T3, causing a transcriptional decrease. In general, T-screen studies in rat Gh3 cells elicited that PFAS antagonized the T3-induced GH3 cell growth, whereas PFOS or its substitute exposure alone could increase cell growth. PFOS and PFOA increased the T4 level in rat hepatic cells. Some PFAS such as PFOS, PFOA, and PFHxS inhibit iodine uptake in both human and rodent cells. In human carcinoma cells, PFOS and PFOA inhibited TPO activity, an enzyme important for TH biosynthesis. PFOS elicited an altered steroidogenic gene expression in human H295R cells. Moreover, our review includes reports on PFAS molecular docking by fitting into the receptor pocket

of thyroid receptors. Only two study evaluated effect of PFAS (PFOS, PFOA, PFNA, PFHxS, and PFDA) on thyroxine-binding globulin (TBG) and found no significant in vitro effect (Tables 11 and S7).

Table 9. In vitro results related to PFAS exposures and estrogen and androgen pathways PFAS-associated thyroid hormone effect.

Authors	Species	Estrogen activity (reporter gene)	Estrogen activity (E-screen)	ERα Expression	ERβ Expression	E2 Secretion/Production	Androgen activity	AR Protein	T Secretion/Production	CYP Enzyme Activities	Other Estrogen-Related Effects
PFOS											
Xin et al., 2020 [113]	Human	↑	↑								
Gogola et al., 2020 [126]	Human					↓					↑2-OHE1/E2 ratio
Gogola et al., 2020 [126]	Human					↑					↓ 2-OHE1, 16-OHE1, 2OHE1/E2 ratio, 16-OHE1/E2 ratio
Halsne et al., 2016 [127]	Human										Normal acini maturation affected, ER-independent mechanisms to normal development of glandular breast tissue
Xu et al., 2017 [114]	Human				↑						
Benninghoff et al., 2011 [104]	Human	↑									
Maras et al., 2006 [128]	Human		–								Altered expression of estrogen-responsive biomarker genes
Li et al., 2020 [129]	Human	↑									Altered expression of estrogen-responsive biomarker genes
Ishibashi et al., 2008 [130]	Yeast	–									
Behr et al., 2018 [101]	Human	↑	–				–				Increased progesterone and estrone, no effect on estrogen- or androgen-responsive genes,
Du et al., 2013 [107]	Monkey and human	↑				↑	–		↓	Altered gene expression	
Rosen et al., 2017 [112]	Human	↑					–				
Biegel et al., 1995 [105]	Rat						–		↓		
Kjeldsen et al., 2013 [99]	Human and hamster	↑					↓			Aromatase unchanged	

Table 9. *Cont.*

Authors	Species	Estrogen activity (reporter gene)	Estrogen activity (E-screen)	ERα Expression	ERβ Expression	E2 Secretion/Production	Androgen activity	AR Protein	T Secretion/Production	CYP Enzyme Activities	Other Estrogen-Related Effects
Kang et al., 2016 [100]	Human	↓				↑	–		↓	↑CYP17, 3b-hsd2, cyp19	↑ Estrone
PFOA											
Xin et al., 2019 [120]	Human	↑									
Yao et al., 2014 [121]	Human	–									
Gogola et al., 2020 [126]	Human					↓					↑2-OHE1/E2 ratio
Gogola et al., 2020 [126]	Human					↑					↓2-OHE1, 16-OHE1, 2OHE1/E2 ratio, 16-OHE1/E2 ratio
Halsne et al., 2016 [127]	Human										Normal acini maturation not affected
Benninghoff et al., 2011 [104]	Human	↑									
Maras et al., 2006 [128]	Human			–							Altered expression of estrogen-responsive biomarker genes
Li et al., 2020 [129]	Human	↑									Altered expression of estrogen-responsive biomarker genes
Ishibashi et al., 2008 [130]	Yeast	–									
Behr et al., 2018 [101]	Human	↑		–			–			↑CYP21A2	↑ Estrone, no effect on estrogen- or androgen-responsive genes
Buhrke et al., 2015 [131]	Human			↑↓							
Rosen et al., 2017 [112]	Human	↑					–				
Rosenmai et al., 2013 [132]	Human and hamster					↑↓	–	–		Unchanged CYP11A, CYP17 or CYP21	↑Estrone, ↓ androstenedione, no effect on production of progesterone, 17-OH progesterone, or DHEA
Kjeldsen et al., 2013 [99]	Human and hamster	↑					↓			Unchanged Aromatase	
Kang et al., 2016 [100]	Human	↓				↑	–		↓	↑CYP17, 3b-hsd2, cyp19	↑ Estrone

Table 9. *Cont.*

Authors	Species	Estrogen activity		ERα Expression	ERβ Expression	E2 Secretion/Production	Androgen activity	AR Protein	T Secretion/Production	CYP Enzyme Activities	Other Estrogen-Related Effects
		reporter gene	E-screen								
PFNA											
Halsne et al., 2016 [127]	Human										Normal acini maturation affected, ER-independent mechanisms to normal development of glandular breast tissue
Benninghoff et al., 2011 [104]	Human	↑									
Maras et al., 2006 [128]	Human										
Li et al., 2020 [129]	Human	↑									Altered expression of estrogen-responsive biomarker genes
Ishibashi et al., 2008 [130]	Yeast	–									
Rosen et al., 2017 [112]	Human	↑					–				
Kjeldsen et al., 2013 [99]	Human and hamster	–					↓			Unchanged Aromatase	
PFHxS											
Li et al., 2020 [129]	Human	↑									Altered expression of estrogen-responsive biomarker genes
Behr et al., 2018 [101]	Human	–	–				–			No effect on steroidogenesis	–: No effect on estrogen- or androgen-responsive genes
Rosen et al., 2017 [112]	Human	↑					–				
Kjeldsen et al., 2013 [99]	Human and hamster	↑					↓			Unchanged Aromatase	
PFDA											
Halsne et al., 2016 [127]	Human										Normal acini maturation affected, ER-independent mechanisms to normal development of glandular breast tissue
Benninghoff et al., 2011 [104]	Human	↑									

Table 9. *Cont.*

Authors	Species	Estrogen activity (reporter gene / E-screen)	ERα Expression	ERβ Expression	E2 Secretion/Production	Androgen activity	AR Protein	T Secretion/Production	CYP Enzyme Activities	Other Estrogen-Related Effects
Li et al., 2020 [129]	Human	↑								Altered expression of estrogen-responsive biomarker genes
Ishibashi et al., 2008 [130]	Yeast	–								
Kjeldsen et al., 2013 [99]	Human and hamster	–				↓			↓ Aromatase	
MIXTURE										
Gogola et al., 2020 [126]	Human				↓				↑↓	↑ 2-OHE1/E2 ratio
	Human				↑				–	↓2-OHE1, 16-OHE1, 2OHE1/E2 ratio, 16-OHE1/E2 ratio
Gogola et al., 2020 [133]	Human									
	Human									Effect on IGF1 though ERα
Gogola et al., 2020 [133]	Human		–	–						–: Effects were independent of ER pathway
	Human		–	–						–: Effects were independent of ER pathway
Kjeldsen et al., 2013 [99]	Human and hamster	↑				↓			Unchanged Aromatase	
Dairkee et al., 2018 [134]	Human		↑	↓						

Green cell (↑), increase; orange cell (↓), decrease; blue cell, alteration or change in different directions; grey cell (–), no effect. For more details, see Supplementary Table S5. ER, estrogen receptor; E2, 17-beta-estradiol; AR, androgen receptor; CYP, cytochromes P450; 2-OHE1, 2-Hydroxyestrone; 16-OHE1, 6-hydroxyestrone; IGF1, Insulin-Like Growth Factor I; T, testosterone.

Table 10. In vivo results related to PFAS exposures and thyroid hormone pathway.

Authors	Species	Body Weight	Organ Weight	Thyroid Hormone Level			Protein Expression/Level			Thyroid Cell Histology	Gene Expression
				T3	T4	TSH	TG	TSHR	TPO		
PFOS											
Chen et al., 2018 [135]	Zebrafish embryos				↓					↓nuclear area	↓ thyroid function-related gene expression
Du et al., 2013 [107]	Zebrafish embryos										↑gene related to early thyroid development (hhex and pax8)
Kim et al., 2011 [136]	Zebrafish embryos	↓length		↑	↓						↓TRα, TRβ, hhex, and pax8

Table 10. *Cont.*

Authors	Species	Body Weight	Organ Weight	Thyroid Hormone Level			Protein Expression/Level			Thyroid Cell Histology	Gene Expression
				T3	T4	TSH	TG	TSHR	TPO		
Ren et al., 2015 [137]	Amphibians (X. laevis)										↑TH upregulated genes; ↓TH downregulated genes
Shi et al., 2009 [138]	Zebrafish embryos	↓		↑							alter genes in HPT system (↓TSH, TTR, TRα, ↑TRβ)
Yu et al., 2011 [139]	Adult female Wistar rat			↓	↓						↑hepatic genes related to T4 uptake and regulation
PFOS potassium salt (PFOS-K)											
Chang et al., 2008 [140]	Female adult SD rat			↓	↓TT4, transit ↑FT4	↓					
F-53B (PFOS substitute)											
Deng et al., 2018 [141]	Zebrafish embryos	↓			↑		↓				↑ttr, ↓tg
Hong et al., 2020 [142]	Adult female SD rat			↓	↓			↑	↑	Thyroid follicular hyperplasia	
PFOA											
Blake et al., 2020 [43]	Pregnant CD-1 mice	↓embryo	↑placenta								
Godfrey et al., 2019 [143]	Japanese medaka embryo										↑ thyroid-related genes
Kim et al., 2021 [144]	Zebrafish embryos										↑genes related to activation or metabolism
HFPO-DA (PFOA substitute)											
Blake et al., 2020 [43]	Pregnant CD-1 mice		↑placenta		↑placenta						
Conley et al., 2021 [145]	SD rat (dam)	↓pup		↓	↓						
PFNA											
Liu et al., 2011 [146]	Zebrafish embryos			↑							alter genes related to TH synthesis and metabolism in F1 larvae
PFHxS											
Ramhøj et al., 2020 [147]	Wistar rat (dam and offspring)			↓	↓	–					
Cassone et al., 2012 [148]	Chicken embryos	↓embryo			↓						↑TH-response genes
PFDA											
Harris et al., 1989 [149]	Adult female C57BL/6 mice	↓	↓Thymus	↑	↑						

Green cell (↑), increase; orange cell (↓), decrease; blue cell, alteration or change in different directions; grey cell (–), no effect. For more details, see Supplementary Table S6. TTR, transthyretin; TPO, thyroperoxidase; TR, thyroid hormone receptor; TG, thyroglobulin; TR, thyroid hormone receptor; TSH, thyroid-stimulating hormone; TSHR, TSH receptor; TH, thyroid hormone; T4, thyroxine; T3, triiodothyronine; FT4, free T4; FT3, free T3; TT4, total T4; TT3, total T3; hhex, hematopoietically expressed homeobox; pax8, paired box gene 8.

Table 11. In vitro results related to PFAS exposures and thyroid hormone pathway.

Authors	Cell Species	Protein				T-Screen		NIS	RAIU	Gene Expression	Molecular Docking
		TTR Binding	TBG	TPO	TR		T4				
PFOS											
Ren et al., 2016 [150]	Human	B									
Song et al., 2012 [151]	Human			↓							
Selano et al., 2019 [152]	Rat male						↑FT4, ↑hepatic uptake				
Xin et al., 2018 [153]	Human	B	–		B: TRα, TRβ						
	Human				↑Transactivity						Fit into pocket of TTR and TRs
	Rat					↑Compound alone					
Weiss et al., 2009 [154]	Human	B									
Long et al., 2013 [155]	Rat					↓Compound alone and +T3					
Buckalew et al., 2020 [156]	Rat							↓			
	Human							↓			
Wang et al., 2019 [157]	Human								↓		
Song et al., 2011 [158]	Human			↓							
Du et al., 2013 [107]	Monkey				↓Transactivity +T3						
	Human									Altered steroidogenic genes	
Ren et al., 2015 [137]	Human				B: TRα						Fit into T3-binding pocket of TRα-LBD
	Rat					↑Compound alone and +T3					
PFOS potassium salt PFOS-K											
Buckalew et al., 2020 [156]	Rat							↓			
	Human							↓			
Wang et al., 2019 [157]	Human								↓		
F-53B PFOS substitute											
Deng et al., 2018 [141]	Rat					↑Compound alone					
PFOA											
Ren et al., 2016 [150]	Human	B	–								
Song et al., 2012 [151]	Human			↓							
Selano et al., 2019 [152]	Rat male						↑FT4, ↑hepatic uptake				
Weiss et al., 2009 [154]	Human	B									

Table 11. *Cont.*

Authors	Cell Species	Protein				T-Screen	NIS	RAIU	Gene Expression	Molecular Docking
		TTR Binding	TBG	TPO	TR	T4				
Long et al., 2013 [155]	Rat					↓Compound alone				
Buckalew et al., 2020 [156]	Rat						↓			
	Human						↓			
Ren et al., 2015 [137]	Human				B: TRα					Fit into T3-binding pocket of TRα-LBD
	Rat					–				
Kim and Lee et al., 2021 [144]	Rat								*Dio2*↓	
PFOA-ammonium										
Buckalew et al., 2020 [156]	Rat									
	Human						↓			
Wang et al., 2019 [157]	Human						↓	–		
PFNA										
Ren et al., 2016 [150]	Human	B	–							
Weiss et al., 2009 [154]	Human	B								
Long et al., 2013 [155]	Rat					↓Compound alone and +T3				
Wang et al., 2019 [157]	Human							–		
Ren et al., 2015 [137]	Human				B: TRα					Fit into T3-binding pocket of TRα-LBD
	Rat					–				
PFHxS										
Ren et al., 2016 [150]	Human	B	–							
Weiss et al., 2009 [154]	Human	B								
Long et al., 2013 [155]	Rat					↓Compound alone and +T3				
Ren et al., 2015 [137]	Human				B: TRα weakly					Fit into T3-binding pocket of TRα-LBD
	Rat					–				
PFHxS potassium PFHxS-K										
Buckalew et al., 2020 [156]	Rat						↓			
	Human						↓			
PFDA										
Long et al., 2013 [155]	Rat					↓Compound alone				
Wang et al., 2019 [157]	Human							–		

Table 11. *Cont.*

Authors	Cell Species	Protein				T-Screen	NIS	RAIU	Gene Expression	Molecular Docking
		TTR Binding	TBG	TPO	TR	T4				
Ren et al., 2015 [137]	Human				B: TRα					Fit into T3-binding pocket of TRα-LBD
	Rat					–		–		
Ren et al., 2016 [150]	Human	B	–							

Green cell (↑), increase; orange cell (↓), decrease; blue cell, alteration or change in different directions; grey cell (–), no effect; purple cell (B), binding to the receptor. For more details, see Supplementary Table S7. TTR, transthyretin; TPO, thyroperoxidase; TR, thyroid hormone receptor; TG, thyroglobulin; TR, thyroid hormone receptor; TSH, thyroid-stimulating hormone; TSHR, TSH receptor; TH, thyroid hormone; T4, thyroxine; T3, triiodothyronine; FT4, free T4; FT3, free T3; TT4, total T4; TT3, total T3; NIS, sodium iodide symporter; RAIU, radioactive iodide uptake; LBD, ligand binding domain; TH, thyroid hormone.

4. Discussion

Exposure to endocrine-disrupting chemicals can affect maternal and fetal health, including long-term health effects later in life [159,160] (Figure 2). The underlying mechanisms are not yet well-understood. This review aimed to identify and further describe mechanisms that may underlie fetal growth reduction to better understand prenatal PFAS exposures and contribute to the establishment of potential new AOPs.

4.1. Experimental Studies on Oxidative Stress and Cytotoxicity

The dose-dependent lethality of PFAS might stem from cytotoxic properties that were found in in vitro studies for PFOA and PFOS but also for PFNA and PFHxS (Table 5). Although all these PFAS seem to be toxic to cells, the molecular mechanism behind this is unknown. It is not fully understood if and how PFAS enter a cell. Due to their amphiphilic structure, passive diffusion across a cellular membrane seems unlikely, indicating an active transport mechanism for this substance class [161]. It has been suggested that PFAS could be substrates for several transporter proteins, including organic anion transporters (OATs) and ATP-binding cassette (ABC) transporters [76,162,163]. In addition, PFAS could enter cells bound to protein ligands, such as albumin and fatty acid binding proteins [161].

PFAS-induced oxidative stress is a well-documented and likely mechanism explaining cytotoxicity (reviewed by [164]). However, it remains unknown whether PFAS directly generate oxidative stress or if PFAS-associated oxidative stress is an indirect effect.

Oxidative stress results from an imbalance between production and accumulation of oxidizing species (most importantly, reactive oxygen reactive species (ROS) such as hydroxyl radicals (●OH), superoxide radicals ($O2•-$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2)) in cells or tissues and the inability of a biological system to detoxify these reactive products. ROS are naturally produced in mitochondria and crucial mediators of many physiological processes. They become toxic, when present in excess, by oxidizing macromolecules such as DNA, proteins, and lipids. Therefore, cells have developed various antioxidant defensive mechanisms, including enzymes such as superoxide dismutase (SOD), catalase (CAT), and those constituting the glutathione system, to be protected from ROS-induced cellular damage [165,166].

Excessive ROS can activate different cellular signaling pathways, including the MAPKs JNK, ERK, and p38. MAPK signaling pathways have fundamental roles in the induction or inhibition of apoptosis. Constant ROS-mediated activation eventually leads to apoptosis [167]. Global induction of ROS-mediated apoptosis via PFAS is very unlikely to explain the relatively mild effects on birth weight, as the PFAS concentrations required to induce this apoptosis do not resemble the in vivo situation, as they are hyper-physiological.
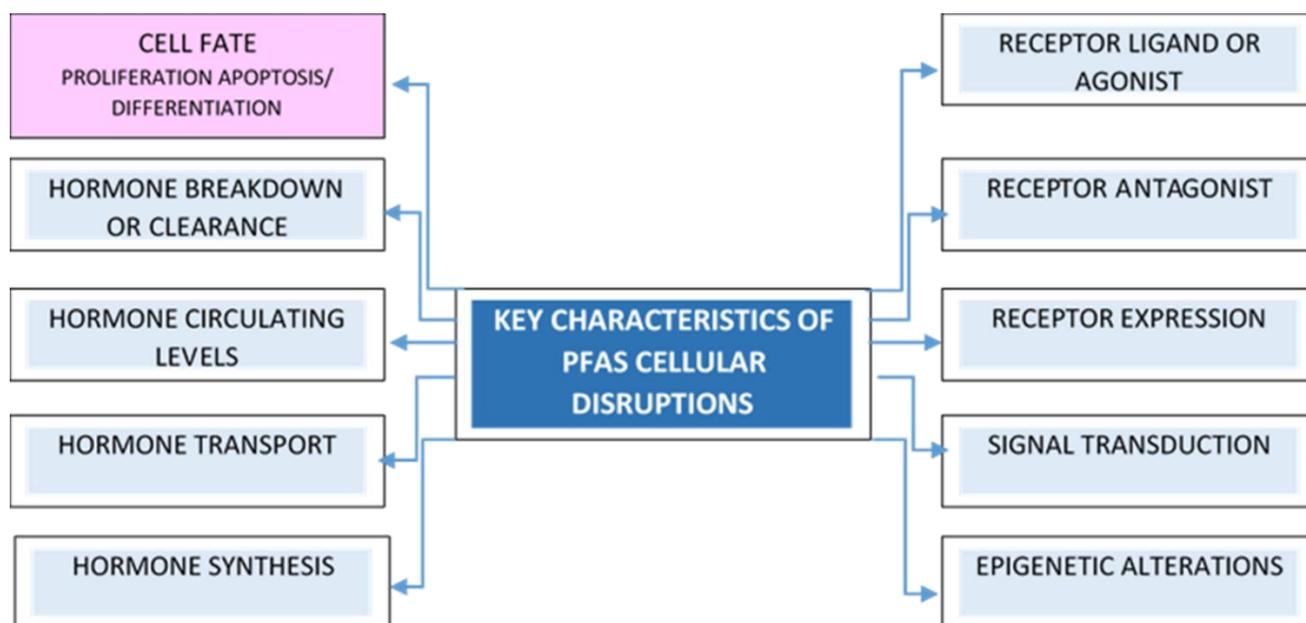
Figure 2. The key characteristics of potential PFAS cellular disruptions for hazard identification.

It is possible that reduced birth weight is not the consequence of cell death but mass loss from reduced cell proliferation. One possibility to reduce cell proliferation is to differentiate cells (e.g., pre-adipocytes) into a non-dividing state (e.g., mature adipocytes) [168]. Adipogenesis, the maturation of adipocytes from adipose tissue derived mesenchymal stem cells, is an ROS-regulated differentiation process [169,170]. During adipogenesis, mesenchymal stem cells undergo a first differentiation step to pre-adipocytes, which still have proliferation potential. A second differentiation step turns pre-adipocytes into mature adipocytes, which no longer have the ability to proliferate [171].

During adipogenesis, AKT is activated by recruitment to the cell membrane, where it is phosphorylated by different proteins, e.g., phosphatidylinositol 3-kinase (PI3K) or mTORC2 or PDK1/2 [172,173]. Activated AKT increases the expression of transcription factor PPARγ, which enhances the expression of adipogenic genes [174]. In addition, MAPKs JNK, ERK, and p38 are activated during adipogenesis [174]. The universal activation mechanism of MAPKs is that MAPK-kinase-kinases (MAPKKKs) phosphorylate and activate MAPK-kinases (MAPKKs). These MAPKKs in turn activate the MAPKs (p38, JNK, and ERK) by phosphorylation [175].

From the studies reviewed here, both oxidative stress and activation of AKT and MAPK signaling increase in response to PFOA and PFOS exposure (Tables 6, 7 and S3). Interestingly, a decrease in total body weight is accompanied by structural changes in adipose tissue [67] and a specific decrease in adipose tissue weight after PFOA treatment [75].

Taken together, we propose a mechanism by which PFAS could lower adipose tissue weight (Figure 3) and thereby reduce birth weight. This model is based on several facts: (I) physiological PFAS concentrations are sufficient to generate ROS [164]; (II) ROS activate PPARγ, AKT, and MAPKs [176–178]; and (III) activation of these proteins is involved in adipogenesis [174].
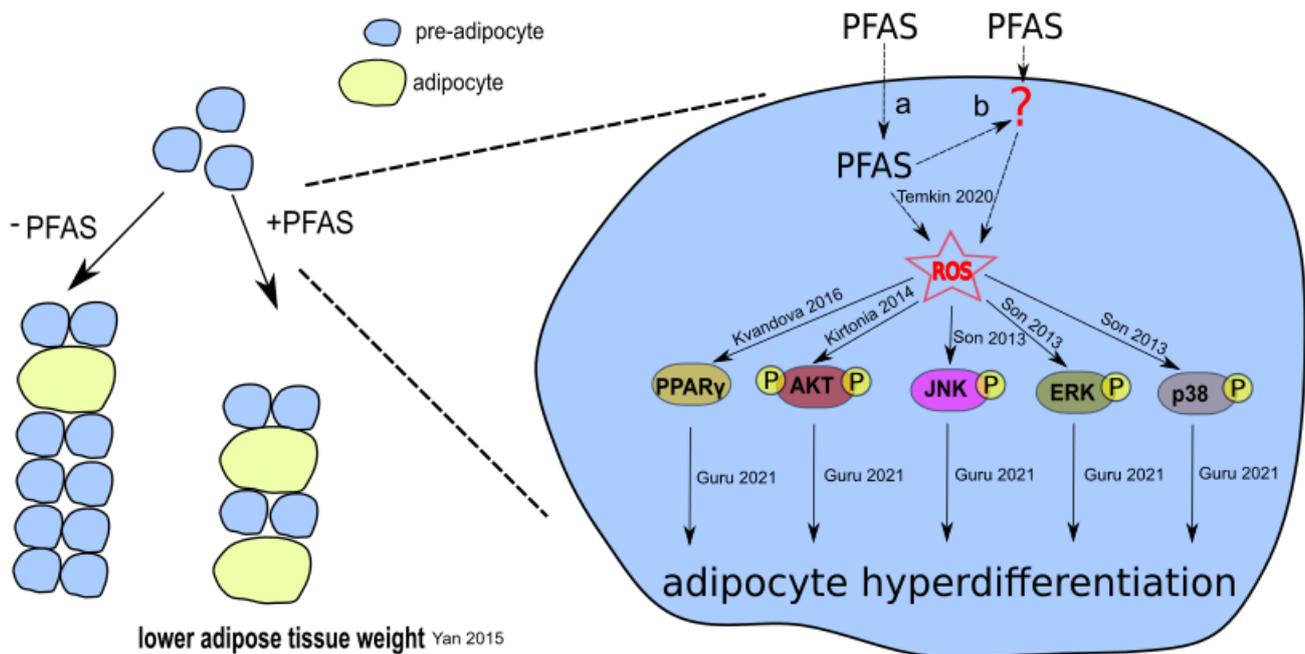
Figure 3. Possible mechanism of PFAS-associated reduction in adipose tissue. PFAS generate ROS directly (a) or indirectly (b). ROS trigger elevated PPARγ expression as well as activation of AKT, JNK, ERK, and p38 by phosphorylation, resulting in hyper-differentiation of pre-adipocytes. As a result, fewer proliferating pre-adipocytes remain after PFAS exposure, causing lower adipose tissue weight. Temkin 2020 [164]; Kvandova 2016 [177]; Kirtonia 2020 [176]; Son 2013 [178]; Guru 2021 [174]; Yan 2015 [75]. The proposed mechanism is not related to existing AOPs (AOP-Wiki).

It is conceivable that PFAS exposure could lead to differentiation of more pre-adipocytes to mature adipocytes. Indeed, it was demonstrated that PFOA, PFOS, PFHxS, and PFNA could differentiate the 3T3-L1 pre-adipocyte cell line into adipocytes in vitro [179].

The molecular mechanism could be PFAS-associated ROS production that triggers adipogenesis via increased expression of PPARγ-related genes and activation of MAPKs ERK, JNK, and p38 as well as AKT. Interestingly, AKT phosphorylation actually decreased and not increased (as expected during adipogenesis) in adipose tissue of PFOA-treated mice [67,75]. This could be caused by the insulin-resistant phenotype of these mice, as decreased AKT phosphorylation was described during insulin resistance [180,181]. Why AKT phosphorylation was specifically reduced in adipose tissue but not muscle or liver tissue should be clarified in future studies.

Nevertheless, the upregulation of some or all of these aforementioned pathways could lead to more differentiated adipocytes and fewer proliferating pre-adipocytes in response to PFAS. Fewer pre-adipocytes would over time lead to a reduced number of adipocytes and overall lesser adipose tissue weight. Thus, PFAS exposure would result in less adipose tissue mass and, in this way, may reduce birth weight. In studies on rodents, a decrease in adipose tissue mass after PFOS and PFOA treatment has already been found [75,182,183]. In women, an inverse relationship between PFOA, especially PFNA and PFDA, exposure and body fat mass was observed [184]. Whether a reduction in adipose tissue mass in response to PFAS exposure in general (or at least for PFOA, PFOS, PFHxS, PFNA, and PDFA) could result in lower birth weight is a matter for future studies. It is noteworthy that a prospective cohort showed a negative association between maternal PFOA and PFNA concentrations and adiposity at birth [185]. However, this effect could be age-dependent, as prenatal PFAS exposure has negative associations with body mass index in early life (first 2 years) and positive associations in childhood and adolescence [32]. However, no clear sex-specific differences were found, which may indicate the involvement of endocrine influences [186–189].

4.2. PFAS-Associated Endocrine Effects

A few reproductive and developmental toxicity studies have been conducted that were primarily focused on long-chain PFAS, including PFOS, PFOA, and PFNA in mice and rats [190]. In Sprague–Dawley rats, GenX (industrial replacement of PFOA) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly [145]. Neonatal morbidity and mortality with exposure to high doses of PFAS and growth deficits and developmental delays were noted in offspring exposed to lower doses [190]. Lactation impairment was observed in mice [191], which led to an increased offspring mortality [192]. Studies have indicated a role of placental dysfunction in these adverse developmental outcomes [43]. Systematic reviews [190] support a relationship between in utero exposure to PFOA and PFOS and reduced fetal growth in animals and humans, and the relationship between PFOA and reduced fetal growth in mice was validated [43,193]. In addition, PFAS are reported to have reproductive effects such as ovulation failure in mice [194]. However, the research primary focus on single-compound exposures does not really reflect the real-life exposure to complex mixtures of PFAS. Future studies must be designed to reflect the real-life mixtures exposures.

There is evidence for PFAS affecting ER signaling in humans and animals although it is not consistent [190]. Study reports suggest an ability of PFAS to modulate and/or further activate ER-mediated effects [36,99,104,109,195,196] with some effects only observed in aquatic organisms [106,119,197]. Microarray analyses of human primary hepatocytes confirmed that PFOA activated the ER pathway [131]. The PFAS in general elicits estrogenic effects, mainly mediated via the estrogen receptor. There are indications of anti-androgenic effects as well (e.g., decrease testosterone level) even though only one of six reviewed in vitro studies found significant anti-androgenic through the AR receptor [99]. PFAS may influence human sex hormone biosynthesis, serum, and tissue hormone levels and receptor expression and function and thereby fetal growth (Figure 4). Whether the effect on fetal growth is mediated through the alteration of the sex hormone system is unknown, but a possible mechanism could be by an alteration in the placental development and function. As already mentioned, we previously found that the serum PFAS-induced ER activity was associated with decreased birth weight and length [36].

There are some suggested sex differences in the effects of PFAS on fetal growth—although the data are not consistent. The effects on the sex hormone system might provide possible explanations for the sexual dimorphism to PFAS exposure. Two of the reviewed studies also support the sex differences, as CYP19A expression in zebra fish increased in female gonads and decreased in male gonads [106], and Rosen et al. [112] found decreased expression of male-specific genes and increased expression of female-specific genes after PFAS exposure in mice. Interestingly, the sex-specific results are not only seen for fetal growth, but epidemiological studies found associations between prenatal PFAS exposure and adiposity and overweight for females, but not males, later in life [198,199].

Thyroid hormones are essential for normal fetal growth and development. The fetus is completely reliant on maternal T4 during the first trimester; thereafter, the fetal thyroid gland begins to function [200,201]. At birth, approximately 30% of T4 in cord blood originates from the mother [202]. Thus, there are concerns about the potential effect of in utero PFAS exposure on thyroid hormone homeostasis in pregnant women and their fetuses [203]. Therefore, thyroid hormones are of critical importance to both pregnant women and their offspring. Decreased maternal provision of T4 to the fetus leads to an increased risk of poor cognition, behavior, and growth [204–206].
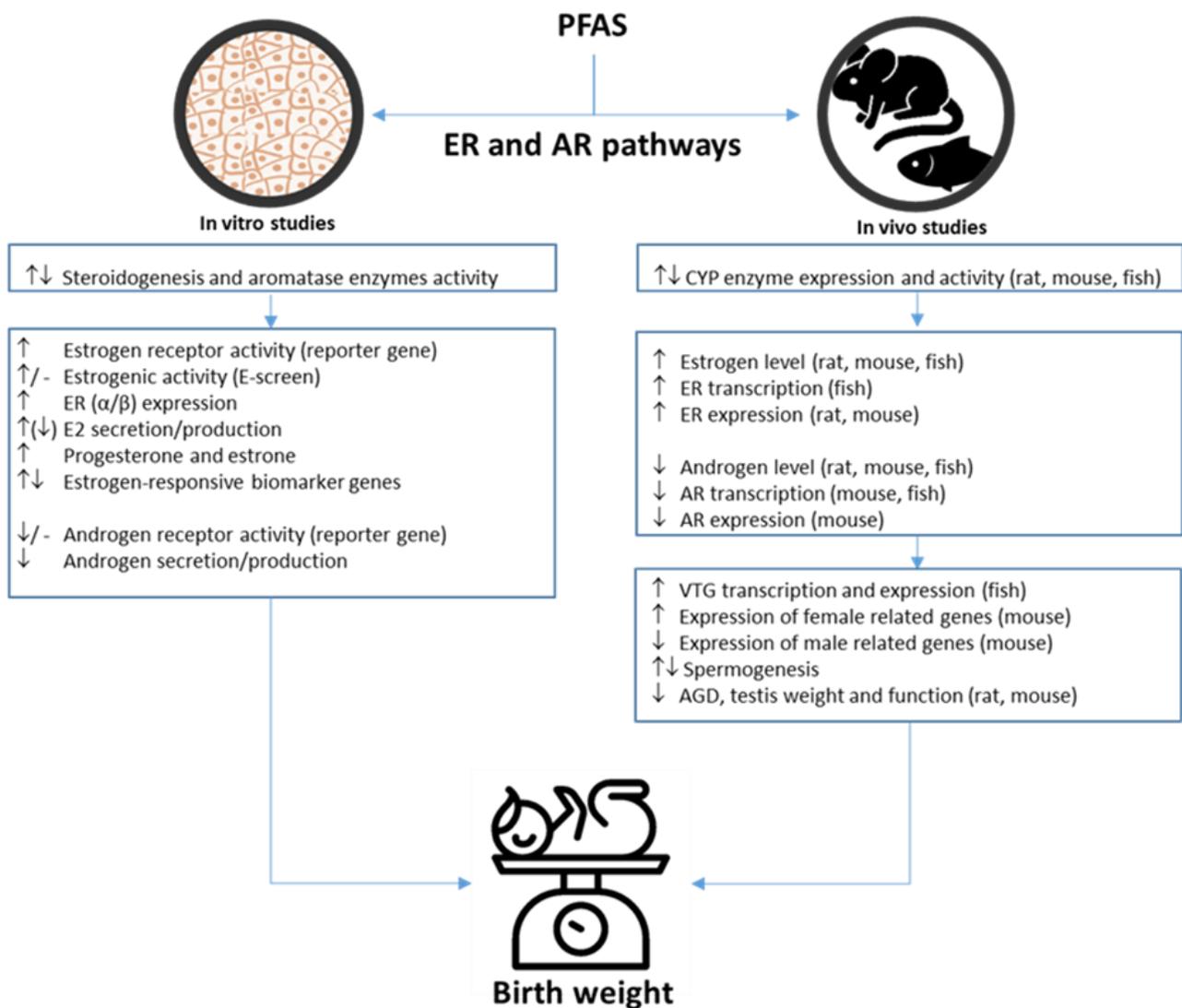
Figure 4. Possible PFAS-induced estrogenic and androgenic effects involved in birth weight. PFAS, per- and polyfluoroalkyl substances; ER, estrogen receptor; AR, androgen receptor; VTG, vitellogenin; E2, estradiol; AGD, anogenital distance.

The in vitro and in vivo studies evaluated in this review elicited that PFAS can interfere with thyroid hormone levels and functions in synthesis, cell levels, transport, binding to receptor, and receptor function (Figure 5). Several epidemiological studies have investigated the association between PFAS and TSH levels, and the majority of the findings are significant positive [206]. However, only two of the reviewed in vivo studies investigated TSH level, with one finding a decrease in TSH [140] and one finding no effect in rats after PFAS exposure [147]. For T3 and T4, both epidemiological studies [206] and the reviewed in vivo studies generally found inverse associations with PFAS exposure, but the results are conflicting. Overall, this review suggests some evidence for thyroid-disrupting effects in in vitro and animal models, whereas human studies provide some conflicting results. Further research including more longitudinal and long-term follow-up on population studies might give further knowledge about the detailed pathways involved in the impact on fetal growth.
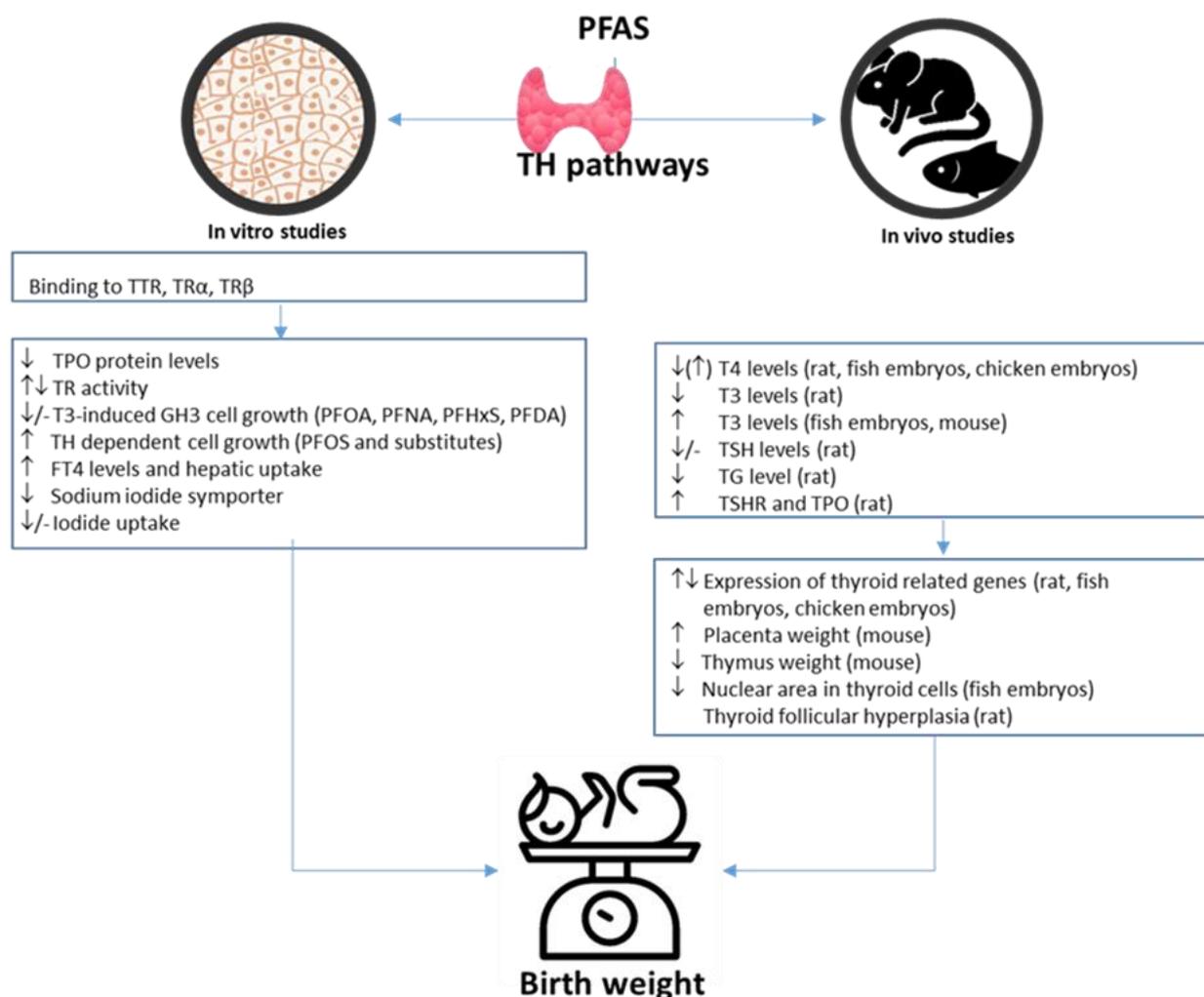
Figure 5. Possible PFAS-induced thyroid effects involved in birth weight. PFAS, per- and polyfluoroalkyl substances; TH, thyroid hormone; TPO, thyroperoxidase; TR, thyroid hormone receptor; T4, thyroxine; T3, triiodothyronine; FT4, free T4; TSH, thyroid-stimulating hormone; TG, thyroglobulin; TSHR, TSH receptor.

5. Strengths and Limitations of the Study

The review provides an overview of different PFAS-affected pathways both at the molecular level and at the functional level (e.g., receptor activity). There are specific differences in human and rodent biology and health outcomes that deserve further investigation. The extent to which results from in vitro studies and in vivo animal studies are transferable to human health needs to be verified and confirmed in further studies. The use of an automatic tool such as AOP-helpFinder to screen and extract information from the literature is an advantage, as the researcher does not have to perform each search independently. It is therefore less time consuming and allows to obtain a good overview of the existing data that have been published. Nevertheless, results obtained by such approach need validation by experts. The present study was limited to exploration of the literature from the *PubMed* database to identify stressor–event linkage. The AOP-helpFinder tool is currently under optimization in order to be able to also decipher relationships between key events, which will allow a more complete exploration of available data and will require less manual curation by experts. Information from various databases will also be screened and added following an integrative systems biology pipeline [48].

6. Conclusions and Future Experimental Model Studies

The described PFAS-induced changes in ROS signaling or endocrine system and their respective influence on birth weight appear unrelated. Although interconnection of individual parts, somehow, is evident (e.g., estrogen receptors have been identified as redox sensors [207]), it remains to be elucidated whether and how oxidative stress and/or adipocyte differentiation and/or general endocrine dysfunction (mediated through the estrogenic, androgenic, and/or thyroid hormone systems) interact to affect birth weight.

Our search did not reveal any study testing a model for pregnancy-related diseases such as animal models for SGA [208]. However, it can be assumed that the basic cellular signaling pathways are evolutionarily conserved. In general, AOPs are developed using all available data from different cellular models complemented by animal studies, as data from in vitro studies should be further supported by in vivo animal studies (e.g., [209]). Genes/proteins described in animal studies may be named differently but are mostly functionally analogous to those in humans [210,211].

Experimental studies in cell systems or animals are critical for elucidating the human health effects of PFAS, e.g., on liver, thyroid, and lipid homeostasis. Some effects in cell systems/animals were not identical to those in humans, and new targets were identified, e.g., mammary gland and immune system changes. Long-term exposure to relevant doses of PFAS, e.g., in animal models, could help elucidate PFAS-induced fetal growth restriction. Experimental in vitro and in vivo studies are needed to confirm key molecular events involved in potential novel AOPs. Future studies also need to examine the effects of complex PFAS mixtures to account for real-life exposure. Another future research direction may be to investigate the interactions of PFAS with other chemical/non-chemical stressors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics10110684/s1, Table S1: In vivo results on PFAS, oxidative stress, and other adverse outcomes; Table S2: In vitro results on PFAS- induced oxidative stress and cellular health; Table S3: In vivo and in vitro results related to PFAS exposures and cellular signaling pathways; Table S4: In vivo results related to estrogen and androgen pathways; Table S5: In vitro results related to estrogen and androgen pathways; Table S6: In vivo studies related to thyroid hormone pathways; Table S7: In vitro studies related to thyroid hormone pathways.

Author Contributions: Work conceptualization and design of search strategy, K.A., F.J., M.U., C.G. and E.C.B.-J.; article screening and selection, C.G., R.W., S.G., M.F., M.W., M.L. and E.C.B.-J.; data extraction, C.G., R.W., S.G., M.W. and M.L.; original draft preparation, C.G., R.W., S.G., E.C.B.-J., M.W., T.I.H. and M.L.; critical review of the manuscript, edition, and provision of important intellectual content, C.G. and all authors; manuscript revision and final version approval, all authors. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Savitz, D.A. Guest editorial: Biomarkers of perfluorinated chemicals and birth weight. *Environ. Health Perspect.* **2007**, *115*, A528–A529. [CrossRef] [PubMed]
2. Beck, J.J.; Pool, R.; van de Weijer, M.; Chen, X.; Krapohl, E.; Gordon, S.D.; Nygaard, M.; Debrabant, B.; Palviainen, T.; van der Zee, M.D.; et al. Genetic meta-analysis of twin birth weight shows high genetic correlation with singleton birth weight. *Hum. Mol. Genet.* **2021**, *30*, 1894–1905. [CrossRef] [PubMed]
3. Brundtland, G.H. From the World Health Organization. Reducing risks to health, promoting healthy life. *JAMA* **2002**, *288*, 1974. [CrossRef] [PubMed]
4. Finken, M.J.J.; van der Steen, M.; Smeets, C.C.J.; Walenkamp, M.J.E.; de Bruin, C.; Hokken-Koelega, A.C.S.; Wit, J.M. Children Born Small for Gestational Age: Differential Diagnosis, Molecular Genetic Evaluation, and Implications. *Endocr. Rev.* **2018**, *39*, 851–894. [CrossRef]
5. Ludvigsson, J.F.; Lu, D.; Hammarström, L.; Cnattingius, S.; Fang, F. Small for gestational age and risk of childhood mortality: A Swedish population study. *PLoS Med.* **2018**, *15*, e1002717. [CrossRef]
6. Govarts, E.; Iszatt, N.; Trnovec, T.; de Cock, M.; Eggesbø, M.; Murinova, L.P.; van de Bor, M.; Guxens, M.; Chevrier, C.; Koppen, G.; et al. Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: Pooled analysis of seven European birth cohorts. *Environ. Int.* **2018**, *115*, 267–278. [CrossRef]
7. Jensen, E.A.; Foglia, E.E.; Dysart, K.C.; Simmons, R.A.; Aghai, Z.H.; Cook, A.; Greenspan, J.S.; DeMauro, S.B. Adverse effects of small for gestational age differ by gestational week among very preterm infants. *Arch. Dis. Child. Fetal Neonatal Ed.* **2019**, *104*, F192–F198. [CrossRef]
8. He, H.; Miao, H.; Liang, Z.; Zhang, Y.; Jiang, W.; Deng, Z.; Tang, J.; Liu, G.; Luo, X. Prevalence of small for gestational age infants in 21 cities in China, 2014–2019. *Sci. Rep.* **2021**, *11*, 7500. [CrossRef]
9. Lee, A.C.; Katz, J.; Blencowe, H.; Cousens, S.; Kozuki, N.; Vogel, J.P.; Adair, L.; Baqui, A.H.; Bhutta, Z.A.; Caulfield, L.E.; et al. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. *Lancet Glob. Health* **2013**, *1*, e26–e36, Erratum in *Lancet Glob. Health* **2013**, *1*, e76. [CrossRef]
10. Magnus, P. Causes of variation in birth weight: A study of offspring of twins. *Clin. Genet.* **1984**, *25*, 15–24. [CrossRef]
11. Järvelin, M.R.; Elliott, P.; Kleinschmidt, I.; Martuzzi, M.; Grundy, C.; Hartikainen, A.L.; Rantakallio, P. Ecological and individual predictors of birthweight in a northern Finland birth cohort 1986. *Paediatr. Perinat. Epidemiol.* **1997**, *11*, 298–312. [CrossRef] [PubMed]
12. Workalemahu, T.; Grantz, K.L.; Grewal, J.; Zhang, C.; Louis, G.M.B.; Tekola-Ayele, F. Genetic and Environmental Influences on Fetal Growth Vary during Sensitive Periods in Pregnancy. *Sci. Rep.* **2018**, *8*, 7274. [CrossRef] [PubMed]
13. Fowden, A.L. The insulin-like growth factors and feto-placental growth. *Placenta* **2003**, *24*, 803–812. [CrossRef]
14. White, V.; Jawerbaum, A.; Mazzucco, M.B.; Gauster, M.; Desoye, G.; Hiden, U. IGF2 stimulates fetal growth in a sex- and organ-dependent manner. *Pediatr. Res.* **2018**, *83*, 183–189. [CrossRef] [PubMed]
15. Murphy, V.E.; Smith, R.; Giles, W.B.; Clifton, V.L. Endocrine regulation of human fetal growth: The role of the mother, placenta, and fetus. *Endocr. Rev.* **2006**, *27*, 141–169. [CrossRef]
16. Salafia, C.M.; Zhang, J.; Miller, R.K.; Charles, A.K.; Shrout, P.; Sun, W. Placental growth patterns affect birth weight for given placental weight. *Birth Defects Res. Part A Clin. Mol. Teratol.* **2007**, *79*, 281–288. [CrossRef]
17. Burton, G.J.; Jauniaux, E. Pathophysiology of placental-derived fetal growth restriction. *Am. J. Obstet. Gynecol.* **2018**, *218*, S745–S761. [CrossRef]
18. Chellakooty, M.; Vangsgaard, K.; Larsen, T.; Scheike, T.; Falck-Larsen, J.; Legarth, J.; Andersson, A.M.; Main, K.M.; Skakkebaek, N.E.; Juul, A. A longitudinal study of intrauterine growth and the placental growth hormone (GH)-insulin-like growth factor I axis in maternal circulation: Association between placental GH and fetal growth. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 384–391. [CrossRef]
19. Benton, S.J.; McCowan, L.M.; Heazell, A.E.; Grynspan, D.; Hutcheon, J.A.; Senger, C.; Burke, O.; Chan, Y.; Harding, J.E.; Yockell-Lelièvre, J.; et al. Placental growth factor as a marker of fetal growth restriction caused by placental dysfunction. *Placenta* **2016**, *42*, 1–8. [CrossRef]
20. Chisholm, K.M.; Folkins, A.K. Placental and Clinical Characteristics of Term Small-for-Gestational-Age Neonates: A Case-Control Study. *Pediatr. Dev. Pathol.* **2016**, *19*, 37–46. [CrossRef]
21. Tachibana, M.; Nakayama, M.; Ida, S.; Kitajima, H.; Mitsuda, N.; Ozono, K.; Miyoshi, Y. Pathological examination of the placenta in small for gestational age (SGA) children with or without postnatal catch-up growth. *J. Matern. Fetal Neonatal Med.* **2016**, *29*, 982–986. [CrossRef] [PubMed]
22. Svensson, K.; Tanner, E.; Gennings, C.; Lindh, C.; Kiviranta, H.; Wikström, S.; Bornehag, C.G. Prenatal exposures to mixtures of endocrine disrupting chemicals and children's weight trajectory up to age 5.5 in the SELMA study. *Sci. Rep.* **2021**, *11*, 11036. [CrossRef] [PubMed]
23. De Silva, A.O.; Armitage, J.M.; Bruton, T.A.; Dassuncao, C.; Heiger-Bernays, W.; Hu, X.C.; Kärrman, A.; Kelly, B.; Ng, C.; Robuck, A.; et al. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Environ. Toxicol. Chem.* **2021**, *40*, 631–657. [CrossRef]

24. EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel); Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; Del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.R.; Leblanc, J.C.; et al. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA J.* **2020**, *18*, e06223. [CrossRef] [PubMed]

25. Poothong, S.; Papadopoulou, E.; Padilla-Sánchez, J.A.; Thomsen, C.; Haug, L.S. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. *Environ. Int.* **2020**, *134*, 105244. [CrossRef]

26. US EPA (United States Environmental Protection Agency). EPA's Per- and Polyfluoroalkyl Substances (PFAS) Action Plan. 2019. Available online: https://www.epa.gov/sites/default/files/2019-02/documents/pfas_action_plan_021319_508compliant_1.pdf (accessed on 6 November 2022).

27. ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological Profile for Perfluoroalkyls*; Department of Health and Human Services, Public Health Service: Atlanta, GA, USA, 2021. Available online: https://www.atsdr.cdc.gov/ToxProfiles/tp200-c2.pdf (accessed on 6 November 2022).

28. Bach, C.C.; Bech, B.H.; Brix, N.; Nohr, E.A.; Bonde, J.P.; Henriksen, T.B. Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: A systematic review. *Crit. Rev. Toxicol.* **2015**, *45*, 53–67. [CrossRef] [PubMed]

29. Negri, E.; Metruccio, F.; Guercio, V.; Tosti, L.; Benfenati, E.; Bonzi, R.; La Vecchia, C.; Moretto, A. Exposure to PFOA and PFOS and fetal growth: A critical merging of toxicological and epidemiological data. *Crit. Rev. Toxicol.* **2017**, *47*, 482–508, Erratum in *Crit. Rev. Toxicol.* **2017**, *47*, i. [CrossRef]

30. Dzierlenga, M.W.; Crawford, L.; Longnecker, M.P. Birth weight and perfluorooctane sulfonic acid: A random-effects meta-regression analysis. *Environ. Epidemiol.* **2020**, *4*, e095. [CrossRef]

31. Cao, T.; Qu, A.; Li, Z.; Wang, W.; Liu, R.; Wang, X.; Nie, Y.; Sun, S.; Zhang, X.; Liu, X. The relationship between maternal perfluoroalkylated substances exposure and low birth weight of offspring: A systematic review and meta-analysis. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 67053–67065. [CrossRef]

32. Lee, Y.J.; Jung, H.W.; Kim, H.Y.; Choi, Y.-J.; Lee, Y.A. Early-Life Exposure to Per- and Poly-Fluorinated Alkyl Substances and Growth, Adiposity, and Puberty in Children: A Systematic Review. *Front. Endocrinol.* **2021**, *12*, 683297. [CrossRef]

33. Fan, X.; Tang, S.; Wang, Y.; Fan, W.; Ben, Y.; Naidu, R.; Dong, Z. Global Exposure to Per- and Polyfluoroalkyl Substances and Associated Burden of Low Birthweight. *Environ. Sci. Technol.* **2022**, *56*, 4282–4294. [CrossRef] [PubMed]

34. Rokoff, L.B.; Rifas-Shiman, S.L.; Coull, B.A.; Cardenas, A.; Calafat, A.M.; Ye, X.; Gryparis, A.; Schwartz, J.; Sagiv, S.K.; Gold, D.R.; et al. Cumulative exposure to environmental pollutants during early pregnancy and reduced fetal growth: The Project Viva cohort. *Environ. Health* **2018**, *17*, 19. [CrossRef] [PubMed]

35. Chang, C.J.; Barr, D.B.; Ryan, P.B.; Panuwet, P.; Smarr, M.M.; Liu, K.; Kannan, K.; Yakimavets, V.; Tan, Y.; Ly, V.; et al. Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach. *Environ. Int.* **2022**, *158*, 106964. [CrossRef] [PubMed]

36. Bjerregaard-Olesen, C.; Bach, C.C.; Long, M.; Wielsøe, M.; Bech, B.H.; Henriksen, T.B.; Olsen, J.; Bonefeld-Jørgensen, E.C. Associations of Fetal Growth Outcomes with Measures of the Combined Xenoestrogenic Activity of Maternal Serum Perfluorinated Alkyl Acids in Danish Pregnant Women. *Environ. Health Perspect.* **2019**, *127*, 17006. [CrossRef] [PubMed]

37. Bommarito, P.A.; Ferguson, K.K.; Meeker, J.D.; McElrath, T.F.; Cantonwine, D.E. Maternal Levels of Perfluoroalkyl Substances (PFAS) during Early Pregnancy in Relation to Preeclampsia Subtypes and Biomarkers of Preeclampsia Risk. *Environ. Health Perspect.* **2021**, *129*, 107004. [CrossRef]

38. Burton, G.J.; Redman, C.W.; Roberts, J.M.; Moffett, A. Pre-eclampsia: Pathophysiology and clinical implications. *BMJ* **2019**, *366*, l2381. [CrossRef]

39. Talia, C.; Connolly, L.; Fowler, P.A. The insulin-like growth factor system: A target for endocrine disruptors? *Environ. Int.* **2021**, *147*, 106311. [CrossRef]

40. Forsthuber, M.; Widhalm, R.; Granitzer, S.; Kaiser, A.M.; Moshammer, H.; Hengstschläger, M.; Dolznig, H.; Gundacker, C. Perfluorooctane sulfonic acid (PFOS) inhibits vessel formation in a human 3D co-culture angiogenesis model (NCFs/HUVECs). *Environ. Pollut.* **2022**, *293*, 118543. [CrossRef]

41. Fei, C.; McLaughlin, J.K.; Tarone, R.E.; Olsen, J. Fetal growth indicators and perfluorinated chemicals: A study in the Danish National Birth Cohort. *Am. J. Epidemiol.* **2008**, *168*, 66–72. [CrossRef]

42. Jiang, W.; Deng, Y.; Song, Z.; Xie, Y.; Gong, L.; Chen, Y.; Kuang, H. Gestational Perfluorooctanoic Acid Exposure Inhibits Placental Development by Dysregulation of Labyrinth Vessels and uNK Cells and Apoptosis in Mice. *Front. Physiol.* **2020**, *11*, 51. [CrossRef]

43. Blake, B.E.; Cope, H.A.; Hall, S.M.; Keys, R.D.; Mahler, B.W.; McCord, J.; Scott, B.; Stapleton, H.M.; Strynar, M.J.; Elmore, S.A.; et al. Evaluation of Maternal, Embryo, and Placental Effects in CD-1 Mice following Gestational Exposure to Perfluorooctanoic Acid (PFOA) or Hexafluoropropylene Oxide Dimer Acid (HFPO-DA or GenX). *Environ. Health Perspect.* **2020**, *128*, 27006. [CrossRef] [PubMed]

44. Zhang, N.; Wang, W.S.; Li, W.J.; Liu, C.; Wang, Y.; Sun, K. Reduction of progesterone, estradiol and hCG secretion by perfluorooctane sulfonate via induction of apoptosis in human placental syncytiotrophoblasts. *Placenta* **2015**, *36*, 575–580. [CrossRef] [PubMed]

45. HBM4EU. Available online: https://www.hbm4eu.eu (accessed on 6 November 2022).

46. AOP-helpFinder. Available online: http://aop-helpfinder.u-paris-sciences.fr (accessed on 6 November 2022).

47. Carvaillo, J.C.; Barouki, R.; Coumoul, X.; Audouze, K. Linking Bisphenol S to Adverse Outcome Pathways Using a Combined Text Mining and Systems Biology Approach. *Environ. Health Perspect.* **2019**, *127*, 47005. [CrossRef] [PubMed]

48. Rugard, M.; Coumoul, X.; Carvaillo, J.C.; Barouki, R.; Audouze, K. Deciphering Adverse Outcome Pathway Network Linked to Bisphenol F Using Text Mining and Systems Toxicology Approaches. *Toxicol. Sci.* **2020**, *173*, 32–40. [CrossRef] [PubMed]

49. Benoit, L.; Jornod, F.; Zgheib, E.; Tomkiewicz, C.; Koual, M.; Coustillet, T.; Barouki, R.; Audouze, K.; Vinken, M.; Coumoul, X. Adverse outcome pathway from activation of the AhR to breast cancer-related death. *Environ. Int.* **2022**, *165*, 107323. [CrossRef]

50. Jaylet, T.; Quintens, R.; Benotmane, M.A.; Luukkonen, J.; Tanaka, I.B., 3rd; Ibanez, C.; Durand, C.; Sachana, M.; Azimzadeh, O.; Adam-Guillermin, C.; et al. Development of an adverse outcome pathway for radiation-induced microcephaly via expert consultation and machine learning. *Int. J. Radiat. Biol.* **2022**, 1–11. [CrossRef]

51. Kaiser, A.M.; Zare Jeddi, M.; Uhl, M.; Jornod, F.; Fernandez, M.F.; Audouze, K. Characterization of Potential Adverse Outcome Pathways Related to Metabolic Outcomes and Exposure to Per- and Polyfluoroalkyl Substances Using Artificial Intelligence. *Toxics* **2022**, *10*, 449. [CrossRef]

52. Jornod, F.; Jaylet, T.; Blaha, L.; Sarigiannis, D.; Tamisier, L.; Audouze, K. AOP-helpFinder webserver: A tool for comprehensive analysis of the literature to support adverse outcome pathways development. *Bioinformatics* **2021**, *38*, 1173–1175. [CrossRef]

53. Kim, H.M.; Long, N.P.; Yoon, S.J.; Anh, N.H.; Kim, S.J.; Park, J.H.; Kwon, S.W. Omics approach reveals perturbation of metabolism and phenotype in *Caenorhabditis elegans* triggered by perfluorinated compounds. *Sci. Total Environ.* **2020**, *703*, 135500. [CrossRef]

54. Kim, J.H.; Barbagallo, B.; Annunziato, K.; Farias-Pereira, R.; Doherty, J.J.; Lee, J.; Zina, J.; Tindal, C.; McVey, C.; Aresco, R.; et al. Maternal preconception PFOS exposure of Drosophila melanogaster alters reproductive capacity, development, morphology and nutrient regulation. *Food Chem. Toxicol.* **2021**, *151*, 112153. [CrossRef]

55. Lee, Y.Y.; Wong, C.K.; Oger, C.; Durand, T.; Galano, J.-M.; Lee, J.C.-Y. Prenatal exposure to the contaminant perfluorooctane sulfonate elevates lipid peroxidation during mouse fetal development but not in the pregnant dam. *Free Radic. Res.* **2015**, *49*, 1015–1025. [CrossRef] [PubMed]

56. Li, J.; Quan, X.-J.; Chen, G.; Hong, J.-W.; Wang, Q.; Xu, L.-L.; Wang, B.-H.; Yu, Z.-H.; Yu, H.-M. PFOS-induced placental cell growth inhibition is partially mediated by lncRNA H19 through interacting with miR-19a and miR-19b. *Chemosphere* **2020**, *261*, 127640. [CrossRef] [PubMed]

57. Li, Z.; Yu, Z.; Gao, P.; Yin, D. Multigenerational effects of perfluorooctanoic acid on lipid metabolism of *Caenorhabditis elegans* and its potential mechanism. *Sci. Total Environ.* **2020**, *703*, 134762. [CrossRef]

58. Ortiz-Villanueva, E.; Jaumot, J.; Martínez, R.; Navarro-Martín, L.; Piña, B.; Tauler, R. Assessment of endocrine disruptors effects on zebrafish (*Danio rerio*) embryos by untargeted LC-HRMS metabolomic analysis. *Sci. Total Environ.* **2018**, *635*, 156–166. [CrossRef] [PubMed]

59. Park, K.; Kim, W.-S.; Choi, B.; Kwak, I.-S. Expression Levels of the Immune-Related p38 Mitogen-Activated Protein Kinase Transcript in Response to Environmental Pollutants on *Macrophthalmus japonicus* Crab. *Genes* **2020**, *11*, 958. [CrossRef]

60. Qiu, L.; Qian, Y.; Liu, Z.; Wang, C.; Qu, J.; Wang, X.; Wang, S. Perfluorooctane sulfonate (PFOS) disrupts blood-testis barrier by down-regulating junction proteins via p38 MAPK/ATF2/MMP9 signaling pathway. *Toxicology* **2016**, *373*, 1–12. [CrossRef]

61. Seyoum, A.; Pradhan, A.; Jass, J.; Olsson, P.E. Perfluorinated alkyl substances impede growth, reproduction, lipid metabolism and lifespan in *Daphnia magna*. *Sci. Total Environ.* **2020**, *737*, 139682. [CrossRef]

62. Wan, H.T.; Wong, A.-Y.; Feng, S.; Wong, C.-K. Effects of In Utero Exposure to Perfluorooctane Sulfonate on Placental Functions. *Environ. Sci. Technol.* **2020**, *54*, 16050–16061. [CrossRef]

63. Wang, B.; Li, D.; Yuan, Z.; Zhang, Y.; Ma, X.; Lv, Z.; Xiao, Y.; Zhang, J. Evaluation of joint effects of perfluorooctane sulfonate and wood vinegar on planarians, *Dugesia japonica*. *Environ. Sci. Pollut. Res. Int.* **2020**, *27*, 18089–18098. [CrossRef]

64. Xia, X.; Yu, R.; Li, M.; Liu, L.; Zhang, K.; Wang, Y.; Li, B.; Zhang, L.; Song, G.; Zheng, X.; et al. Molecular cloning and characterization of two genes encoding peroxiredoxins from freshwater bivalve Anodonta woodiana: Antioxidative effect and immune defense. *Fish Shellfish Immunol.* **2018**, *82*, 476–491. [CrossRef]

65. Yue, Y.; Li, S.; Qian, Z.; Pereira, R.F.; Lee, J.; Doherty, J.J.; Zhang, Z.; Peng, Y.; Clark, J.M.; Timme-Laragy, A.R.; et al. Perfluorooctanesulfonic acid (PFOS) and perfluorobutanesulfonic acid (PFBS) impaired reproduction and altered offspring physiological functions in *Caenorhabditis elegans*. *Food Chem. Toxicol.* **2020**, *145*, 111695. [CrossRef] [PubMed]

66. Zhang, L.; Sun, W.; Chen, H.; Tian, F.; Cai, W. Transcriptome analysis of acute exposure of the Manila clam, *Ruditapes philippinarum* to perfluorooctane sulfonate (PFOS). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2020**, *231*, 108736. [CrossRef] [PubMed]

67. Du, G.; Sun, J.; Zhang, Y. Perfluorooctanoic acid impaired glucose homeostasis through affecting adipose AKT pathway. *Cytotechnology* **2018**, *70*, 479–487. [CrossRef] [PubMed]

68. Guruge, K.S.; Yeung, L.W.; Yamanaka, N.; Miyazaki, S.; Lam, P.K.; Giesy, J.P.; Jones, P.D.; Yamashita, N. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). *Toxicol. Sci.* **2006**, *89*, 93–107. [CrossRef]

69. Li, D.; Zhang, L.; Zhang, Y.; Guan, S.; Gong, X.; Wang, X. Maternal exposure to perfluorooctanoic acid (PFOA) causes liver toxicity through PPAR-α pathway and lowered histone acetylation in female offspring mice. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 18866–18875. [CrossRef]

70. Li, F.; Yu, Y.; Guo, M.; Lin, Y.; Jiang, Y.; Qu, M.; Sun, X.; Li, Z.; Zhai, Y.; Tan, Z. Integrated analysis of physiological, transcriptomics and metabolomics provides insights into detoxication disruption of PFOA exposure in *Mytilus edulis*. *Ecotoxicol. Environ. Saf.* **2021**, *214*, 112081. [CrossRef]

71. Liu, W.; Yang, B.; Wu, L.; Zou, W.; Pan, X.; Zou, T.; Liu, F.; Xia, L.; Wang, X.; Zhang, D. Involvement of NRF2 in Perfluorooctanoic Acid-Induced Testicular Damage in Male Mice. *Biol. Reprod.* **2015**, *93*, 41. [CrossRef]

72. Liu, R.C.M.; Hurtt, M.E.; Cook, J.C.; Biegel, L.B. Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. *Fundam. Appl. Toxicol.* **1996**, *30*, 220–228. [CrossRef]

73. Salimi, A.; Nikoosiar Jahromi, M.; Pourahmad, J. Maternal exposure causes mitochondrial dysfunction in brain, liver, and heart of mouse fetus: An explanation for perfluorooctanoic acid induced abortion and developmental toxicity. *Environ. Toxicol.* **2019**, *34*, 878–885. [CrossRef]

74. Wang, J.; Li, Y.; Liu, Y.; Zhang, H.; Dai, J. Disturbance of perfluorooctanoic acid on development and behavior in *Drosophila larvae*. *Environ. Toxicol. Chem.* **2010**, *29*, 2117–2122. [CrossRef]

75. Yan, S.; Zhang, H.; Zheng, F.; Sheng, N.; Guo, X.; Dai, J. Perfluorooctanoic acid exposure for 28 days affects glucose homeostasis and induces insulin hypersensitivity in mice. *Sci. Rep.* **2015**, *5*, 11029. [CrossRef] [PubMed]

76. Yang, C.-H.; Glover, K.P.; Han, X. Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. *Toxicol. Sci.* **2010**, *117*, 294–302. [CrossRef] [PubMed]

77. Chiu, W.A.; Guyton, K.Z.; Martin, M.T.; Reif, D.M.; Rusyn, I. Use of high-throughput in vitro toxicity screening data in cancer hazard evaluations by IARC Monograph Working Groups. *Altex* **2018**, *35*, 51–64. [CrossRef] [PubMed]

78. Gorrochategui, E.; Pérez-Albaladejo, E.; Casas, J.; Lacorte, S.; Porte, C. Perfluorinated chemicals: Differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. *Toxicol. Appl. Pharmacol.* **2014**, *277*, 124–130. [CrossRef] [PubMed]

79. Gorrochategui, E.; Lacorte, S.; Tauler, R.; Martin, F.L. Perfluoroalkylated Substance Effects in Xenopus laevis A6 Kidney Epithelial Cells Determined by ATR-FTIR Spectroscopy and Chemometric Analysis. *Chem. Res. Toxicol.* **2016**, *29*, 924–932. [CrossRef] [PubMed]

80. Reistad, T.; Fonnum, F.; Mariussen, E. Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. *Toxicol. Lett.* **2013**, *218*, 56–60. [CrossRef]

81. Sun, P.; Nie, X.; Chen, X.; Yin, L.; Luo, J.; Sun, L.; Wan, C.; Jiang, S. Nrf2 Signaling Elicits a Neuroprotective Role against PFOS-mediated Oxidative Damage and Apoptosis. *Neurochem. Res.* **2018**, *43*, 2446–2459. [CrossRef]

82. Sun, P.; Gu, L.; Luo, J.; Qin, Y.; Sun, L.; Jiang, S. ROS-mediated JNK pathway critically contributes to PFOS-triggered apoptosis in SH-SY5Y cells. *Neurotoxicol. Teratol.* **2019**, *75*, 106821. [CrossRef]

83. Tang, L.-L.; Wang, J.-D.; Xu, T.-T.; Zhao, Z.; Zheng, J.-J.; Ge, R.-S.; Zhu, D.-Y. Mitochondrial toxicity of perfluorooctane sulfonate in mouse embryonic stem cell-derived cardiomyocytes. *Toxicology* **2017**, *382*, 108–116. [CrossRef]

84. Wang, C.; Nie, X.; Zhang, Y.; Li, T.; Mao, J.; Liu, X.; Gu, Y.; Shi, J.; Xiao, J.; Wan, C.; et al. Reactive oxygen species mediate nitric oxide production through ERK/JNK MAPK signaling in HAPI microglia after PFOS exposure. *Toxicol. Appl. Pharmacol.* **2015**, *288*, 143–151. [CrossRef]

85. Wei, Y.; Shi, X.; Zhang, H.; Wang, J.; Zhou, B.; Dai, J. Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (*Gobiocypris rarus*) using toxicogenomic analysis. *Aquat. Toxicol.* **2009**, *95*, 27–36. [CrossRef]

86. Xu, J.; Shimpi, P.; Armstrong, L.; Salter, D.; Slitt, A.L. PFOS induces adipogenesis and glucose uptake in association with activation of Nrf2 signaling pathway. *Toxicol. Appl. Pharmacol.* **2016**, *290*, 21–30. [CrossRef] [PubMed]

87. Zarei, M.H.; Shirazi, S.F.H.; Aghvami, M.; Pourahmad, J. Perfluorooctanesulfonate (PFOS) Induces Apoptosis Signaling and Proteolysis in Human Lymphocytes through ROS Mediated Mitochondrial Dysfunction and Lysosomal Membrane Labialization. *Iran. J. Pharm. Res.* **2018**, *17*, 995–1007. [PubMed]

88. Lu, Y.; Pan, Y.; Sheng, N.; Zhao, A.Z.; Dai, J. Perfluorooctanoic acid exposure alters polyunsaturated fatty acid composition, induces oxidative stress and activates the AKT/AMPK pathway in mouse epididymis. *Chemosphere* **2016**, *158*, 143–153. [CrossRef] [PubMed]

89. Mashayekhi, V.; Tehrani, K.H.; Hashemzaei, M.; Tabrizian, K.; Shahraki, J.; Hosseini, M.J. Mechanistic approach for the toxic effects of perfluorooctanoic acid on isolated rat liver and brain mitochondria. *Hum. Exp. Toxicol.* **2015**, *34*, 985–996. [CrossRef] [PubMed]

90. Suh, K.S.; Choi, E.M.; Kim, Y.J.; Hong, S.M.; Park, S.Y.; Rhee, S.Y.; Oh, S.; Kim, S.W.; Pak, Y.K.; Choe, W.; et al. Perfluorooctanoic acid induces oxidative damage and mitochondrial dysfunction in pancreatic β-cells. *Mol. Med. Rep.* **2017**, *15*, 3871–3878. [CrossRef]

91. Tang, J.; Lu, X.; Chen, F.; Ye, X.; Zhou, D.; Yuan, J.; He, J.; Chen, B.; Shan, X.; Jiang, J.; et al. Effects of Perfluorooctanoic Acid on the Associated Genes Expression of Autophagy Signaling Pathway of *Carassius auratus* Lymphocytes in vitro. *Front. Physiol.* **2018**, *9*, 1748. [CrossRef]

92. Tian, J.; Hong, Y.; Li, Z.; Yang, Z.; Lei, B.; Liu, J.; Cai, Z. Immunometabolism-modulation and immunotoxicity evaluation of perfluorooctanoic acid in macrophage. *Ecotoxicol. Environ. Saf.* **2021**, *215*, 112128. [CrossRef]

93. Lee, Y.J.; Choi, S.-Y.; Yang, J.-H. PFHxS induces apoptosis of neuronal cells via ERK1/2-mediated pathway. *Chemosphere* **2014**, *94*, 121–127. [CrossRef]

94. Lee, Y.J.; Choi, S.-Y.; Yang, J.-H. NMDA receptor-mediated ERK 1/2 pathway is involved in PFHxS-induced apoptosis of PC12 cells. *Sci. Total Environ.* **2014**, *491–492*, 227–234. [CrossRef]

95. Dong, T.; Peng, Y.; Zhong, N.; Liu, F.; Zhang, H.; Xu, M.; Liu, R.; Han, M.; Tian, X.; Jia, J.; et al. Perfluorodecanoic acid (PFDA) promotes gastric cell proliferation via sPLA2-IIA. *Oncotarget* **2017**, *8*, 50911–50920. [CrossRef] [PubMed]

96. Kleszczyński, K.; Składanowski, A.C. Mechanism of cytotoxic action of perfluorinated acids. III. Disturbance in Ca^{2+} homeostasis. *Toxicol. Appl. Pharmacol.* **2011**, *251*, 163–168. [CrossRef] [PubMed]

97. Xu, M.; Zhang, T.; Lv, C.; Niu, Q.; Zong, W.; Tang, J.; Liu, R. Perfluorodecanoic acid-induced oxidative stress and DNA damage investigated at the cellular and molecular levels. *Ecotoxicol. Environ. Saf.* **2019**, *185*, 109699. [CrossRef] [PubMed]

98. Yang, J.-H. Perfluorooctanoic acid induces peroxisomal fatty acid oxidation and cytokine expression in the liver of male Japanese medaka (*Oryzias latipes*). *Chemosphere* **2010**, *81*, 548–552. [CrossRef] [PubMed]

99. Kjeldsen, L.S.; Bonefeld-Jørgensen, E.C. Perfluorinated compounds affect the function of sex hormone receptors. *Environ. Sci. Pollut. Res. Int.* **2013**, *20*, 8031–8044. [CrossRef]

100. Kang, J.S.; Choi, J.-S.; Park, J.-W. Transcriptional changes in steroidogenesis by perfluoroalkyl acids (PFOA and PFOS) regulate the synthesis of sex hormones in H295R cells. *Chemosphere* **2016**, *155*, 436–443. [CrossRef]

101. Behr, A.C.; Lichtenstein, D.; Braeuning, A.; Lampen, A.; Buhrke, T. Perfluoroalkylated substances (PFAS) affect neither estrogen and androgen receptor activity nor steroidogenesis in human cells in vitro. *Toxicol. Lett.* **2018**, *291*, 51–60. [CrossRef]

102. Bao, M.; Huang, W.; Au, W.W.; Zheng, S.; Liu, C.; Huang, Y.; Wu, K. Exposure to perfluorooctane sulfonate based on circadian rhythm changes the fecundity and expression of certain genes on the hypothalamic-pituitary-gonadal-liver axis of female zebrafish. *Toxicol. Appl. Pharmacol.* **2019**, *381*, 114715. [CrossRef]

103. Bao, M.; Zheng, S.; Liu, C.; Huang, W.; Xiao, J.; Wu, K. Perfluorooctane sulfonate exposure alters sexual behaviors and transcriptions of genes in hypothalamic-pituitary-gonadal-liver axis of male zebrafish (*Danio rerio*). *Environ. Pollut.* **2020**, *267*, 115585. [CrossRef]

104. Benninghoff, A.D.; Bisson, W.H.; Koch, D.C.; Ehresman, D.J.; Kolluri, S.K.; Williams, D.E. Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. *Toxicol. Sci.* **2011**, *120*, 42–58. [CrossRef]

105. Biegel, L.B.; Liu, R.C.; Hurtt, M.E.; Cook, J.C. Effects of ammonium perfluorooctanoate on Leydig cell function: In vitro, in vivo, and ex vivo studies. *Toxicol. Appl. Pharmacol.* **1995**, *134*, 18–25. [CrossRef] [PubMed]

106. Chen, J.; Wang, X.; Ge, X.; Wang, D.; Wang, T.; Zhang, L.; Tanguay, R.L.; Simonich, M.; Huang, C.; Dong, Q. Chronic perfluorooctanesulphonic acid (PFOS) exposure produces estrogenic effects in zebrafish. *Environ. Pollut.* **2016**, *218*, 702–708. [CrossRef] [PubMed]

107. Du, G.; Hu, J.; Huang, H.; Qin, Y.; Han, X.; Wu, D.; Song, L.; Xia, Y.; Wang, X. Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of endocrine-related genes in vitro and in vivo. *Environ. Toxicol. Chem.* **2013**, *32*, 353–360. [CrossRef] [PubMed]

108. Qu, J.-H.; Lu, C.-C.; Xu, C.; Chen, G.; Qiu, L.-L.; Jiang, J.-K.; Ben, S.; Wang, Y.-B.; Gu, A.-H.; Wang, X.-R. Perfluorooctane sulfonate-induced testicular toxicity and differential testicular expression of estrogen receptor in male mice. *Environ. Toxicol. Pharmacol.* **2016**, *45*, 150–157. [CrossRef] [PubMed]

109. Qiu, Z.; Qu, K.; Luan, F.; Liu, Y.; Zhu, Y.; Yuan, Y.; Li, H.; Zhang, H.; Hai, Y.; Zhao, C. Binding specificities of estrogen receptor with perfluorinated compounds: A cross species comparison. *Environ. Int.* **2020**, *134*, 105284. [CrossRef]

110. Qiu, L.; Wang, H.; Dong, T.; Huang, J.; Li, T.; Ren, H.; Wang, X.; Qu, J.; Wang, S. Perfluorooctane sulfonate (PFOS) disrupts testosterone biosynthesis via CREB/CRTC2/StAR signaling pathway in Leydig cells. *Toxicology* **2021**, *449*, 152663. [CrossRef]

111. Rodríguez-Jorquera, I.A.; Colli-Dula, R.C.; Kroll, K.; Jayasinghe, B.S.; Parachu Marco, M.V.; Silva-Sanchez, C.; Toor, G.S.; Denslow, N.D. Blood Transcriptomics Analysis of Fish Exposed to Perfluoro Alkyls Substances: Assessment of a Non-Lethal Sampling Technique for Advancing Aquatic Toxicology Research. *Environ. Sci. Technol.* **2019**, *53*, 1441–1452. [CrossRef]

112. Rosen, M.B.; Das, K.P.; Rooney, J.; Abbott, B.; Lau, C.; Corton, J.C. PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. *Toxicology* **2017**, *387*, 95–107. [CrossRef]

113. Xin, Y.; Wan, B.; Yu, B.; Fan, Y.; Chen, D.; Guo, L.-H. Chlorinated Polyfluoroalkylether Sulfonic Acids Exhibit Stronger Estrogenic Effects than Perfluorooctane Sulfonate by Activating Nuclear Estrogen Receptor Pathways. *Environ. Sci. Technol.* **2020**, *54*, 3455–3464. [CrossRef]

114. Xu, C.; Jiang, Z.-Y.; Liu, Q.; Liu, H.; Gu, A. Estrogen receptor beta mediates hepatotoxicity induced by perfluorooctane sulfonate in mouse. *Environ. Sci. Pollut. Res. Int.* **2017**, *24*, 13414–13423. [CrossRef]

115. Zhang, H.; Lu, H.; Chen, P.; Chen, X.; Sun, C.; Ge, R.-S.; Su, Z.; Ye, L. Effects of gestational Perfluorooctane Sulfonate exposure on the developments of fetal and adult Leydig cells in F1 males. *Environ. Pollut.* **2020**, *262*, 114241. [CrossRef] [PubMed]

116. Zhao, B.; Li, L.; Liu, J.; Li, H.; Zhang, C.; Han, P.; Zhang, Y.; Yuan, X.; Ge, R.-S.; Chu, Y. Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal Leydig cells. *PLoS ONE* **2014**, *9*, e78888. [CrossRef]

117. Zhong, S.-Q.; Chen, Z.-X.; Kong, M.-L.; Xie, Y.-Q.; Zhou, Y.; Qin, X.-D.; Paul, G.; Zeng, X.-W.; Dong, G.-H. Testosterone-Mediated Endocrine Function and TH1/TH2 Cytokine Balance after Prenatal Exposure to Perfluorooctane Sulfonate: By Sex Status. *Int. J. Mol. Sci.* **2016**, *17*, 1509. [CrossRef] [PubMed]

118. Lu, H.; Zhang, H.; Gao, J.; Li, Z.; Bao, S.; Chen, X.; Wang, Y.; Ge, R.; Ye, L. Effects of perfluorooctanoic acid on stem Leydig cell functions in the rat. *Environ. Pollut.* **2019**, *250*, 206–215. [CrossRef] [PubMed]

119. Wei, Y.; Dai, J.; Liu, M.; Wang, J.; Xu, M.; Zha, J.; Wang, Z. Estrogen-like properties of perfluorooctanoic acid as revealed by expressing hepatic estrogen-responsive genes in rare minnows (*Gobiocypris rarus*). *Environ. Toxicol. Chem.* **2007**, *26*, 2440–2447. [CrossRef]

120. Xin, Y.; Ren, X.-M.; Wan, B.; Guo, L.-H. Comparative In Vitro and In Vivo Evaluation of the Estrogenic Effect of Hexafluoropropylene Oxide Homologues. *Environ. Sci. Technol.* **2019**, *53*, 8371–8380. [CrossRef]

121. Yao, P.-L.; Ehresman, D.J.; Rae, J.M.; Chang, S.-C.; Frame, S.R.; Butenhoff, J.L.; Kennedy, G.L.; Peters, J.M. Comparative in vivo and in vitro analysis of possible estrogenic effects of perfluorooctanoic acid. *Toxicology* **2014**, *326*, 62–73. [CrossRef]

122. Zhao, Y.; Tan, Y.S.; Haslam, S.Z.; Yang, C. Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6 mice. *Toxicol. Sci.* **2010**, *115*, 214–224. [CrossRef]

123. Feng, Y.; Shi, Z.; Fang, X.; Xu, M.; Dai, J. Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis. *Toxicol. Lett.* **2009**, *190*, 224–230. [CrossRef]

124. Singh, S.; Singh, S.K. Prepubertal exposure to perfluorononanoic acid interferes with spermatogenesis and steroidogenesis in male mice. *Ecotoxicol. Environ. Saf.* **2019**, *170*, 590–599. [CrossRef]

125. Singh, S.; Singh, S.K. Acute exposure to perfluorononanoic acid in prepubertal mice: Effect on germ cell dynamics and an insight into the possible mechanisms of its inhibitory action on testicular functions. *Ecotoxicol. Environ. Saf.* **2019**, *183*, 109499. [CrossRef] [PubMed]

126. Gogola, J.; Hoffmann, M.; Nimpsz, S.; Ptak, A. Disruption of 17β-estradiol secretion by persistent organic pollutants present in human follicular fluid is dependent on the potential of ovarian granulosa tumor cell lines to metabolize estrogen. *Mol. Cell. Endocrinol.* **2020**, *503*, 110698. [CrossRef] [PubMed]

127. Halsne, R.; Tandberg, J.I.; Lobert, V.H.; Østby, G.C.; Thoen, E.; Ropstad, E.; Verhaegen, S. Effects of perfluorinated alkyl acids on cellular responses of MCF-10A mammary epithelial cells in monolayers and on acini formation in vitro. *Toxicol. Lett.* **2016**, *259*, 95–107. [CrossRef] [PubMed]

128. Maras, M.; Vanparys, C.; Muylle, F.; Robbens, J.; Berger, U.; Barber, J.L.; Blust, R.; De Coen, W. Estrogen-like properties of fluorotelomer alcohols as revealed by mcf-7 breast cancer cell proliferation. *Environ. Health Perspect.* **2006**, *114*, 100–105. [CrossRef]

129. Li, J.; Cao, H.; Feng, H.; Xue, Q.; Zhang, A.; Fu, J. Evaluation of the Estrogenic/Antiestrogenic Activities of Perfluoroalkyl Substances and Their Interactions with the Human Estrogen Receptor by Combining In Vitro Assays and In Silico Modeling. *Environ. Sci. Technol.* **2020**, *54*, 14514–14524. [CrossRef]

130. Ishibashi, H.; Yamauchi, R.; Matsuoka, M.; Kim, J.-W.; Hirano, M.; Yamaguchi, A.; Tominaga, N.; Arizono, K. Fluorotelomer alcohols induce hepatic vitellogenin through activation of the estrogen receptor in male medaka (*Oryzias latipes*). *Chemosphere* **2008**, *71*, 1853–1859. [CrossRef]

131. Buhrke, T.; Krüger, E.; Pevny, S.; Rößler, M.; Bitter, K.; Lampen, A. Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. *Toxicology* **2015**, *333*, 53–62. [CrossRef]

132. Rosenmai, A.K.; Nielsen, F.K.; Pedersen, M.; Hadrup, N.; Trier, X.; Christensen, J.H.; Vinggaard, A.M. Fluorochemicals used in food packaging inhibit male sex hormone synthesis. *Toxicol. Appl. Pharmacol.* **2013**, *266*, 132–142. [CrossRef]

133. Gogola, J.; Hoffmann, M.; Ptak, A. Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate IGF1 secretion by adult ovarian granulosa cell tumor spheroids and thereby increase proliferation of non-cancer ovarian granulosa cells. *Toxicol. In Vitro* **2020**, *65*, 104769. [CrossRef]

134. Dairkee, S.H.; Luciani-Torres, G.; Moore, D.H.; Jaffee, I.M.; Goodson, W.H., 3rd. A Ternary Mixture of Common Chemicals Perturbs Benign Human Breast Epithelial Cells More Than the Same Chemicals Do Individually. *Toxicol. Sci.* **2018**, *165*, 131–144. [CrossRef]

135. Chen, J.; Zheng, L.; Tian, L.; Wang, N.; Lei, L.; Wang, Y.; Dong, Q.; Huang, C.; Yang, D. Chronic PFOS Exposure Disrupts Thyroid Structure and Function in Zebrafish. *Bull. Environ. Contam. Toxicol.* **2018**, *101*, 75–79. [CrossRef] [PubMed]

136. Kim, S.; Ji, K.; Lee, S.; Lee, J.; Kim, J.; Kim, S.; Kho, Y.; Choi, K. Perfluorooctane sulfonic acid exposure increases cadmium toxicity in early life stage of zebrafish, *Danio rerio*. *Environ. Toxicol. Chem.* **2011**, *30*, 870–877. [CrossRef] [PubMed]

137. Ren, X.-M.; Zhang, Y.-F.; Guo, L.-H.; Qin, Z.-F.; Lv, Q.-Y.; Zhang, L.-Y. Structure-activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. *Arch. Toxicol.* **2015**, *89*, 233–242. [CrossRef] [PubMed]

138. Shi, X.; Liu, C.; Wu, G.; Zhou, B. Waterborne exposure to PFOS causes disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. *Chemosphere* **2009**, *77*, 1010–1018, Erratum in *Chemosphere* **2010**, *81*, 821. [CrossRef] [PubMed]

139. Yu, W.-G.; Liu, W.; Liu, L.; Jin, Y.-H. Perfluorooctane sulfonate increased hepatic expression of OAPT2 and MRP2 in rats. *Arch. Toxicol.* **2011**, *85*, 613–621. [CrossRef]

140. Chang, S.C.; Thibodeaux, J.R.; Eastvold, M.L.; Ehresman, D.J.; Bjork, J.A.; Froehlich, J.W.; Lau, C.; Singh, R.J.; Wallace, K.B.; Butenhoff, J.L. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology* **2008**, *243*, 330–339. [CrossRef]

141. Deng, M.; Wu, Y.; Xu, C.; Jin, Y.; He, X.; Wan, J.; Yu, X.; Rao, H.; Tu, W. Multiple approaches to assess the effects of F-53B, a Chinese PFOS alternative, on thyroid endocrine disruption at environmentally relevant concentrations. *Sci. Total Environ.* **2018**, *624*, 215–224. [CrossRef]

142. Hong, S.-H.; Lee, S.H.; Yang, J.Y.; Lee, J.H.; Jung, K.K.; Seok, J.H.; Kim, S.-H.; Nam, K.T.; Jeong, J.; Lee, J.K.; et al. Orally Administered 6:2 Chlorinated Polyfluorinated Ether Sulfonate (F-53B) Causes Thyroid Dysfunction in Rats. *Toxics* **2020**, *8*, 54. [CrossRef]

143. Godfrey, A.; Hooser, B.; Abdelmoneim, A.; Sepúlveda, M.S. Sex-specific endocrine-disrupting effects of three halogenated chemicals in Japanese medaka. *J. Appl. Toxicol.* **2019**, *39*, 1215–1223. [CrossRef]

144. Kim, J.; Lee, G.; Lee, Y.-M.; Zoh, K.-D.; Choi, K. Thyroid disrupting effects of perfluoroundecanoic acid and perfluorotridecanoic acid in zebrafish (*Danio rerio*) and rat pituitary (GH3) cell line. *Chemosphere* **2021**, *262*, 128012. [CrossRef]

145. Conley, J.M.; Lambright, C.S.; Evans, N.; McCord, J.; Strynar, M.J.; Hill, D.; Medlock-Kakaley, E.; Wilson, V.S.; Gray, L.E., Jr. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. *Environ. Int.* **2021**, *146*, 106204. [CrossRef] [PubMed]

146. Liu, Y.; Wang, J.; Fang, X.; Zhang, H.; Dai, J. The thyroid-disrupting effects of long-term perfluorononanoate exposure on zebrafish (*Danio rerio*). *Ecotoxicology* **2011**, *20*, 47–55. [CrossRef] [PubMed]

147. Ramhøj, L.; Hass, U.; Gilbert, M.E.; Wood, C.; Svingen, T.; Usai, D.; Vinggaard, A.M.; Mandrup, K.; Axelstad, M. Evaluating thyroid hormone disruption: Investigations of long-term neurodevelopmental effects in rats after perinatal exposure to perfluorohexane sulfonate (PFHxS). *Sci. Rep.* **2020**, *10*, 2672. [CrossRef] [PubMed]

148. Cassone, C.G.; Vongphachan, V.; Chiu, S.; Williams, K.L.; Letcher, R.J.; Pelletier, E.; Crump, D.; Kennedy, S.W. In ovo effects of perfluorohexane sulfonate and perfluorohexanoate on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. *Toxicol. Sci.* **2012**, *127*, 216–224. [CrossRef]

149. Harris, M.W.; Uraih, L.C.; Birnbaum, L.S. Acute toxicity of perfluorodecanoic acid in C57BL/6 mice differs from 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam. Appl. Toxicol.* **1989**, *13*, 723–736. [CrossRef]

150. Ren, X.-M.; Qin, W.-P.; Cao, L.-Y.; Zhang, J.; Yang, Y.; Wan, B.; Guo, L.-H. Binding interactions of perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological implications. *Toxicology* **2016**, *366–367*, 32–42. [CrossRef]

151. Song, M.; Kim, Y.-J.; Park, Y.-K.; Ryu, J.-C. Changes in thyroid peroxidase activity in response to various chemicals. *J. Environ. Monit.* **2012**, *14*, 2121–2126. [CrossRef]

152. Selano, J.; Richardson, V.; Washington, J.; Mazur, C. Characterization of non-radiolabeled Thyroxine (T4) uptake in cryopreserved rat hepatocyte suspensions: Pharmacokinetic implications for PFOA and PFOS chemical exposure. *Toxicol. In Vitro* **2019**, *58*, 230–238. [CrossRef]

153. Xin, Y.; Ren, X.-M.; Ruan, T.; Li, C.-H.; Guo, L.-H.; Jiang, G. Chlorinated Polyfluoroalkylether Sulfonates Exhibit Similar Binding Potency and Activity to Thyroid Hormone Transport Proteins and Nuclear Receptors as Perfluorooctanesulfonate. *Environ. Sci. Technol.* **2018**, *52*, 9412–9418. [CrossRef]

154. Weiss, J.M.; Andersson, P.L.; Lamoree, M.H.; Leonards, P.E.; van Leeuwen, S.P.; Hamers, T. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. *Toxicol. Sci.* **2009**, *109*, 206–216. [CrossRef]

155. Long, M.; Ghisari, M.; Bonefeld-Jørgensen, E.C. Effects of perfluoroalkyl acids on the function of the thyroid hormone and the aryl hydrocarbon receptor. *Environ. Sci. Pollut. Res. Int.* **2013**, *20*, 8045–8056. [CrossRef] [PubMed]

156. Buckalew, A.R.; Wang, J.; Murr, A.S.; Deisenroth, C.; Stewart, W.M.; Stoker, T.E.; Laws, S.C. Evaluation of potential sodium-iodide symporter (NIS) inhibitors using a secondary Fischer rat thyroid follicular cell (FRTL-5) radioactive iodide uptake (RAIU) assay. *Arch. Toxicol.* **2020**, *94*, 873–885. [CrossRef] [PubMed]

157. Wang, J.; Hallinger, D.R.; Murr, A.S.; Buckalew, A.R.; Lougee, R.R.; Richard, A.M.; Laws, S.C.; Stoker, T.E. High-throughput screening and chemotype-enrichment analysis of ToxCast phase II chemicals evaluated for human sodium-iodide symporter (NIS) inhibition. *Environ. Int.* **2019**, *126*, 377–386. [CrossRef] [PubMed]

158. Song, M.; Kim, Y.-J.; Song, M.-K.; Choi, H.-S.; Park, Y.-K.; Ryu, J.-C. Identification of classifiers for increase or decrease of thyroid peroxidase activity in the FTC-238/hTPO recombinant cell line. *Environ. Sci. Technol.* **2011**, *45*, 7906–7914. [CrossRef] [PubMed]

159. Chambers, W.S.; Hopkins, J.G.; Richards, S.M. A Review of Per- and Polyfluorinated Alkyl Substance Impairment of Reproduction. *Front. Toxicol.* **2021**, *3*, 732436. [CrossRef] [PubMed]

160. Kiess, W.; Häussler, G.; Vogel, M. Endocrine-disrupting chemicals and child health. *Best Pract. Res. Clin. Endocrinol. Metab.* **2021**, *35*, 101516. [CrossRef]

161. Bangma, J.; Guillette, T.C.; Bommarito, P.A.; Ng, C.; Reiner, J.L.; Lindstrom, A.B.; Strynar, M.J. Understanding the dynamics of physiological changes, protein expression, and PFAS in wildlife. *Environ. Int.* **2022**, *159*, 107037. [CrossRef]

162. Dankers, A.C.; Roelofs, M.J.; Piersma, A.H.; Sweep, F.C.; Russel, F.G.; van den Berg, M.; van Duursen, M.B.; Masereeuw, R. Endocrine disruptors differentially target ATP-binding cassette transporters in the blood-testis barrier and affect Leydig cell testosterone secretion in vitro. *Toxicol. Sci.* **2013**, *136*, 382–391. [CrossRef]

163. Kummu, M.; Sieppi, E.; Koponen, J.; Laatio, L.; Vähäkangas, K.; Kiviranta, H.; Rautio, A.; Myllynen, P. Organic anion transporter 4 (OAT 4) modifies placental transfer of perfluorinated alkyl acids PFOS and PFOA in human placental ex vivo perfusion system. *Placenta* **2015**, *36*, 1185–1191. [CrossRef]

164. Temkin, A.M.; Hocevar, B.A.; Andrews, D.Q.; Naidenko, O.V.; Kamendulis, L.M. Application of the Key Characteristics of Carcinogens to Per and Polyfluoroalkyl Substances. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1668. [CrossRef]

165. Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V.; Squadrito, F.; Altavilla, D.; Bitto, A. Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Med. Cell. Longev.* **2017**, *2017*, 8416763. [CrossRef] [PubMed]

166. Sinenko, S.A.; Starkova, T.Y.; Kuzmin, A.A.; Tomilin, A.N. Physiological Signaling Functions of Reactive Oxygen Species in Stem Cells: From Flies to Man. *Front. Cell Dev. Biol.* **2021**, *9*, 714370. [CrossRef] [PubMed]

167. Redza-Dutordoir, M.; Averill-Bates, D.A. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim. Biophys. Acta* **2016**, *1863*, 2977–2992. [CrossRef] [PubMed]

168. Marcon, B.H.; Shigunov, P.; Spangenberg, L.; Pereira, I.T.; de Aguiar, A.M.; Amorín, R.; Rebelatto, C.K.; Correa, A.; Dallagiovanna, B. Cell cycle genes are downregulated after adipogenic triggering in human adipose tissue-derived stem cells by regulation of mRNA abundance. *Sci. Rep.* **2019**, *9*, 5611. [CrossRef]

169. Castro, J.P.; Grune, T.; Speckmann, B. The two faces of reactive oxygen species (ROS) in adipocyte function and dysfunction. *Biol. Chem.* **2016**, *397*, 709–724. [CrossRef]

170. de Villiers, D.; Potgieter, M.; Ambele, M.A.; Adam, L.; Durandt, C.; Pepper, M.S. The Role of Reactive Oxygen Species in Adipogenic Differentiation. *Adv. Exp. Med. Biol.* **2018**, *1083*, 125–144. [CrossRef]

171. Zhang, Y.; Khan, D.; Delling, J.; Tobiasch, E. Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. *Sci. World J.* **2012**, *2012*, 793823. [CrossRef]

172. Rinne, N.; Christie, E.L.; Ardasheva, A.; Kwok, C.H.; Demchenko, N.; Low, C.; Tralau-Stewart, C.; Fotopoulou, C.; Cunnea, P. Targeting the PI3K/AKT/mTOR pathway in epithelial ovarian cancer, therapeutic treatment options for platinum-resistant ovarian cancer. *Cancer Drug Resist.* **2021**, *4*, 573–595. [CrossRef]

173. Yudushkin, I. Control of Akt activity and substrate phosphorylation in cells. *IUBMB Life* **2020**, *72*, 1115–1125. [CrossRef]

174. Guru, A.; Issac, P.K.; Velayutham, M.; Saraswathi, N.T.; Arshad, A.; Arockiaraj, J. Molecular mechanism of down-regulating adipogenic transcription factors in 3T3-L1 adipocyte cells by bioactive anti-adipogenic compounds. *Mol. Biol. Rep.* **2021**, *48*, 743–761. [CrossRef]

175. Morrison, D.K. MAP kinase pathways. *Cold Spring Harb. Perspect. Biol.* **2012**, *4*, a011254. [CrossRef] [PubMed]

176. Kirtonia, A.; Sethi, G.; Garg, M. The multifaceted role of reactive oxygen species in tumorigenesis. *Cell. Mol. Life Sci.* **2020**, *77*, 4459–4483. [CrossRef] [PubMed]

177. Kvandová, M.; Majzúnová, M.; Dovinová, I. The role of PPARgamma in cardiovascular diseases. *Physiol. Res.* **2016**, *65* (Suppl. 3), S343–S363. [CrossRef] [PubMed]

178. Son, Y.; Kim, S.; Chung, H.-T.; Pae, H.-O. Reactive oxygen species in the activation of MAP kinases. *Methods Enzymol.* **2013**, *528*, 27–48. [CrossRef] [PubMed]

179. Watkins, A.M.; Wood, C.R.; Lin, M.T.; Abbott, B.D. The effects of perfluorinated chemicals on adipocyte differentiation in vitro. *Mol. Cell. Endocrinol.* **2015**, *400*, 90–101. [CrossRef] [PubMed]

180. D'Oria, R.; Laviola, L.; Giorgino, F.; Unfer, V.; Bettocchi, S.; Scioscia, M. PKB/Akt and MAPK/ERK phosphorylation is highly induced by inositols: Novel potential insights in endothelial dysfunction in preeclampsia. *Pregnancy Hypertens.* **2017**, *10*, 107–112. [CrossRef]

181. Shao, J.; Yamashita, H.; Qiao, L.; Friedman, J.E. Decreased Akt kinase activity and insulin resistance in C57BL/KsJ-Leprdb/db mice. *J. Endocrinol.* **2000**, *167*, 107–115. [CrossRef]

182. Shabalina, I.G.; Kramarova, T.V.; Mattsson, C.L.; Petrovic, N.; Rahman Qazi, M.; Csikasz, R.I.; Chang, S.C.; Butenhoff, J.; DePierre, J.W.; Cannon, B.; et al. The Environmental Pollutants Perfluorooctane Sulfonate and Perfluorooctanoic Acid Upregulate Uncoupling Protein 1 (UCP1) in Brown-Fat Mitochondria through a UCP1-Dependent Reduction in Food Intake. *Toxicol. Sci.* **2015**, *146*, 334–343. [CrossRef]

183. Tan, X.; Xie, G.; Sun, X.; Li, Q.; Zhong, W.; Qiao, P.; Sun, X.; Jia, W.; Zhou, Z. High fat diet feeding exaggerates perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic pathways. *PLoS ONE* **2013**, *8*, e61409. [CrossRef]

184. Lind, P.M.; Lind, L.; Salihovic, S.; Ahlström, H.; Michaelsson, K.; Kullberg, J.; Strand, R. Serum levels of perfluoroalkyl substances (PFAS) and body composition—A cross-sectional study in a middle-aged population. *Environ. Res.* **2022**, *209*, 112677. [CrossRef]

185. Starling, A.P.; Adgate, J.L.; Hamman, R.F.; Kechris, K.; Calafat, A.M.; Ye, X.; Dabelea, D. Perfluoroalkyl Substances during Pregnancy and Offspring Weight and Adiposity at Birth: Examining Mediation by Maternal Fasting Glucose in the Healthy Start Study. *Environ. Health Perspect.* **2017**, *125*, 067016. [CrossRef] [PubMed]

186. Chen, M.-H.; Ng, S.; Hsieh, C.-J.; Lin, C.-C.; Hsieh, W.-S.; Chen, P.-C. The impact of prenatal perfluoroalkyl substances exposure on neonatal and child growth. *Sci.Total Environ.* **2017**, *607–608*, 669–675, Erratum in *Sci. Total Environ.* **2018**, *644*, 1653. [CrossRef] [PubMed]

187. Horikoshi, T.; Nishimura, T.; Nomura, Y.; Iwabuchi, T.; Itoh, H.; Takizawa, T.; Tsuchiya, K.J. Umbilical cord serum concentrations of perfluorooctane sulfonate, perfluorooctanoic acid, and the body mass index changes from birth to 5 1/2 years of age. *Sci. Rep.* **2021**, *11*, 19789. [CrossRef] [PubMed]

188. Tanner, E.M.; Bornehag, C.G.; Gennings, C. Dynamic growth metrics for examining prenatal exposure impacts on child growth trajectories: Application to perfluorooctanoic acid (PFOA) and postnatal weight gain. *Environ. Res.* **2020**, *182*, 109044. [CrossRef] [PubMed]

189. Starling, A.P.; Liu, C.; Shen, G.; Yang, I.V.; Kechris, K.; Borengasser, S.J.; Boyle, K.E.; Zhang, W.; Smith, H.A.; Calafat, A.M.; et al. Prenatal Exposure to Per- and Polyfluoroalkyl Substances, Umbilical Cord Blood DNA Methylation, and Cardio-Metabolic Indicators in Newborns: The Healthy Start Study. *Environ. Health Perspect.* **2020**, *128*, 127014. [CrossRef] [PubMed]

190. Fenton, S.E.; Ducatman, A.; Boobis, A.; DeWitt, J.C.; Lau, C.; Ng, C.; Smith, J.S.; Roberts, S.M. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environ. Toxicol. Chem.* **2021**, *40*, 606–630. [CrossRef] [PubMed]

191. White, S.S.; Calafat, A.M.; Kuklenyik, Z.; Villanueva, L.; Zehr, R.D.; Helfant, L.; Strynar, M.J.; Lindstrom, A.B.; Thibodeaux, J.R.; Wood, C.; et al. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol. Sci.* **2007**, *96*, 133–144. [CrossRef]

192. Lau, C.; Thibodeaux, J.R.; Hanson, R.G.; Narotsky, M.G.; Rogers, J.M.; Lindstrom, A.B.; Strynar, M.J. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol. Sci.* **2006**, *90*, 510–518. [CrossRef]

193. Koustas, E.; Lam, J.; Sutton, P.; Johnson, P.I.; Atchley, D.S.; Sen, S.; Robinson, K.A.; Axelrad, D.A.; Woodruff, T.J. The Navigation Guide—Evidence-based medicine meets environmental health: Systematic review of nonhuman evidence for PFOA effects on fetal growth. *Environ. Health Perspect.* **2014**, *122*, 1015–1027. [CrossRef]

194. Zhang, Y.; Cao, X.; Chen, L.; Qin, Y.; Xu, Y.; Tian, Y.; Chen, L. Exposure of female mice to perfluorooctanoic acid suppresses hypothalamic kisspeptin-reproductive endocrine system through enhanced hepatic fibroblast growth factor 21 synthesis, leading to ovulation failure and prolonged dioestrus. *J. Neuroendocrinol.* **2020**, *32*, e12848. [CrossRef]

195. Wang, X.; Bai, Y.; Tang, C.; Cao, X.; Chang, F.; Chen, L. Impact of Perfluorooctane Sulfonate on Reproductive Ability of Female Mice through Suppression of Estrogen Receptor α-Activated Kisspeptin Neurons. *Toxicol. Sci.* **2018**, *165*, 475–486. [CrossRef] [PubMed]

196. Bjerregaard-Olesen, C.; Ghisari, M.; Bonefeld-Jørgensen, E.C. Activation of the estrogen receptor by human serum extracts containing mixtures of perfluorinated alkyl acids from pregnant women. *Environ. Res.* **2016**, *151*, 71–79. [CrossRef] [PubMed]

197. Chen, P.; Wang, Q.; Chen, M.; Yang, J.; Wang, R.; Zhong, W.; Zhu, L.; Yang, L. Antagonistic Estrogenic Effects Displayed by Bisphenol AF and Perfluorooctanoic Acid on Zebrafish (*Danio rerio*) at an Early Developmental Stage. *Environ. Sci. Technol. Lett.* **2018**, *5*, 655–661. [CrossRef]

198. Mora, A.M.; Oken, E.; Rifas-Shiman, S.L.; Webster, T.F.; Gillman, M.W.; Calafat, A.M.; Ye, X.; Sagiv, S.K. Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood. *Environ. Health Perspect.* **2017**, *125*, 467–473. [CrossRef]

199. Halldorsson, T.I.; Rytter, D.; Haug, L.S.; Bech, B.H.; Danielsen, I.; Becher, G.; Henriksen, T.B.; Olsen, S.F. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: A prospective cohort study. *Environ. Health Perspect.* **2012**, *120*, 668–673. [CrossRef]

200. Contempré, B.; Jauniaux, E.; Calvo, R.; Jurkovic, D.; Campbell, S.; de Escobar, G.M. Detection of thyroid hormones in human embryonic cavities during the first trimester of pregnancy. *J. Clin. Endocrinol. Metab.* **1993**, *77*, 1719–1722. [CrossRef]

201. Vulsma, T.; Gons, M.H.; de Vijlder, J.J. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. *N. Engl. J. Med.* **1989**, *321*, 13–16. [CrossRef]

202. Fisher, D.A. Fetal thyroid function: Diagnosis and management of fetal thyroid disorders. *Clin. Obstet. Gynecol.* **1997**, *40*, 16–31. [CrossRef]

203. Wang, Y.; Rogan, W.J.; Chen, P.-C.; Lien, G.-W.; Chen, H.-Y.; Tseng, Y.-C.; Longnecker, M.P.; Wang, S.-L. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. *Environ. Health Perspect.* **2014**, *122*, 529–534. [CrossRef]

204. Idris, I.; Srinivasan, R.; Simm, A.; Page, R.C. Maternal hypothyroidism in early and late gestation: Effects on neonatal and obstetric outcome. *Clin. Endocrinol.* **2005**, *63*, 560–565. [CrossRef]

205. Sahu, M.T.; Das, V.; Mittal, S.; Agarwal, A.; Sahu, M. Overt and subclinical thyroid dysfunction among Indian pregnant women and its effect on maternal and fetal outcome. *Arch. Gynecol. Obstet.* **2010**, *281*, 215–220. [CrossRef] [PubMed]

206. Boesen, S.A.H.; Long, M.; Wielsøe, M.; Mustieles, V.; Fernandez, M.F.; Bonefeld-Jørgensen, E.C. Exposure to Perflouroalkyl acids and foetal and maternal thyroid status: A review. *Environ. Health* **2020**, *19*, 107. [CrossRef] [PubMed]

207. Vernier, M.; Dufour, C.R.; McGuirk, S.; Scholtes, C.; Li, X.; Bourmeau, G.; Kuasne, H.; Park, M.; St-Pierre, J.; Audet-Walsh, E.; et al. Estrogen-related receptors are targetable ROS sensors. *Genes Dev.* **2020**, *34*, 544–559. [CrossRef] [PubMed]

208. Vuguin, P.M. Animal models for small for gestational age and fetal programming of adult disease. *Horm. Res. Paediatr.* **2007**, *68*, 113–123. [CrossRef]

209. Halappanavar, S.; van den Brule, S.; Nymark, P.; Gaté, L.; Seidel, C.; Valentino, S.; Zhernovkov, V.; Høgh Danielsen, P.; De Vizcaya, A.; Wolff, H.; et al. Adverse outcome pathways as a tool for the design of testing strategies to support the safety assessment of emerging advanced materials at the nanoscale. *Part. Fibre Toxicol.* **2020**, *17*, 16. [CrossRef]

210. Schaefer, M.H.; Yang, J.-S.; Serrano, L.; Kiel, C. Protein conservation and variation suggest mechanisms of cell type-specific modulation of signaling pathways. *PLoS Comput. Biol.* **2014**, *10*, e1003659. [CrossRef]

211. Peiris, T.H.; Ramirez, D.; Barghouth, P.G.; Oviedo, N.J. The Akt signaling pathway is required for tissue maintenance and regeneration in planarians. *BMC Dev. Biol.* **2016**, *16*, 7. [CrossRef]